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Review Article

Impact of vermicompost and Rhizobacteria in organic agriculture on plant growth and soil health: A review

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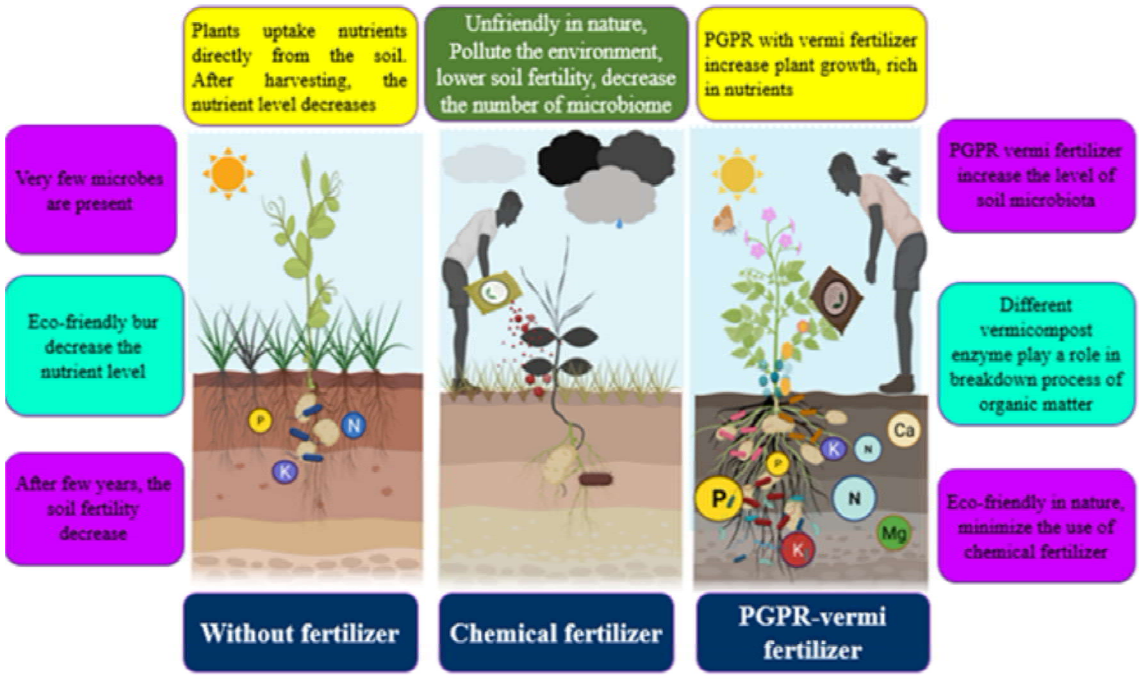
GRAPHICAL ABSTRACT



ABSTRACT

One of the consequences of climate change is a decline in land productivity, which leads to a decrease in agricultural output. The usage of inorganic fertilizers will result in a greater reduction in land quality. Vermicompost and plant growth-promoting rhizobacteria (PGPR) are two other answers to these issues. These manures include organically bonded micro and macro elements that impact the growth and development of plants, as well as soil sustainability. The favourable consequences of PGPR on soil and crop were improved by vermicompost, with the level of promotion dependent on vermicompost dosage and crop type. The PGPR dramatically decreased soil carbon and nitrogen while increasing the soil's microbe-biomass nitrogen and carbon in the presence of vermicompost. Vermicomposting is a low-tech, ecologically benign method of treating organic waste. Application of vermicompost with PGPR in an effort to mitigate climate change by promoting crop growth and production. This study, which also serves as a review, will show how PGPR and vermicompost can be utilized to promote sustainable agriculture while reducing the usage of synthetic manure and the role of vermicomposting in salt conditions and their effects on plants. The major goal of this review is to give information on the earthworm-microbe-soil-vermicompost interaction dynamics.

***Keywords*:** Organic farming, soil fertility, vermicompost, PGPR, sustainable agriculture.

Today, increasing community concerns have pushed many nations to utilize pesticides and fertilizers to boost agriculture production in an effort to satisfy their rising food demands (Yadav *et al*., 2013). Nonetheless, the extended and inappropriate need for synthetic manure has culminated in health concerns for humans and soil, in addition to environmental damage. Farmers in wealthy nations are thus pushed to upgrade their current farms to "natural or organic farming (OF's)" (Yadav *et al*., 2013). The OF's is a farming method that eliminates or limits the usage of chemical fertilizers, herbicides, regulators for growth, and feed additives for livestock. The fundamental principles of organic farming are sustainability in terms of the environment, society, and economy (Stockdale *et al*., 2001). One of the most important aspects of organic farming is that it maintains soil fertility over time by sustaining organic-matter content, and nitrogen self-sufficiency via leguminous, and biological nitrogen fixation (BNF). It is also effective for organic matter recycling and pest, or disease control based mostly on rotational crop systems (Yadav *et al*., 2013). The biological community of soils generally includes soil's living organisms, such as insects, earthworms, nematodes, mammals, microbes, and plant roots (Park *et al*., 2010).

The cycling of nitrogen (N) and carbon (C), the breakdown of agricultural residues, and the promotion of plant development are all important functions performed by soil microflora (bacteria, archaea, and fungi. Ranchers are used to directly control soil physicochemical components (such as pH, soil texture, and nutrient availability), but precisely managing soil's biological component, the microorganism component, presents barriers (Pathma and Sakthivel, 2012). Beneficial microorganisms are essential in organic agricultural soils for nutrient supply, bio-control activity, and decomposition of organic matter (Yadav *et al*., 2013). In addition, harmful microbes could have a deleterious effect on crop yield, soil health, and, in severe situations, harvest damage. PGPR plays an important function in root and shoot development, (Triharyanto *et al*., 2021) providing essential nutrients to plants, helping in phosphate solubilization, N-fixing, and yield increases in this danger (Katiyar *et al*., 2016). PGPR are helpful rhizobacteria that are found in the rhizosphere of roots. When used as a biofertilizer, PGPR promotes plant development and offers all required nutrients to the plants (Kumar *et al*., 2022) while also functioning as a bio-control agent (Sharma *et al*., 2017). Vermicompost increased the favorable effects of PGPR on soil nutrients, microbial biomass, crop production, and crop quality. Earthworms and bacteria collaborate throughout the biodegradation process to generate vermicompost, which is worm feces mixed with worm castings. According to research on earthworm microorganisms, the soil macro fauna, particularly earthworm's is an important component, along with microbes, across a wide range of climate, and soil that are engaged directly or indirectly in organic matter breakdown and soil stability (Munnoli, 2015). Vermicompost improves crop performance by increasing the quantity of branches and fruits (Singh *et al*., 1997). This review study discusses vermicompost's' bacterial biodiversity (PGPR) and nutritional status, as well as their usefulness in agriculture and agricultural productivity.

Meaning of vermicomposting and how it assists plants to absorb nutrients from the soil

The term "vermicomposting" refers to the biological oxidation process, which is non-thermophilic in nature (Munnoli, 2015). In this process, the organic matter is transformed into vermicompost through interactions between earthworms and beneficial microflora like bacteria. The Vermicompost is a peat-like substance with abundant microbial activity, aeration, high permeability, and water-holding capacity (Arancon *et al*., 2004a). Earthworms are commonly referred to as "farmers' friends" or "nature's ploughman." Vermicompost includes plant-accessible minerals such as soluble potassium (K), magnesium (Mg), nitrates (NO_3), calcium (Ca), and exchangeable phosphorus (P), as well as a huge specific surface community that offers multiple micro or mini-sites for bacterial performance and good nutrient possession (Pathma and Sakthivel, 2012). Earthworms and bacteria collaborate in the biodegradation process to generate vermicompost, which is worm feces mixed with worm castings. The gut of earthworms includes a widely varying spectrum of microbes, hormones, and enzymes which help in the fast breakdown of partially eaten food, converting this into vermicompost in about 4-8 weeks (Nagavallemma *et al*., 2004). Figure 1 depicts earthworms, which are found in farmyard manure.

Fig. 1. Earthworms are commonly referred to be "farmers' friends".

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Vermicompost provides macroelements including P, N, K, Mg, and Ca as well as micro-elements like Mo, Fe, Cu, and Zn (Khan and Ishaq, 2011). The nitrogen, phosphorus, and potassium content of the vermicompost were 0.74, 0.97, and 0.45 per cent, respectively. Earthworms have an impact on the soil's microbial ecology, as well as its physical and chemical qualities (Pathma and Sakthivel, 2012). Utilizing earthworms as adaptable organic bioprocesses for the breakdown of organic waste, vermicomposting is a successful technique for the recycling of nutrients. Because of its high microbial activity and availability of nutrients, vermicompost with PGPR bacteria improves soil health, promotes crop production and plant development, and reduces the number of pests and plant diseases (Lazcano and Dominguez, 2011). The capacity of nature's ploughman to transform waste material into gold. "Aristotle referred to them as the intestine of the earth because they could metabolize a wide range of biological or organic matter and Earthworms were regarded by Charles Darwin as the unheralded warriors of mankind" (Bonney, 1903). Plant growth was greater in soil supplemented with vermicompost than in soils treated with synthetic or harmful manures or animal compost (Arancon *et al*., 2004). Vermicompost increased the favourable effects of PGPR on soil nutrients, microbial biomass, crop production, and crop quality (Pathma and Sakthivel, 2012). Vermicompost and PGPR had a considerable synergistic effect on crop quality, with crop nitrate concentrations dramatically decreasing while vitamin C and soluble protein in spinach greatly increased (Song *et al*., 2015).

Vermicompost has been statistically proven to be a miracle plant growth enhancer. They decompose plant litter and heavy soil particles, increasing the levels of natural or organic materials for the breakdown of microbials and converting biological wastes into useful vermicompost (Kumar *et al*., 2022). It is an aerobic, biological process that is effective at converting organic materials that resemble humus for the environment (Chanda *et al*., 2011). Vermicompost enhances nutrient content in the soil, boosts O_2availability, influences soil microbial activity, maintains normal temperature in the soil, increases water infiltration, and soil porosity, and promotes plant growth, and yield (Arora *et al*., 2011). Furthermore, compost improves the soil's ability to hold nutrients while also supplying much-needed nutrients to the plants. Increase the cation-exchange-capacity (CEC) of the soil, it raising nutrient retention and then provides the plants with the potassium, nitrogen, and phosphate, they needed. Although the nutrients in compost products vary greatly, biosolids and composts made with animal dung often have higher nutritional values overall. During the first 6 to 12 months after its application, the usage of specific composts may minimize or eliminate the need to fertilize plants (Rekha *et al*., 2018).

Vermicompost-amended soil offers the necessary nutrients, which chemically treated soil lacks. Although, vermicompost-treated plants may have grown the most due to this improved nutrient uptake by plants, and increased NPK concentration. Pressmud can be vermicomposted utilizing the following earthworm species: *Eudrilus eugeniae, Eisenia fetida,* and *Perionyx excavatus*. This pressmud vermicompost exhibits a high nutritional content and enzymatic microbial activities that make it simple for plants to absorb nutrients (Parthasarthi and Ranganathan, 2002). Vermicomposted soil's carbon concentration is observed to limit the release of nutrients into the soil, assisting plants in absorbing the available nutrients (Rekha *et al*., 2018). They improve the organic matter content of the soil, including the "humic compounds," which affect nutrient accumulation and encourage root growth. They also provide balanced nutrients to plant roots that drive growth (Sinha *et al*., 2009). Additionally, a substantial amount of zinc (Zn), calcium (Ca), manganese (Mn), and magnesium (Mg) are present. Furthermore, vermicompost contains enzymes such as cellulase, amylase, chitinase, and lipase, which break down organic materials such as waste in the soil (to release nutrients and make them available to plant roots) even after they have been expelled. Additionally, they enrich the soil with beneficial microorganisms and feed the already present ones, enhancing their biological characteristics and the ability for soil renewal (Benjamin and Ohja, 2019).

Vermicomposting and the significance of PGPR in sustainable agriculture

The capacity of earthworms to boost plants' availability of nutrients is most probably reliant on the "earthworm gut" bacterial activity. The angleworm (earthworm) promotes and stimulates microbial activity by enhancing soil microorganisms, microbial, and biomass populations, as well as boosting aeration through burrowing (Pathma and Sakthivel, 2012). Earthworm-microorganism interactions appear to be complicated. Angleworms consume PGPRs such as *Rhizobium, Pseudomonas, Bacillus, Azotobacter, Azospirillum*, and others, which may be triggered or amplified due to the gut's favourable micro-habitats. Earthworms have been proposed as key agents capable of regulating the generation of PGPRs by microorganisms (Gopal *et al*., 2009) via the stimulation and encouragement of microbial activity in organic substrates. Earthworm activity, therefore, boosts the community of PGPR (Sinha *et al*., 2010). This particular bacterial community boosts plant development directly

through nutrient solubility (Naik *et al*., 2008), synthesis of growth hormones, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, nitrogen fixation (Han *et al*., 2005), and indirectly by reducing fungal infections. Because of its exceptional nutritional quality and heightened microorganism and antagonism activity, the use of PGPR-based vermicompost has lately increased (Bhardwaj *et al*., 2022). Table 1 displays the biological diversity of vermicompost with the activity of microbes such as PGPR and their positive features.

Microbial-based vermicompost addition in the field

By modifying the soil qualities over time, organic farming has been able to sustain better crop output and improve soil quality and productivity (Yadav *et al*., 2013). Compost comprises bacterium, fungi, and actinomycetes; as a result, a new supply of humic increased but promoted microorganisms (Balasubramanian *et al*., 1972). Farmers may introduce microorganisms to their soils for a variety of reasons. In principle, these additional microorganisms can help with nutrient availability (biofertilizers or bio-stimulants), insect control (biopesticides), or plant growth stimulation via hormone signaling (plant growth promoters [PGPs]; possibly also bio-stimulants.

Farmers may use microbial additions to increase the number of beneficial bacteria in their soils, introduce specific microorganisms directly connected to the benefit of a certain crop (Benjamin and Ohja, 2019), or improve nutrient availability (Sharma and Namdeo, 1999). Arbuscular-mycorrhizal fungus (AMF) may primarily help plants by improving phosphate absorption, water and nitrogen may also be delivered in modest amounts by the fungus (Hijri, 2016). In these interactions, a plant will give a microbe certain advantages, such as sugars as food, in return for the bacterium's resources, such as nutrients or water (Gaskin *et al*., 2013).

Nitrogen-fixing bacteria were also more abundant in

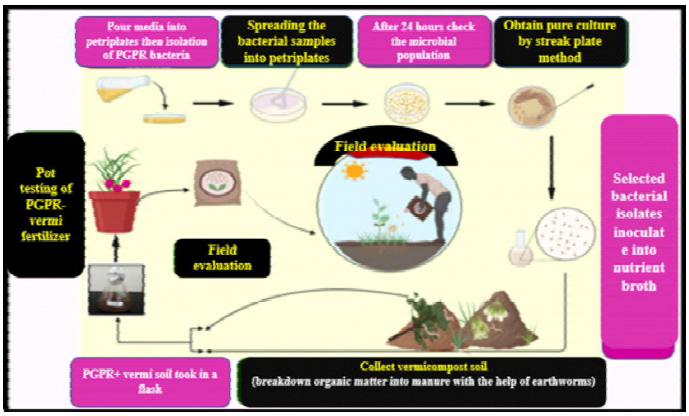

Fig. 2. The vermi-based microbial inoculation into the environment.

areas where vermicompost was sprayed following crop harvest (Munnoli, 2015). Legumes, for example, grow nodules on their roots to house rhizobia bacteria; the bacteria absorb nitrogen from the atmosphere and supply it to the plant, while the plant offers sugars and a perfect habitat for the rhizobia to live in. When planting, these inoculants can be used as a seed coat or directly applied to the soil (Fig. 2) (Gaskin *et al*., 2013). Another frequent practice is to inoculate the soil with mycorrhizal fungus. These fungi exist partly within the roots and partly in the soil. They get access to soil nutrients like nitrogen and phosphate, which they subsequently pass on to the plant. The AMF seems to be the most common form of mycorrhizal fungus that develop partnerships with agricultural plants. Glomus species are typically found in commercial AMF inoculants. While agricultural activities have an effect on the biophysiochemical characteristics of soils. Arthropod, bacterium, protozoans, and nematode densities were greater in organic agricultural soil than in traditionally farmed soils. According to Bulluck *et al.* (2002), natural or organic fertilizer additions increased helpful soil microflora, decreased the

Table 1: The biological diversity of vermicompost with the activity of microbes such as PGPR and their positive features.

Vermicompost Angleworms	PGPR bacterial species found in vermicompost	Properties	References
Unspecified	*Rhizobium trifolii*	Nitrogen (N) fixing and plant development in leguminous plants	Pathma and Sakthivel 2012
Lumbricus rubellus	*Pseudomonas putida, and Rhizobium japonicum*	Help in plant growth	Madsen and Alexander 1982
Aporrectodea trapezoids	*Rhizobium meliloti* 'L5.30'	Root nodulation in leguminous plants, and Enhancing N-fixation	Pathma and Sakthivel 2012
Eudrilus sp.	Free-living Nitrogen fixing bacteria, *Azotobacter, Azospirillum, Nitrobacter, Nitrosomonas,* Ammonifying bacteria, *Fluorescent Pseudomonads,* and Phosphate (P) solubilizing bacteria.	Phosphate solubilization, Nitrification, and control plant diseases by PGPR.	Gopal *et al.,* 2009 and Surekha 2007

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number of pathogen communities, total carbon (C), and exchange capacity of cations, and decreased bulk densities, all of which improved soil quality (Kumari *et al*., 2022).

Earthworms' role in soil biological, chemical, and physical quality

"The term soil conditioner refers to earthworms, which are also regarded as a bioindicator of soil fertility" (Sinha *et al*., 2010). Angleworms are an important indicator of the quality of the soil and land where they live. The angleworm (earthworm) promotes and stimulates microbial activity by enhancing soil microorganisms, microbial, and biomass populations, as well as boosting aeration through burrowing (Pathma and Sakthivel, 2012). Earthworms have an impact on the soil's microbial ecology, as well as its physical and chemical qualities (Pathma and Sakthivel, 2012). They result in a complete progression in the soil's biological characteristics (helpful soil bacteria such as PGPR and other living organisms such as plants, fungi, nematodes, and earthworms), physical features such as (flexibility and absorbency), and chemical properties like pH levels and required plant nutrition. Every day, they consume a considerable quantity of soil including living organisms, and metabolize them in their intestines with the help of enzymes (for example amylase, cellulase, lipase, and chitinase). The gut of earthworms includes a widely varying spectrum of microbes, hormones, and enzymes which help in the fast breakdown of partially eaten food, converting this into vermicomposts in about 4-8 weeks (Nagavallemma *et al*., 2004). Hardly 5 to 10 per cent of the material that has been chemically decomposed and consumed is taken into the body (Scheu, 1987); the remainder is expelled out as 'vermicastings,' which are fine 'mucus-coated granular aggregates' that are high in KNP (potassium, nitrate, and phosphorous), micro-minerals like Mo, Fe, Cu, and Zn (Khan and Ishaq, 2011), and advantageous soil bacteria (Sinha *et al*., 2010). Utilizing earthworms as adaptable organic bioprocesses for the breakdown of organic waste, vermicomposting is a successful technique for the recycling of nutrients and increasing the physical, biological, and chemical quality of the soil (Lazcano and Dominguez, 2011).

Vermicomposting's effects on salt stress and how it benefits the environment

Soil salinization is a crucial issue for agriculture and threatens sustainable development goals related to nutrition, food security, and resource preservation. The detrimental effects of rising salinity-stress levels are felt by soil physio-chemical properties as well as plant metabolism (Kumari *et al*., 2022). Sodium chloride is the most prevalent of these salts, and salinity is a mechanism that raises concentrations of salts in the soil. Salinity also has an adverse effect on the distribution and quantity of soil-dwelling microbes. Broad bean production, lipid content, nucleic acid, protein content, and enzyme activities of nitrogen and carbon metabolism are all severely impacted by the salinity problem (Dhanalakshmi *et al*., 2014). The long-term effects of salinity on plant growth, development, seed germination, viability, and yield output include electrolyte homeostasis, the toxicity of ions, nutritional disorders, osmotic stress, a decrease of cell division, cell- differentiation, and cell expansion, activation and synthesis of anti-oxidant enzymes, creation of nitric oxides and polyamines, and reduction in cell division, differentiation, and expansion. Due to its negative effects on agricultural growth and output, saline soil has become a global problem, especially in arid and semi-dry climates (Romero-Munar *et al*., 2019). Salinity has an impact on a variety of biological processes, including photosynthesis, respiration, nitrogen fixation, carbohydrate metabolism, nutrient absorption, and defense systems, and it eventually leads to plant death (Hanin *et al*., 2016). Reduced seed yield as well as also decrease fresh and dried plant weight are results of an increase in salty conditions. Salinity has a water-deficit effect on plants that makes it harder for them to absorb water.

There are two stages to the reactions of plants to salinity. Immediately following salt application, the first ionic independent phase (osmotic phase) can be seen, which inhibits cell division, reduces the intake of mineral nutrients like Ca^{2+}, K^{+}, and closes stomata (Isayenkov and Maathuis, 2019) Both salt-tolerant and salt-sensitive plant growth is impacted by a notable decline in shoot growth relative to root growth. The capacity of a plant to tolerate and overcome excessive salt in order to sustain its production is known as

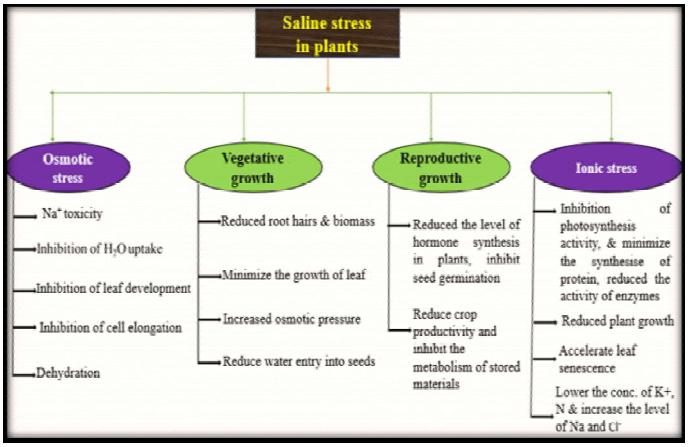

Fig. 3. The effects of salinity stress on ionic, osmotic, vegetative, and reproductive stresses in plants.

salt tolerance (Kumari *et al*., 2022). The ion-specific phase is focused on the buildup of cytotoxic ions, which block metabolic processes, result in early senescence, and ultimately lead to cell death (Fig. 3). The leaf blades become deposited with Na^+ as a result of this prolonged salt contact.

Additionally, it results in the premature senescence of leaves, decreased energy production, a reduction in the area used for photosynthetic activity, changes in the activity of different plant enzymes, and an increase in the production of reactive oxygen species (ROS), which can result in crop mortality (Chanda *et al*., 2011). In molecular analyses of plants' reactions to salt stress, reactive oxygen species (ROS) including superoxide (O_2), singlet oxygen (O_2), peroxide (H_2O_2), and radical (OH), have been identified (Ahmad *et al*., 2011). Plants possess a variety of antioxidant enzymes that help them protect themselves from the harmful effects of oxidative stress (catalase, peroxidase, and glutathione). Under stressful circumstances, glycine, proline, and betaine work together as suitable osmoprotectants and ROS scavengers (Kumari *et al*., 2022).

Brassica oleraceae is one family of species that can tolerate salt, other examples of species that tolerate salts are *Allium cepa* (Triharyanto *et al*., 2021), *Triticum aestivum*, *barley*, *Agave americana, Quercus, Evolvulus glomeratus*, and *Amaranthus tricolor* (Jha *et al*., 2019). Rice and cotton are salt-sensitive species. There is an urgent need to develop crops that can withstand high salt concentrations while still producing optimal yields.

To reduce the effects of salinity on plants, a variety of methods, including transgenic strategies, conventional breeding, and chemical use, were investigated. However, traditional methods do not mesh well with the research trials to manage the salinity issue. Traditional methods of managing agroecosystems mostly concentrate on supplying nutrients and water to make up for nutrient deficits rather than removing salt buildup. Proper use of organic fertilizer (such as vermicomposting), soil microbes, water-management programs, and environmental conditions can help to lessen the damaging consequences of salinization on plants (Fig. 4) (Kumari *et al*., 2022). Significant amounts of carbon are added to salt-affected soil by organic matter, which also enhances the quality of soil, reduces the bulk density of soil, increases water penetration, and also enhances microorganism activity in soil (Gopinath *et al*., 2008). Agriculture programs like organic farming have the ability to reduce soil salinity as knowledge of salt stress has developed. Beneficial microorganisms (PGPR, *Pseudomonas, Rhizobium*), P- solubilizer bacteria, and free-living soil bacteria such as (*Azotobacter* spp. and *Bacillus megatherium*), and arbuscular mycorrhizal fungi are a few examples of organic fertilizers. Alternative agricultural methods, including organic farming, have consequently opened up new chances to increase soil resources and sustainably use them. This tactic boosts plant output by supplying the macro- and micronutrients required for healthy growth (Kumari *et al*., 2022).

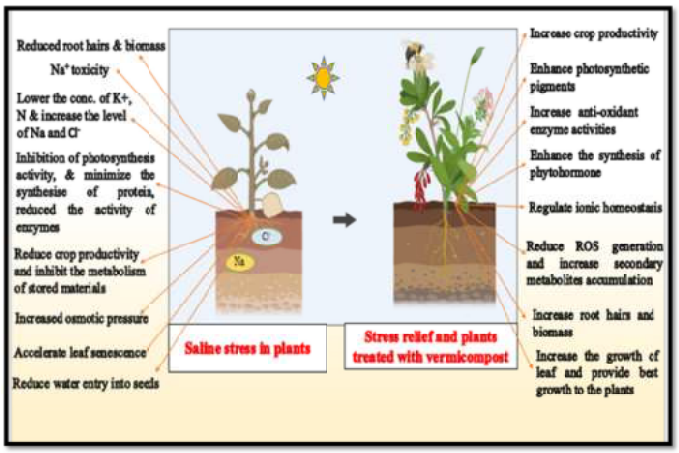

Fig. 4. The impacts of salt stress on plants and how vermicompost can help plants under stressful conditions.

The background story and movement in India to expend landfill waste via vermicomposting

Commercial-scale vermicomposting of Municipal waste solid (MSW), including "sewage sludge," is a growing trend to remove solid waste from landfills (Sinha *et al*., 2010). Municipal governments and composting businesses are both involved in the vermicomposting industry, decomposing a variety of organic wastes on a large scale, and selling the finished products to farmers. This has two advantages. reducing the cost of waste disposal in landfills while making money from the sale of worms and vermicompost (Sinha, 2009). In 1970, the Netherlands developed the first significant experiments for the treatment of municipal and industrial organic wastes. Later, England and Canada followed suit. America, Italy, the Philippines, Thailand, China, Korea, Japan, Brazil, France, Australia, Israel, and Russia later adopted vermiculture.

Vermicompost program in India

In the 1990s, India also started a vermicomposting program for municipal solid waste (MSW), and Bhawalkar Earthworms Research Institute (BERI) in Pune was one of the leading institutions at the time. The Delhi-based Tata Energy Research Institute (TERI) likewise accomplishes a remarkable job. It has expanded recently as a result of "sustainable non-chemical agriculture" and "poverty

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eradication" programs. Vermicompost is being used widely by farmers, and a revolution is underway. The vermicomposting industry has improved the lives of the underprivileged in India and created chances for independent work for the unemployed. The NGO's are encouraging men and women to collect garbage from villages and farmers, vermicompost them, and sell both worms and vermicompost to farmers in a number of Indian communities where they are freely distributing cement tanks and 1,000 worms. Every year, people who sell worms and their vermicompost to farmers make between 5 and 6 lakh rupees in income. In the vermiculture revolution, Bihar, Karnataka, Tamil Nadu, Gujarat, and Maharashtra are major states. To handle all municipal urban solid waste, the Karnataka Compost Development Corporation developed the first vermicomposting unit in the nation. This operation currently produces 150 to 200 tons of vermicompost daily from municipal waste (Kale, 2005). She has cited a number of farmers whose transformation from "poor farm labourer's" to "wealthy farmers" after adopting vermiculture. Composting is an environmentally acceptable and effective process of turning organic waste into stable and mature nutrients. Many effective strategies have been identified to enhance composting, including the inclusion of bulking agents, different additives, and microbial inoculants. This is because the emissions of N_2O, CH_4, and NH_3 are significant drawback of conventional composting. More research is needed to increase composting production in India in order to reduce gas emissions, antibiotic resistance genes, and heavy metals mobilization, as well as the role of genetically modified microbes (Sinha, 2009).

New advances, way forward and current research in vermicomposting

In order to get a wide range of natural or organic fertilizers for sustainable agriculture practices and to improve the importance of co-composting and vermicomposting practices throughout the transition to a circular economy, technology development based on research and advancement activities will be essential (Awasthi *et al.*, 2022). Vermicomposting with *Eisenia fetida* earthworms has been widely used to convert sludge into soil-improving fertilizer, according to previous review studies (Ghorbani *et al*., 2021). For the first time, *in-situ* microplastic biodegradation employing hyperthermophilic bacteria was shown to be possible by utilizing sludge composting (Chen *et al.*, 2022). Developing highly efficient microorganisms with the ability to improve the process of composting is an important research problem for bio-waste management, in addition to the creation of organic fertilizers (Chen *et al.*, 2022). Co-composting with microbial inoculants has been used for a long time to improve the quality of natural fertilizers (Guo *et al*., 2021). Because of a lack of understanding of the management of organic waste resources, waste management technologies are not being implemented effectively and efficiently worldwide. This necessitates developing a new system of production, consumption, reuse, recycling, and waste avoidance. The benefits of composting as an eco-friendly technique for managing organic matter such as waste materials and the development of compost in a circular economy should be known to government organizations, nations, and individuals. Genetically modified PGPR bacteria can also play an important role to increase crop productivity. More research is needed to increase composting production in India in order to reduce gas emissions, antibiotic resistance genes, and heavy metal mobilization, as well as the role of genetically modified microbes. Giraddi *et al*. (2014) reviewed the work on earthworms and organic matter recycling from an Indian perspective. Recent trends and advances in composting and vermicomposting technologies have been reviewed by Zhou *et al*. (2022).

Importance of microbial-based worm-vermicompost in sustainable agriculture

The bacterial (PGPR) based vermicompost represents a step forward in the direction of sustainable agriculture. Today, the application of fertilizer in agriculture has expanded into an essential practice. Soil salinization is a crucial issue for agriculture and threatens sustainable development goals related to nutrition, food security, and resource preservation. To reduce the effects of salinity on plants, a variety of methods, including transgenic strategies, conventional breeding, and chemical use, were investigated. Vermicompost and PGPR are the answers to these issues. These manures include organically bonded micro and macro elements that impact the growth and development of plants, as well as soil sustainability. The favorable consequences of PGPR on soil and crop were improved by vermicompost, with the level of promotion dependent on vermicompost dosage and crop type. The benefits of composting as an eco-friendly technique for managing organic matter such as waste materials and the development of compost in a circular economy should be known to government organizations, nations, and individuals. Alternative agricultural methods, including organic farming, have consequently opened up new chances to increase soil resources and sustainably use them. This tactic boosts plant output by supplying the macro- and micronutrients required for healthy growth. Some important advantages of microbial vermicomposting practices in agriculture are as follows:

1. It helps to provide all the essential macro as well as micronutrients to the soils and plants.
2. Helps to reduce the salinity and enhance the quality of soil (Kumar *et al.*, 2021).
3. PGPR helps to increase crop yields and resists the plants from diseases.
4. Increase the antioxidant enzyme activity as well as enhance the photosynthetic pigments and root hairs.
5. Vermicomposting with *Eudrilus eugeniae* and *Eisenia fetida* earthworms has been widely used to convert sludge into soil-improving fertilizer.

Future of vermicomposting

1. Emphasizes the importance of earthworms in plant and soil health, as well as their involvement in organic waste disposal.
2. Covers everything there is to know about eating habits, food preferences, dietary needs, and how earthworms and microbial populations interact.
3. It also manages environmental pollution and soil issues. We must therefore understand how it aids in both soil and environmental pollution control.
4. Provides fresh viewpoints on vermicomposting research.
5. Earthworms efficiently use the good bacteria in the soil, eliminate soil pathogens, and transform organic waste into enzymes, protein-rich products, vitamins, antibiotics, and other organic compounds. More knowledge is required in this area.

Limitations

1. The process of vermicomposting requires time. To prepare vermicompost, the organic waste must first decompose for about six months.
2. Vermicomposting requires more upkeep than the conventional composting method.

Application of PGPR and vermicompost in sustainable agriculture

The Fig. 5 illustrates the various uses for PGPR-based vermicompost and their significance for sustainable agriculture.

Vermicompost has been statistically proven to be a miracle plant growth enhancer. They decompose plant litter and heavy soil particles, increasing the levels of natural or organic materials for the breakdown of microbials and

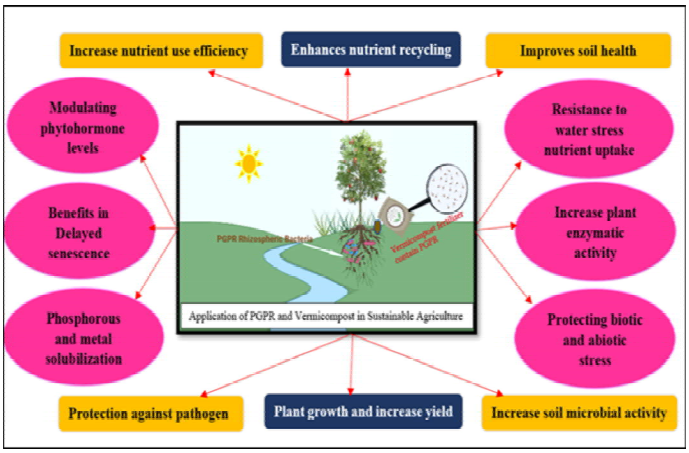

Fig. 5. Application of vermicompost PGPR-based biofertilizer in sustainable agriculture.

converting biological wastes into useful vermicompost (Kumar *et al.*, 2022). According to Chen *et al.* (2022), earthworm effects on carbon mineralization were quantified using models of their respiration and ingestion. Earthworm tissue C/N increased somewhat whereas substrate C/N decreased. Carbon content in earthworm biomass first rose and then fell. As the experiment came to a close, fungus proliferated and kept their biomass carbon level consistent while bacteria dropped. Also, the study reported by Triharyanto *et al.* (2021) said vermicomposting is used in combination with PGPR to promote the development and yield of shallots while attempting to reduce climate change. The largest number of bulbils were formed when PGPR and 5 tons of vermicompost per hectare were combined. It is not necessary to use PGPR because the 5 tons per hectare of vermicompost fertilizer is adequate to sustain the growth and output of shallot bulbs.

CONCLUSION

Several research groups have reported the practices of vermicomposting in agriculture, but there are few research literatures available on carrier-based bioformulation, their effect on salt conditions, also the effect of fertilizer, chemical fertilizer, and vermicomposting in the soil. Vermicompost promotes germination, growth, blooming, and fruit production in a diverse variety of plant genera when applied to soil or plant-growing media. Vermicompost has high levels of biofertilizers and enhances the physiochemical and biological qualities of agricultural soil. Plant growth stimulation can be considerably affected by the biological properties of vermicompost, the species of plants employed, as well as the culture circumstances. There is an urgent need to do a study on the biodegradation of organic materials by earthworms and microorganisms. According to some

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accounts, earthworms distribute a few hazardous bacteria such as *Pythium* and *Fusarium* spores. The existence and proliferation of antagonist-suppressing diseases and other PGP bacteria outweigh these negative effects. Application of vermicompost with PGPR in an effort to mitigate climate change by promoting crop growth and production. This review is primarily intended to offer information on the dynamics of the earthworm-microbe-soil-vermicompost interface; nevertheless, precise experimental research is required to standardize microbial advances. This review also focused on saline stress and its effects on plants, and on Indian programmes that promote vermicomposting. More research is needed to increase composting production in India in order to reduce gas emissions, antibiotic resistance genes, and heavy metal mobilization, as well as the role of genetically modified microbes. We can boost vermicomposting by using sludge-based composting, using genetically modified PGPR bacteria, and techniques like rhizo-engineering and nanotechnology can also help increase crop productivity. However, much more research is needed in the future to boost vermicomposting and promote organic farming.

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Review Article

Repellent and fumigant actions of the essential oil from *Salvia officinalis* against storage pests: A review

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ABSTRACT

Stored food grains are greatly damaged by many pests all over the globe. Even though the usage of synthetic pesticides yields good results, they are non-biodegradable and are of high cost. Synthetic pesticides are even harmful to the humans and environment, which leads to the search and usage of plant-based volatile oil, which exhibits insecticidal properties. Due to this property, they are safe for the population and the environment. Sage (*Salvia officinalis* L.) is a popular herb in the mint family (Labiatae/Lamiaceae), this can be used as an alternative to synthetic pesticides. This review is focused on the use of sage in controlling stored product pests. The important aspect discussed in this review is the action of sage (essential oil) on the stored pests for instance the repellent and fumigant actions on the stored pests. The article also stresses the importance of the extensive use of biopesticides.

***Keywords*:** *Salvia officinalis*, essential oil, storage insects.

Salvia officinalis L., the common sage or just sage, is a perennial, evergreen subshrub, with woody stems, grayish leaves, and blue to purplish flowers. It is a member of the mint family Lamiaceae or Labiatae. *Salvia officinalis* is one of the widely grown genera of this family which includes 900 species. It is grown all over the globe and is native to the Middle East and Mediterranean region (Bisset and Wichtl, 2001; Miura *et al*., 2001; Anonymous, 2004). The aerial part of the plant is used in traditional medicine, cookery, and many other foods because of its seasoning properties. As mentioned in the folk literature this plant has been widely used in treating different kinds of disorders like ulcers, rheumatism, seizure, gout, dizziness, inflammation, paralysis, tremor, hyperglycemia, and diarrhea (Zargari, 1990; Garcia *et al*., 2016). It is used as a traditional medicine in Europe to treat sweating, inflammation, dyspepsia, and age-related cognitive disorders. In Germany, it is used for dyspepsia.

In the past few years plant with antimicrobial activity have earned special interest due to their resistance to antibiotics that microorganisms have acquired (Essawi and Srour, 2000). There has been an increase in concern about food safety and the possible outcome of synthetic additives on health (Reische *et al.,* 1998). The herbal extracts of aromatic plants have been used for different purposes since ancient times (Heath, 1981). The sage essential oil of sage contains antimicrobial agents, antioxidant and anti-inflammatory activities. The volatile oils consist of a complex mixture, primarily monoterpenes, sesquiterpenes, and their oxygenated derivatives such as alcohols, esters, aldehydes, ethers, ketones, oxides, and phenols (Cowan, 1999). Few other volatile oils consist of phenylpropenes and specific nitrogen or sulphur-containing substances. Mainly it consists of many compounds, one constituent dominates the other in many species (Cowan, 1999).

There are many negative effects on health caused by synthetic pesticides and those include dermatological, gastrointestinal, carcinogenic, neurological, reproductive, respiratory, and endocrine effects. *Salvia officinalis* has been acknowledged in many literatures for its antioxidant activity and its effectiveness against various species of bacteria that includes *Bacillus megatherium, Bacilus cereus, B. subtilis, Aeromonas sobria, A. hydrophila, Staphylococcus aureus,* and *Sterptococcus pneumonia* (Delamare *et al*., 2007; Horiuchi *et al.,* 2007). This review is focused on the use of sage in controlling stored product pests and further discusses the repellent and fumigant actions against storage pests.

Sage: Repellent and fumigant action against *Tribolium castaneum* and *Ulomoides dermestoides*

The sage was evaluated for the fumigant and repellent actions against the *Ulomoides dermestoides* and *Tribolium castaneum*. One of the major compounds of sage was found to be 1,8-cineole, the essential oil of sage was found to be a

promising repellent against *U. dermestoides* and *T. castaneum* and can be used for integrated pest management. Sage essential oil, when treated against *U. dermestoides* had a 97 per cent repellency rate after 2 h of treatment (Maria *et al.,* 2019). The essential oil of sage showed strong fumigant activity against both species at the highest concentration after 48 h of exposure. The essential oil of sage showed better repellency than commercial repellents against *U. dermestoides* (Maria *et al.,* 2019).

The evaluation of sage oil with antibiotics and antioxidant chemicals components was studied further to know the biological activities and properties of components of the oil that need to be investigated for the control of pests (Adrar *et al*., 2016).

Sage: In the control of *Acanthoscelides obtectus* (Bean weevil)

Bean weevil is one of the chief storage pests in the bean crop. The sage was evaluated for insecticidal and repellent action against bean weevil. The essential oil of sage proved to have an insecticidal effect on *Acanthoscelides obtectus*. The mortality rate was high for all the dosages, 95 per cent mortality rate was observed from 6 h of exposure for all doses. The sage essential oil-induced repellency rate was above 90 per cent for *A. obtectus* when applied with 0.5 liter per tonne of grains (Mauricio *et al*., 2016). However, the control of *A. obtectus* was done with different essential oils such as *Schimus terebinthifolius*, here a 95 per cent mortality rate was observed after 48 h of exposure (Santos *et al*., 2007). The same was observed with *Cunila angustifolia* essential oil after 24 h of exposure (Savaris *et al.,* 2012). With *Baccharis articulata* essential oil 95 per cent mortality rate was observed at 5.0 liter ton^{-1} of grains (de Campos *et al*., 2014).

Sage: Efficacy against *Sitophilus oryzae* and *Sitophilus granarius*

The toxicity of sage was evaluated by its vapours against the adult and larvae of storage pests. The effect of sage essential oil was stronger on the stored pests. The sage essential oil showed a good toxic effect against *Sitophilus oryzae* and *Sitophilus granaries.* The fumigant toxicity of the sage on *S. oryzae* was similar to the *S. granaries.* When the comparison was done on *S. granarius* with different essential oils from various sources, essential oil from *Ferula gummosa* showed a stronger effect when compared to the *Elettaria cardamomum* and *S. officinalis.* However, essential oils of *S. officinalis* along with *F. gummosa* and *E. cardamomum* were effective against *S. granarius* as a fumigant toxin. Further, the toxicity of the essential oil of *S. officinalis* against *T. castaneum* was unclear (Mohammad *et al*., 2012). The *T. castaneum* when treated with *S. officinalis,* was more susceptible when compared to the *Sitophilus* species (Rastegar *et al.,* 2009).

Sage: Evaluation of essential oil on *Sitophilus oryzae, Rhizopertha dominica, Tribolium castaneum, Oryzaephilus surinamensis,* and *Ephestia cautella*

The components of sage essential oil were extracted and evaluated against *S. oryzae, Rhizopertha dominica, T. castaneum, O. surinamensis,* and *Ephestia cautella* along with the cut flowers and quarantine pests. From the fumigant activity, it was found that *S. officinalis* showed more fumigant activity *i.e.,* the percentage of adult mortality towards the *R. dominica*, followed by *O. surinamensis, S. oryzae,* and *T. castaneum* at different concentrations compared to other aromatic plants. In addition to the above experiment, when 15 per cent CO_2 was added, it was noticed that there was an increase in the activity of essential oils in fumigating grains (Kostyukovsky *et al.,* 2001).

Sage: Biological activity of essential oil on *Sytophilus granarius* and *Tribolium confusum*

Salvia officinalis along with *Mentha spicata* were proven for their bio-insecticidal property against the two storage pests *S. granarius* and *Tribolium confusum.* The aerial part of sage showed to have a toxic effect on the *S. granarius* adults that is 90 per cent of mortality rate was seen, and even for *T. confusum* 90 per cent mortality rate was seen with sage essential oil. The application of this essential oil resulted in high mortality to both the stored pest. Bio-insecticidal property of sage essential oil is directly proportional to the concentration of the oil. A mortality rate of 100 per cent was seen after 3 days of application, this proves the insecticidal effect of the sage (Leila *et al*., 2020). It was observed that when 1 ml of the solution was diluted with agar-agar, a higher toxicity rate was seen along with a 100 per cent mortality rate (Benazzeddine, 2010).

Sage: Essential oil fumigant toxicity on the larvae of Khapra beetle (*Trogoderma granarium*)

The fumigant toxicity of the essential oil of sage was tested against *Trogoderma granarium*. After exposing the vapors of essential oil of sage there was 98-100 per cent mortality of the larvae after 48 h, at different concentrations. *Saliva officinalis* essential oil has fumigant toxicity against *T. granarium*. The essential oil of sage showed lethal activity with an LC_{50} value of 50.1b µL/L air (Ghaleb *et al*., 2012). The essential oils of the plants belonging to the family Apiaceae, Lamiaceae, Lauraceae, and Myrtaceae when subjected to test the LC_{50} for fumigant toxicity, they showed a positive result against the stored pests. The essential oil of *S. officinalis* was

potentially useful in the management of *T. granarium* (Rajendran and Sriranjini, 2008).

Sage: Repellent, fumigant, and contact toxicity of essential oil against *Callosobruchus maculatus*

The sage essential oil was analyzed against *Callosobruchus maculatus* for its repellent, fumigant, and contact toxicities. Sage essential oil showed the highest repellency activity and the decreasing order of repellent activity was sage > coriander > rosemary (Dayaram and Khan, 2016). Even at the lowest concentration sage kept constant the higher repellency rate after 48 h. Sage essential oil resulted in 50% mortality of *C. maculatus* along with rosemary essential oil. The sage essential oil proved to be a potent fumigant for *C. maculatus* (Dayaram and Khan, 2016). Against *C. maculatus* the essential oils are said to be impelling due to the presence of mono and sesquiterpenes, where the more effective one is monoterpenes (Ajayi *et al.,* 2014). As the concentration of the essential oil of sage increases, there was a decrease in the number of eggs oviposited (Elhag, 2000).

Sage: Toxic and acetylcholinesterase inhibitory effect of essential oil on *Tribolium confusum*

The evaluation of contact toxicity of sage essential oil against *Tribolium confusum* was carried out. The essential oil proved to exhibit contact toxicity from the biochemical tests against *T. confusum* larvae and adults with LC_{50} values of 0.13 and 0.16 µL/cm^2, respectively. A significant repellent activity was demonstrated from the toxicity tests. The sage essential oil further caused the inhibition in the acetylcholinesterase activity. At the highest concentration, acetylcholinesterase was an effective inhibitor against *T. confusum* after 72 h of exposure (Khemais *et al*., 2017). The major reason for the insecticidal properties exhibited by the sage essential oil against *T. confusum* is due to the presence of chemical constituents such as 1,8- cineole and camphor, which are the monoterpenes present in the plant. The insecticidal activity of essential oils is due to some of their major chemical constituents, which are reported in many studies. Few previous studies report the insecticidal activity of 1,8- cineole and camphor against the stored pests (Lee *et al*., 2004).

Sage: As a bio-insecticide against *Sitophilus granarius*

Earlier it has been mentioned about the efficacy by Mohammad *et al.* (2012) and biological activity by Leila *et al.* (2020). The ethanolic extract of sage was tested against *S. granarius* for its insecticidal property. The sage was an effective repellent with a 60 per cent repellency rate on day 1 and day 2. As the days moved on, there was a decrease in the percentage of repellency. The mortality rate of *S. granarius* was higher on day 10 with a 72 per cent mortality rate. On average the mortality rate of the sage ethanolic extract was about 46.6 per cent (Mehmet, 2017). For the management of *S. granarius* the examination of plant volatile oils, as well as bioassays, is required for the evaluation of fumigant toxicity (Hamza *et al*., 2016). Along with sage, even *Thuja, Eucalyptus*, and *Peppermint* oils showed to be effective for the management of *S. granarius.*

Sage: Repellence effect of volatile oil on *Plodia interpunctella*

The response of the adult *Plodia interpunctella* was evaluated for sage volatile oil for its repellence effect. A significant repellency was seen for the sage essential oil with a 93.33% repellency rate. The *P. interpunctella* showed a different response to the different essential oils among them sage essential exhibited the strongest repellency along with few other essential oils. The repellent study was done using a Y-tube olfactometer where the moths moved towards control more than the treated arm. To be precise the moths moved 33 times towards the control and 17 times towards the treatment. This essential oil can be used for protecting stored products from the injury of *P. interpunctella* (Zahra *et al.,* 2001).

CONCLUSION

Aromatic plants synthesize secondary metabolites, mainly essential oils, which can be further isolated. *S. officinalis* is a medicinal plant and its aromatic compounds occur naturally. This essential oil is toxic to stored pests and possess no harm to mammals, which is moreover, friendly to the environment. Due to their insecticidal efficiency and volatility, they are a good alternative fumigant compared to synthetic fumigants for the control of stored product insects. The use of essential oil in pest management decreases the use of pesticide and justify their environmental effect. This essential oil can further be used for the development of botanical pesticides in the control of stored product insects. This essential oil can also be used to protect the stored products from the injury of many stored pests. So, the sage essential oil has a good potential to replace chemical repellents for many storage pests as it is safe for humans and leaves no residue on stored products.

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Review Article

Significant causative agents that cause toxicity in mushrooms

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ABSTRACT

Worldwide outbreaks of food-related illnesses brought on by wild mushroom poisoning have raised questions about food safety. On an annual basis, we hear a lot of stories from all over the globe concerning people eating toxic mushrooms. Scientists are interested in it, notably in the areas of hazardous substance composition, process, and its use in poisonous mushrooms. The toxins which are present in poisonous mushrooms are quickly taken in by blood vessels and travel straight towards their aimed target despite withstanding the extremes of the temperature of cooking and the digestive acids. However, patients must be aware that in 95 per cent of instances, the nature of fungus and toxins is unknown, thus evaluation and therapy rely on the symptoms they are experiencing at the time of hospitalization. Clinical syndromes associated with mushroom poisoning include fulminate hepatic failure, immediate failure of the renal system, rhabdomyolysis, erythromelalgia, haemorrhage, gastroenteritis, mental disturbance, and irritation of the skin. The aim of this review is to explain poisonous mushrooms, the medical symptoms of toxic mushrooms, different noxious, and how they are used medically.

Key words*:* Poisonous mushroom, toxins, clinical symptoms, gastroentertitis, rhabdomyolysis, erythromelalgia.

Fungi, the second-most significant class of life forms, are believed to consist of 11.7-13.2 million species, however, only 150,000 of them have been thoroughly studied to date (Wu *et al*., 2019). Since ancient times, gastronome dining has praised mushrooms for their distinct flavor and delicate aroma. They are thought to be suppliers of essential elements such as fortified fiber, minerals, and vitamins, especially vitamin D (Ren *et al*., 2012). Particularly as an immunomodulator and anticancer drug, mushrooms are often used. In common, people hold the view that mushrooms are actually nutritious foods that can be grown or generated gradually across the globe (Öztürk *et al*., 2015).

Mushrooms naturally contain antioxidant substances that are employed in pharmaceutical formulations. Mushrooms can be categorized as poisonous, edible, or inedible (Sevindik *et al*., 2017). Additionally, there are many mushrooms that could be harmful if consumed, and it can be challenging to tell which species are "edible" and which are hazardous. Furthermore, several "edible" species could turn hazardous to certain individuals in unpredictable conditions (White *et al*., 2019). The majority of formulations and compounds made from mushrooms are used as innovative dietary supplements (DS) or "nutraceuticals," rather than as pharmaceuticals (Patel and Goyal, 2013). Polysaccharides, proteins, immunoglobulins, conjugated lipids, phenolic components, alpha-tocopherols, provitamin D2, lectins, and other bioactive nutraceuticals have been observed in mushrooms (Ma *et al*., 2018).

Poisoning from mushrooms is a serious and prevalent condition brought on by toxins. Cytotoxic and myotoxic are considered to be deadly mushrooms as they have the majority of devastating poisonous effects (He *et al*., 2022). Amanita phalloides, sometimes known as the "death cap" mushroom is responsible for more than half of lethal poisoning cases nationwide. Other harmful mushroom species including the Amanita verna, Amanita virosa, Gyromitra, Gallerina, and Lepiota species, also possess the toxic amatoxin. Death could result from eating merely a small amount of a single deadly kind of mushroom (Gopinath *et al*., 2011). Raw consumption of certain Gyromitra species has caused serious illnesses along with deaths among people. (Michelot and Toth, 1991). These are thought to trigger diarrhea, and indications of serious illness might involve liver as well as brain damage. Furthermore taking weather conditions into account, the 'brain-like appearance' of the gyromitra mushroom aids in separating it from the other Amanita species. Additionally, this review paper also aims to investigate orellanine's acute toxicity. These species are primarily found in North America and Europe (Flament *et al*., 2020). Prolonged damage to the kidneys through the toxin orellanine, is evident.

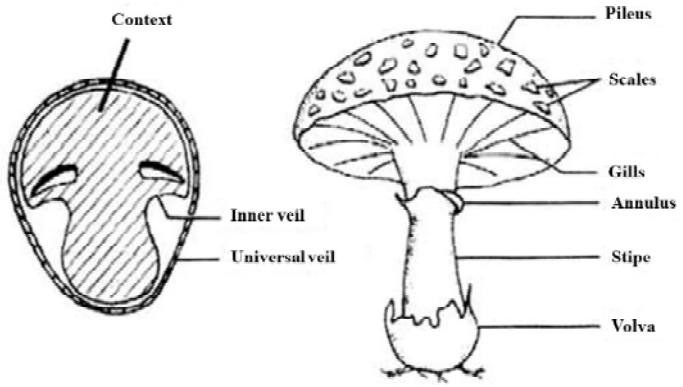
As poisoning from mushrooms is intimately linked to individual well-being and standard of living, understanding the harmful components and deadly varieties of mushrooms that are poisonous is crucial when it comes to the prevention and management of symptoms of mushroom poisoning (Patocka *et al*., 2021).

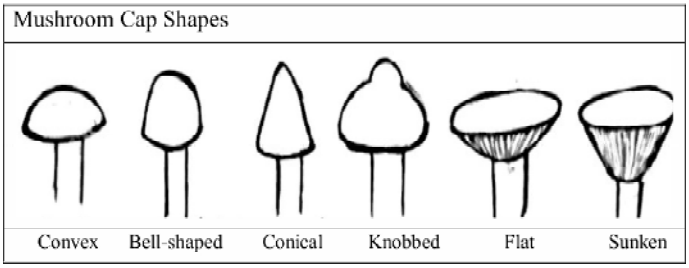
Whereabouts of Mushrooms

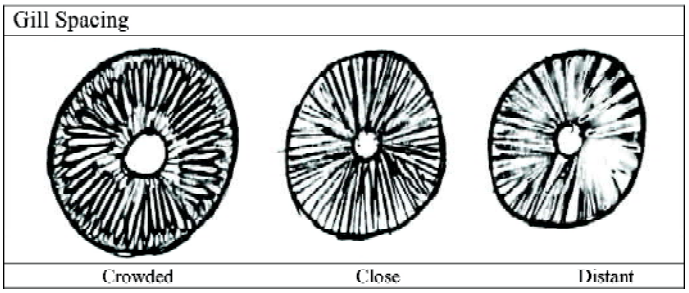
Mushrooms are a well-known source of nutrition that is eaten frequently around the globe (FAOSTAT, 2023). They constitute one of the most well-known edible fungi that belong to the Basidiomycetes family (Sisodiya *et al*., 2023). For the human race, mushrooms are an essential source of wholesome, useful food and revenue. They are frequently cited as a source of a vast variety of physiologically active chemicals, many of which have demonstrated pharmacological qualities such as antioxidants, antibiotics, anticancer, antiphlogistic, and anti-diabetic effects (Dulay *et al*., 2023). They have a high protein and amino acid content but a low fatty acid level. They nevertheless include a large number of vitamins, including B1, B2, B12, C, D, and E (Blumfield *et al*., 2020). As a result of their savoury essence and fibrous appearance, mushrooms complement meat-based foods well, which adds to the benefit of utilizing their use for muscle food products (Guinard *et al*., 2016). A single naturally occurring, non-fortified dietary supplement that contains vitamin D is found in mushrooms, which also include healthy minerals including selenium, potassium, iron, copper, and phosphorus (Kumari, 2020).

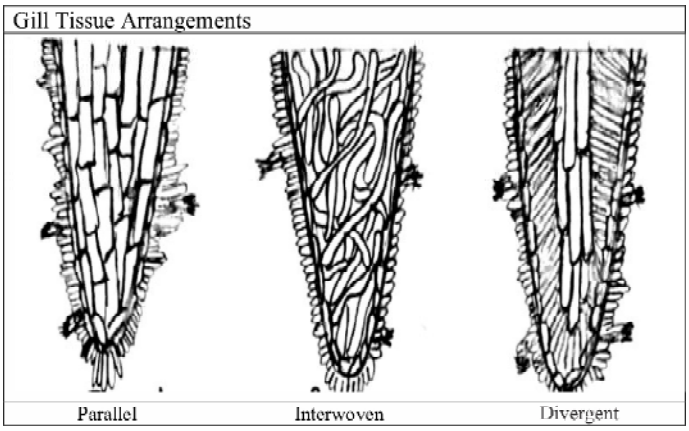
Morphological description

Mushrooms constitute the name for the spore-producing reproductive structures of fungi. Fungi were once classified as belonging to the plant kingdom, but modern taxonomy now recognizes them as a distinct group of organisms under the kingdom Mycota. This is mostly because fungi have chitin in their cell walls (Ayeka, 2018). The essential components of a mushroom's distinctive anatomy include a fruiting body with a cap that is held up by a stem, a thin protective covering called vellum that is produced during its growth process, and lamellae where basidiospores are formed (Shen *et al*., 2017). Except for *Ganoderma lucidum* which has a hard, bareheaded fruiting body, most mushroom fruiting bodies are composed of water,





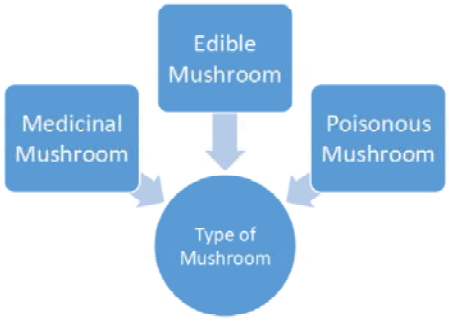




which has around 90% moisture. Its dry weight is mostly made up of amino acids (19-35%) and carbohydrates (50-65%), and very little fats (2-6%) present (Rathore *et al*., 2017).

Types of Mushrooms

Mushrooms are broadly classified into three main categories.



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Edible Mushroom

Due to their abundance of protein, amino acids, and polysaccharides, edible mushrooms constitute a pleasant and nutritious diet (Sun *et al*., 2020). Both the cultivation and consumption of nutrition, berries, and fresh produce have increased globally. Edible mushrooms are a good option to partially meet the aforementioned demand because they have a high protein content (Aguilar-Rivera *et al*., 2017). Over the previous ten years, the output of culinary mushrooms has risen significantly, reaching 10.5 million tonnes in the year 2016 to 11.8 million tonnes by 2019, a 57% increase (FAOSTAT, 2023). In China, there are 1,020 kinds of mushrooms that are edible and 480 species of deadly mushrooms. (Wu *et al*., 2019). According to several writers, some species that are edible must first be treated before they can be consumed safely (Niksic *et al*., 2016). It's important to note that animals can consume these mushrooms naturally or unintentionally as part of their diet. For instance, they can eat macromycetes that they discover in pastures (Sanchez and Mata, 2012).

Table 1. Some examples of edible mushrooms.

Scientific Name	Common Name	Reference
Agaricus bisporous	Button Mushroom	Tian *et al*., 2012
Agaricus campestris	Field Mushroom	Dai *et al*., 2009
Antrodia subserpens	-	Chen and Cui., 2015
Calocera viscosa	Yellow tuning fork	Muszyn´ska *et al*, 2012
Hygrocybe cantharellus	Chanterelle waxy cap	Chittaragi and Naika, 2014

And so on…

Medicinal Mushroom

The global effect of rising dietary supplement expertise is significant. Healing with fungi, or what is commonly referred to as therapeutic mushrooms, is an age-old practice in medicine. Promoting the wellness benefits of mushrooms is a common theme in both Eastern and Western cultures (Wasser, 2011). Though the utilization of mushrooms for medicinal purposes has a centuries-old and ongoing legacy in East Asia, the initially recorded usage of mushrooms as a cure in Europe dates back to "Iceman", Ötzi, who lived approximately 3,500 BC (Wu *et al*., 2019). More Highly Basidiomycetes fungi are also known as "pillar mushrooms" or "stand mushrooms," which have a variety of biologically potent, strong-molecular (such as ß-glucans) and weak-molecular (such as triterpenes, lectins, and steroids) substances in their fleshy bodies, the mycelia are cultured by the spores, and fluid which might have anticarcinogenic effects is also cultured (Wasser, 2017). The primary components of therapeutic mushrooms are polysaccharides, which also have antioxidant, cancer-fighting, antidiabetic, anti-inflammatory, antibacterial, and immunomodulatory properties (Wei *et al*., 2018). They also include a number of enzymes, including peroxidase, glucose oxidase, laccase, and superoxide dismutase. Enzymes therapies have been demonstrated to be crucial for the cure of cancer by reducing the effects of oxidative stress and decreasing cell proliferation (Zaidman *et al*., 2005).

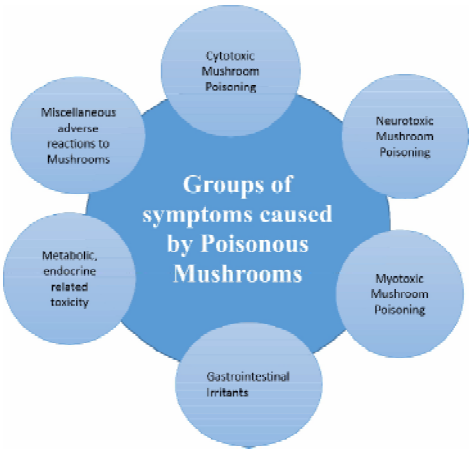
Table 2. Some examples of medicinal mushrooms.

Scientific Name	Common Name	Reference
Coriolus versicolor	Turkey tail	Saleh *et al*., 2017
Ganoderma lucidum	Lingzhi mushroom	Bulam *et al*., 2019
Lentinula edodes	Shiitake mushroom	Ina *et al*., 2013
Grifola frondosa	Maitake mushroom	Alonso *et al*., 2013
Hericium erinaceus	Lion's mane mushroom	Spelman *et al*., 2017

And so on…

Poisonous Mushroom

Annually, poisoning episodes brought on by eating deadly mushrooms happen worldwide. Unintentional poisoning with mushrooms can have negative health effects or could be fatal in rare circumstances. One of the major contributors to food-borne illness mortality and a global safety of food concern is mushroom poisoning (Patocka *et al*., 2021). The two seasons that show the highest cases of mushroom poisoning are spring and autumn because of the cold, damp evenings that encourage the development of mushrooms. The quantity consumed, the mushroom's age, the season, the location, and how the mushroom was prepared before consumption can all affect the level of toxic impacts (Njugi, 2017). Additionally, aside from the inherent biological makeup of the fruiting body, toxic effects may also result from more common causes of infection with food caused by incorrect handling after harvesting or storing mushrooms (Gawlikowski *et al*., 2015).



The key poisonous components found in mushrooms are peptides and protein compounds that can enhance cytotoxic effects that act on particular targets (Yin *et al*., 2019). Mushroom poisonous signs are typically divided into sections based mostly on manifesting symptoms and therapeutic results (Table 3) (Goldfrank, 2019).

Table 3. Outline of the key species that cause mushroom toxicant symptoms, as well as species that people could be confused with.

Syndrome	Poisonous Mushroom species	Edible species with which poisonous species can be confused	Symptoms	Reference
Cytotoxic	*Amanita oscreata* *Amanita virosa*	*Amanita velosa* *Agaricus campestris*	Hepatitis Nausea Vomiting Abdominal pain Irritability Diarrhea	Tavassoli *et al*., 2019
Neurotoxic	*Amanita concentrica*	*Amanita fulva*	Chills Sweating Salivation Diarrhea	Xu *et al*., 2020
Myotoxic	*Russula subnigricans* Gyromitrin (this is a species that come under Myotoxin)	*Tricholoma equestre*	Rhabdomyolysis Acute renal failure Profound low calcium levels mortality from cardiovascular collapse and breathing problems	Parnmen *et al*., 2021
Metabolic, endocrine and related toxicity	*Cantharocybe virosa*	*Termitomyces* R. Heim	Nausea Vomiting Abdominal pain Fatigue Circulatory disturbance Diarrhea	Parnmen *et al*., 2021 Manimohan *et al*., 2010
Gastrointestinal Irritant	*Agaricus xanthoderma* *Tricholoma pardinum*	*Agaricus campestris* *Tricholoma terreum*	Vomiting Diarrhea Hypo-tension	Wennig *et al*., 2020
Miscellaneous adverse reactions to mushrooms	Erythromelalgia (associated with *Clitocybe acromelagia*) *Paxillus involutus*	-	Production of acromelic acid	White *et al*., 2019

Various kinds of toxic substances distinguished by the deadly mushrooms discovered everywhere, exhibit a range of post-consumption medical signs. Despite being a center for biodiversity and being home to numerous classes of fungi and mushrooms, the Indian subcontinent still has a large number of hazardous mushroom varieties whose harmful substances are still not completely characterized (Patra and Mukherjee, 2022). Mushrooms that have amatoxins have been responsible for the majority of the fatal incidents. Among the genera of bicyclic octapeptides known as amatoxins, aren't rendered inactive by high or low pH levels, cooking, or human intestinal enzymes (Bever *et al*., 2002). Although Inocybe species prefer to fruit in urban areas and thus tend to be easier to pick to feed a meal or grazed on by small children, this is likely that some of these species could be confused for or mingled with other species of interest for food (Kosentka *et al*., 2013). Ascomycota is a phylum of fungal filaments that includes taxa having fruiting organs like morels, Helvella, and truffles as well as those that produce mycotoxins. The Basidiomycota phylum includes all other types of macroscopic mushrooms (Alshannaq, 2017). Some of the toxins that are highly poisonous are mentioned below.

Gyromitrin and Orellanine as notorious mushroom toxins

Gyromitrin: toxin from Gyromitra mushroom

In the northern hemisphere, exist ascomycete mushrooms of the genus Gyromitra (Ascomycota phylum). About 18 various species make up the genus Gyromitra (Stephenson, 2010). Raw consumption of certain *Gyromitra* species has caused acute illnesses along with deaths in humans (Michelot and Toth, 1991). Diagnostic statistics show that nausea and vomiting are most common, along with jaundice, convulsions, and coma come next (Hendricks, 1940). *Gyromitra gigas* (Kromb.) and *G. esculenta* are the principal species of concern. Current advances in chromatography, life sciences, and ecology have revealed that further Gyromitra taxa might be hazardous, despite the fact that certain *Gyromitra* species are palatable when it is cooked (Patowary, 2010).

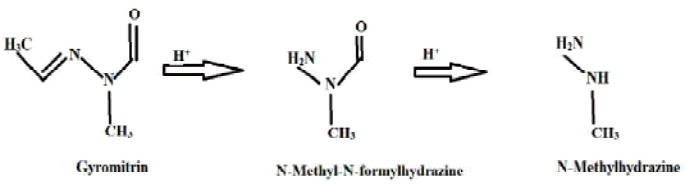


Fig. 1. Gyromitrin is transformed *in vivo* into N-methyl-N-formaldehyde and then into N-methylhydrazine from acetaldehyde N-methyl-N-formylhydrazone (Patocka *et al*. 2012).

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It seems that *Gyromitra* species induce intestinal inflammation, and signs of serious illness can include cerebral and liver problems. Gyromitirn is present in almost every kind of gyromitra mushroom (Fig. 1). The harmful effects may be identified among the other amanita species based on seasonal variations because it grows more frequently in the early stages of summer and spring than the other species do in the autumn. The 'brain-like look' of the gyromitra mushroom (Fig. 2) helps to distinguish it from the *Amanita* species in addition to the seasonal consideration. Gyromitrin poisoning can cause delayed gastroenteritis, headaches, seizures, hepatitis, hemolysis, and a condition called met among other clinical symptoms (Barman *et al*., 2017).

Fig. 2. *Gyromitra* spp. (false morel); source (Google)

Case study: Gyromitrin poisoning was first documented in 1782 and then again at the turn of the 19th century (Flament *et al*. 2020). Between 1782 and 1965, Eastern Europe saw a significant number of poisonings, according to Franke *et al*. (Flament *et al*. 2020). Nevertheless, less instances of intoxication have been detected compared to all other mycotoxins because of this toxin's thermosensitivity. Some patients consumed mushrooms more than once because of the prolonged latency period. Liver failure caused the death of some of these patients (Flament *et al*., 2020).

Treatment: Gyromitrin poisoning with seizures requires the daily use of pyridoxine (70 mg/kg to 5 g) coupled with anticonvulsant medication. Through the poisonous metabolite monomethyl hydrazine, pyridoxine operates by correcting pyridoxal 5 phosphate shortage in the neurological system. When discovered, methemoglobinemia, which is also observed in gyromitrin overdose, should be treated with intravenous methylene blue (Barman *et al*., 2017).

Orellanine as a nephrotoxic toxin

Orellanine is the main toxin found in mushrooms from the family Cortinariaceae and genus *Cortinarius*. *Cortinarius orellanus* (Flament *et al*., 2020) and *C. speciosissimus* [(Flament *et al*., 2020 and Womle *et al*., 2004)] are the two most often reported poisoning cases. Additionally, *C. orellanosus* (Judge *et al*., 2010), *C. armillatus* (Flament *et al*., 2020), and *C. eartoxicus* (Gasparini, 2004) are mentioned in some cases. *Cortinarius splendens* (Flament *et al*., 2020) toxicity is still up for debate. These species are primarily found in North America and Europe. There have also been some reports of poisoning in Australia (Mount *et al*., 2002; Flament *et al*., 2020).

Grzymala discovered orellanine (C10H8N2O6, M = 252.2) for the first time in 1957 following a mass poisoning in Poland that left 19 people dead (Flament *et al*., 2020). In 1962, it was segregated (Flament *et al*., 2020) (Fig. 3). A bipyridine N-oxide, orellanine is also known as 2,22 -bipyridine-3,32 ,4,42 -tetrahydroxy-1,12 -dioxide (Flament *et al*., 2020). It is stable in the mushroom and extremely polar (logP = 1.19). It has the same toxic qualities as orellanine, but is photosensitive. It gets reduced through mono-hydroxylation to orellinine after extraction. (C10H8N2O5, M = 236.2), and subsequently by bi-dehydroxylation to orelline (non-toxic) (2018). As orellanine is not sensitive to temperature, cooking the mushrooms does not lower its toxic effects (Dinis-Oliveira *et al*., 2016). To the best of our understanding, there's not any publicly available information on orellanine's metabolism.

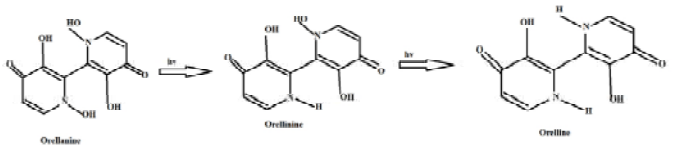

Fig. 3. Structure of Orellanine and its decomposition (Flament *et al*. 2020).

Delayed renal failure: The toxin compounds orellanine, cortinarin A, and cortinarin B, which trigger nephritis of the interstitial space and interstitial fibrosis of the tubules in the mushroom species *Cortinarius orellanus* (Fig. 4), *Omphalatus orarius* as well as *Mycena pura*. Acute renal injury wants medical attention as part of the remedy for Cortinarius ingestion, and dialysis treatment ought to be looked at in a small number of cases (Barman *et al*., 2017).

Case study: Since 1957, numerous instances of orellanine poisoning have been documented in the literature. The majority of poisonings occur accidentally, occasionally as a result of confusion with hallucinogenic mushrooms (Flament

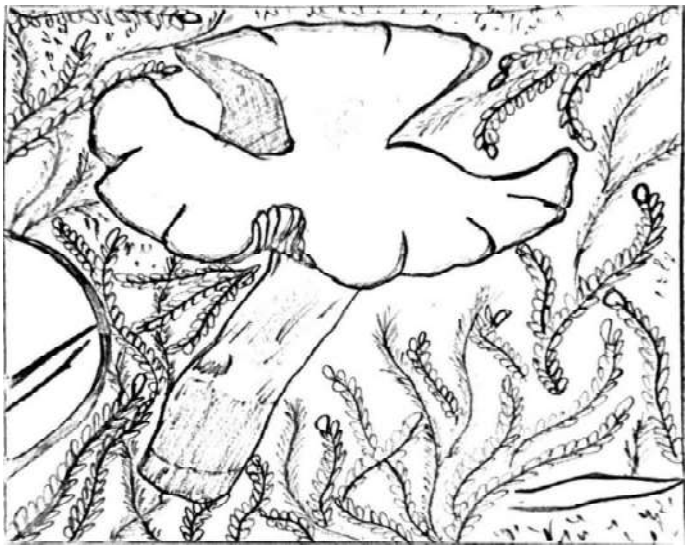

Fig. 4. *Cortinrius orellanus;* source (Google)

et al. 2020). In one instance, a schizophrenic patient voluntarily consumed *Cortinarius orellanus* (Flament *et al*. 2020). Many patients eat mushrooms multiple times because of their prolonged latency period, often days after their initial meal (Flament *et al*. 2020). When they arrive at the hospital, the levels of creatinine in the blood of a majority of sufferers are greater than their normal range. Those who had a greater level had a kidney transplant.

Treatment: Individuals who have kidney damage, excessive fluid intake, difficulty metabolizing diuretics, high potassium levels, acidosis of the metabolic system, and signs of urinary retention (pericarditis, unexpected neurological disability), must be evaluated for dialysis (Barman *et al*., 2017).

Rhabdomyolysis: a disorder caused by myotoxicity

Rhabdomyolysis is the medical term for the degeneration of striated muscle. The connective tissue in muscles degrades and necrotizes, which results in the release of substances within cells into the circulatory system. Usually, it is brought on by cartilage damage, either through trauma or not. This kind of damage might cause cells to collect what's inside to such an extent that they ultimately exceed the human body's normal detoxifying mechanism, considering that muscular tissue accounts for about 40% of the total body's bulk (Cervellin *et al*., 2017). Males, African-Americans, those between the ages of 10 and 60, and those with body mass indices greater than 40 kg/m^2 appear to be more susceptible to the condition (Chavez *et al*., 2016). Depending on the patient's age, the cause of rhabdomyolysis may also change. The reasons cited most often in grownups are narcotics and trauma, but nearly one-third of occurrences among youngsters are caused by illnesses (Chavez *et al*., 2016).

The development of AKI is the rhabdomyolysis situation that causes the most concern. AKI is thought to result in 10-40% of rhabdomyolysis cases, compared to 5-15% of AKI cases, according to estimates. AKI sufferers have a higher mortality rate that might reach 80% (Chatzizisis, 2008).

Rhabdomyolysis is not observed until the initial constriction has subsided due to the discharge of damaged tissue into the bloodstream (Khan, 2009). During surgical procedures, veins may be constrained by the application of tourniquets, splinting, hydraulic pressure tools, and clenching which can result in myocardial hypoxia and impede the delivery of vital nutrients to the cells (Khan, 2009). Rhabdomyolysis can also be brought on by prolonged immobilization (such as a coma, anaesthesia, or surgery) because of the pressure that remains on gravity-dependent tissues. Extracellular volume loss, continuous immobilization or compression for longer than 5 to 6 hours, pre-existing azotemia, diabetes, and hypertension are risk factors (Khan, 2009).

Another medical symptom related to *Tricholoma equestre* species is prolonged rhabdomyolysis. The initial appearance of indications, which include myalgia and increasing weakening, happens between 24 and 72 hours following ingestion. Laboratory test reveals hyperkalemia and an increased kidney phosphokinase level (Barman *et al*., 2017).

Case study: A formerly normal 26-year-old African American guy with sickle cell disorder shows up at a neighboring hospital's emergency room following a casually mixed combat battle. In the third and last round of a gruelling three-round fight, the patient was seen collapsing from weariness (Murugappan *et al.,* 2019). Only minor physical damage was inflicted by the individual throughout the game, and witnesses noted that the fall had nothing to do with any particular strike. To protect the patient, who was unable to effectively protect himself, the struggle was stopped (Murugappan *et al.,* 2019).

The electrocardiogram was indicative of hyperkalemia, and sodium bicarbonate, insulin, and intravenous dextrose were given as the first therapy. Hyperkalemia (5.6 mEq/L, reference range 3.3-5.1), hypernatremia (148 mEq/L, reference range 133-145), and acidemia with serum bicarbonate of 8 mEq/L (reference range 22-32) were notable findings in the basic metabolic panel (Murugappan *et al.,* 2019).

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The patient began to experience scattered myalgias following being transferred to the specialized trauma center for additional care. At the point of movement, significant testing results included a creatinine level in the serum of 2.5 mg/dL, potassium levels in the blood of 9.9 mEq/L, and lactic acid concentrations of 19.9 mmol/L (reference range: 0.5-2.0). In just over two hours, CK (creatine kinase) increased to 17,500 IU/L from an elevated level of 2,271 IU/L (reference range 29-201) (Murugappan *et al.,* 2019).

He developed anuria despite vigorous fluid resuscitation. On entering the trauma intensive care unit, intractable acidosis and hyperkalemia were treated with emergency hemodialysis. respiratory failure and severe hemodynamic instability required mechanical breathing and vasopressor assistance (Murugappan *et al.,* 2019). The patient was identified as having deep volume depletion along with pigment-induced renal failure and acute exertional rhabdomyolysis. Despite receiving the most supportive treatment possible, serum potassium and CK levels progressively declined. Due to worries about developing compartment syndrome, the patient's calves and thighs had bilateral fasciotomies right at his or her bedside. The patient's condition worsened and he started passing bloody faeces and had high bladder pressure. Serum fibrinogen was 55 mg/dL and serum haemoglobin fell from 11 g/dL to 6.3 g/dL (reference range 180-400) (Murugappan *et al.,* 2019).

Treatment: Rhabdomyolysis brought on by the *Tricholoma equestra* species is characterized by elevated creatinine phosphokinase, pigmented granular casts in the urine, and a brownish or reddish urine supernatant colour. Thus, the main course of medication is vigorous intravenous fluid infusion; favoured saline solution 20–40 ml/kg/h up to 1 l/h.

Erythromelalgia

Mitchell suggested the term Erythromelalgia in 1878. The Greek terms "erythros," which means scarlet, "melos," which means extremities, and "algos," which means ache, are the origins of the term "erythromelalgia" (Mann *et al.,* 2019). Since over 150 years ago, erythromelalgia is frequently described in the literature, but its incidence, aetiology, and pathophysiology are still unknown (Friberg *et al*., 2013). In 2004, the NaV1.7 voltage-gated sodium-carrier components are encoded by a gene named SCN9A, was found to be mutated, making erythromelalgia the very first human neuropathic pain illness identified as being associated with an ion channel alteration (Breivik, 2014). On a clinical level erythromelalgia is identified by looking at signs like burning sensations in the extremities, pain that is made worse by warmth, ache that is made better by chilling, *etc* (Kunt Kvernebo, 1998).

The disease can occur in two forms:

- Primary Erythromelalgia: It involves the gene SCN9A, which generates the Nav 1.7 voltage-gated sodium channels component proteins, connected to autosomal dominant inheritance, without any genetic link, and of unknown origin (Klein-Weigel *et al*., 2018). It is the first instance of persistent pain whose molecular causes have been identified (Daniels, 2011).
- Secondary Erythromelalgia: A number of ailments, such as autoimmunity and brain diseases, myeloproliferative medulla disorders, high blood pressure, vein insufficiency, diabetes type 2, lupus erythematosus systemic, chronic lichen planus osteoarthritis, and monosodium urate crystalline buildup, as well as a negative drug response (to bromocriptine or nifedipine), are associated with it (Han *et al*., 2006).

Case Study: A 12-year-old girl who had no noteworthy prior medical records was the person being treated. She went to the ER with complaints of acute burning discomfort along with erythema on her left hand, armpit, and palm of the hand which had been present for four days (Huh *et al*., 2015). Red lines that appeared to be following the outermost veins could be seen as erythema. The experiencing symptoms attack that included burning sensations and erythema (superficial reddening of the skin, typically in patches) intensified and influence a wider region of her body when she had been subjected to the temperature, a steaming shower or bath, and most definitely to the raised temperature of sunlight during the day. The pain proved progressive and highly sensitive to heat (Huh *et al*., 2015).

The feeling of pain initially began in her left lower and upper arm, spread to her right arm, and finally resulted in unilateral stiffness or an "unusual" hot sensation in both of her feet and hands. Checkups revealed that only lightly touching using the fingertips is enough to cause agonizing discomfort. Chilling significantly reduced the discomfort (Huh *et al*., 2015).

To differentiate between autoimmune conditions, reactions to allergens, as well as cardiovascular disease, assessments are being performed. She tested the stiffness of the arteries using a pulsed wave velocity (PWV) technique because she experienced excruciating burning discomfort at the point of the blood vessel whenever the vessel became

apparent due to an elevated temperature or sunlight trigger. PWV and echocardiography produced no particular findings (Huh *et al*., 2015).

Basic tests in laboratories lacked precision: White blood cell count was 9890/uL (neutrophils 68.6%, lymphoid cells 25.5%, monocytes 3.1%, and eosinophils 1.3%), haemoglobin was 13.7 g/dL, hematocrit was 39.6%, platelets were 227 000/uL, and the erythrocyte settling rate was 11 mm/h. C-reactive protein was 0.5 mg/L, and blood urea nitrogen was 15. Both cerebral magnetic resonance imaging (MRI) with angioplasty and pelvic-abdominal radiology were normal (Huh *et al*., 2015).

The high-temperature exposure stimulation tests instantly caused vasculature erythema and excruciating searing pain. Because of religious reasons, both of her parents forbade her from having a skin biopsy (Huh *et al*., 2015).

A couple of days afterwards, she complained of a distinct symptom: heat whenever she urinated, along with a burning sensation similar to vasculitis that also injured her torso. Both ears were still being attacked by pain. Mexiletine (100 mg t.i.d.) was used as a remedy for the erythromelalgia of the ears for two weeks (Huh *et al*., 2015).

Treatment: Dexamethasone (0.1 mg/kg) was administered intravenously for three days before being monitored by swallowed doses of prednisone (10 mg t.i.d.), aspirin (100 mg b.i.d.), and propranolol (10 mg b.i.d.) (Huh *et al*., 2015).

Poisonous Mushroom as a cure for the deadly disease

Though highly appealing, the mushroom nevertheless has a harmful effect on the human body until it is chemically altered. Poor research has been done on the effectiveness of several chemicals in causing cancer. However, some of these cancer-causing mycotoxins discovered in mushrooms are listed (refer to Table 4) (Masroor, 2021).

Table 4. Cancer-causing mycotoxins discovered in mushrooms (Masroor, 2021).

Mushroom Name	Mycotoxin present	Reference
Agaricus bisporous	Agaritine	Schulzova *et al*., 2009
Gyromitra esculenta	Gyromitrin	Dart, 2004
Podostroma cornu-damae	Trichothecenes	Lee *et al*., 2018
Amanita phalloides, *Conocybe, Galerina, Lepiota*	Amanitins	Hechler *et al*., 2015

Presence of Agaritin in Mushrooms

Agaritine is a glutamyl-hydroxymethyl-phenyl hydrazine that is readily soluble in water. It is particularly connected to the family of Agaricaceae and is also present in a few species from the genus Leucoagaricus and Macrolepiota (Schulzova *et al*., 2009). More specifically, pure glucans (1-3, 1-6 linked glucan) regarding *Agaricus* species have been utilized therapeutically when combined with different cancer-fighting agents and they've been reported to trigger amplification of immune system responses (Hong *et al*., 2004) (Fig. 5).

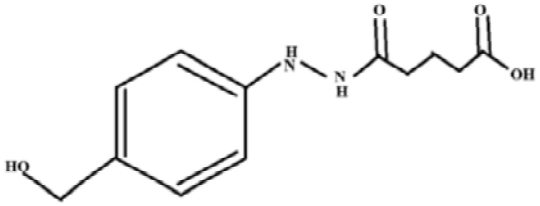

Fig. 5. Skelton Formula of Agaritine

Presence of Gyromitrin in Mushrooms

Gyromitrin is a chemically unsteady compound because it is an oxidizable volatile liquid at ambient temperature. Certain types of Gyromitra contain the toxin gyromitrin (White *et al*., 2003). *Gyromitra esculenta*, often known by the names false morel or lorchel, is well known for its ability to produce the deadly mycotoxin gyromitrin (acetaldehyde N-methyl-N-formylhydrazone) and its homologs, which are paradoxically consumed as a delicacy in many parts of the world (Dirks *et al*., 2023). By creating radical species, N-methyl-N-formylhydrazone and methylhydrazine are recognized as hepatotoxic, however, they are also known to cause cancer in animals (Patocka *et al*., 2012) (Fig. 6). A gastrointestinal prodrome that appears for over five hours following ingesting *G. esculenta* is Gyromitra syndrome. Severe kidney damage may happen to a lesser extent and acute liver damage may happen over the following two days in a large percentage of individuals (Horowitz *et al*., 2017).

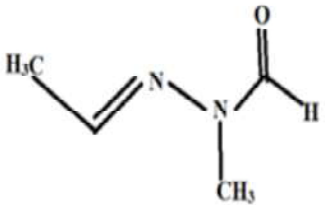

Fig. 6. Skeletal Formula of Gyromirtrin monomethylhydrazine

Presence of Trichothecenes in Mushrooms

The trichothecenes family, which includes nearly 200 distinct toxins, has a tricyclic 12,13-epoxytrichothec-9-ene (EPT) core composition. They can be further categorized into Groups A, B, C, and D, based on the EPT substituting pattern

(Grove, 2007). Threats to the well-being of humans and animals are posed by groupings of chemically similar mycotoxins known as trichothecenes (TCT), they are made by numerous fibrous fungal species, such as Fusarium, Myrothecium, Stachybotrys, Trichoderma, Trichothecium, and Spicellum (Chen *et al*., 2020). Numerous plant species are affected by Fusarium's production of trichothecenes, a few of which are the types of mycotoxins that pose the biggest risk to the integrity of agricultural commodities (Desjardins, 2006). Because of its extra capacity for topical absorption, TCT is dangerously intoxicating, and its derivatives have an impact on the primordial cellular structures of the hematopoietic, immunological, cutaneous, renal system, and hepatic systems. Dairy cattle and pigs are more vulnerable to this type of toxin, with neurological, immunological, hematological, and reproductive impacts constituting among the more susceptible targets (Zhu *et al*., 2020).

Presence of Amanitins in Mushrooms

There has been a long-running problem concerning the very hazardous amanitin-containing mushroom, which has been associated with instances of total death ranging from 1.8% to 22% in patients undergoing supporting treatment or medical care (Liu *et al*., 2020). Amanitins are poisonous bicyclic octapeptides having a molecular mass of roughly 900 g/mol that are found in some fungi and are part of the amatoxin family. At present, it is known that three important families of fungi-the amanita (*Amanita* sp.), galerina (*Galerina* sp.), and lepiota (*Lepiota* sp.)- contain these toxins (Diaz, 2005). It could be challenging to tell apart amatoxin-containing species from one another, less dangerous species of mushrooms. Nevertheless, an annulus (or "ring") located underneath the cap and a volva (or "cup") are two potential indicators of *Amanita* species (Ward *et al*., 2013). Amatoxins are bicyclic octapeptides, and á—amanitin (á-AMA) (Fig. 7), is one of them which are thought to be the main toxins found in amanita mushrooms. Amatoxins are extremely resilient and resilient to exposure to high temperatures, chilly or acids (Bever *et al*., 2018). The medical approach to treating α-amanitin mostly concentrates on symptoms as well as supportive treatment because it has no particular medication used for detoxification (Xue *et al*., 2023). Following consumption, gastrointestinal disinfection is a necessary first step. The gastric phase is marked by hematuria, cramping in the stomach, diarrhea, nausea, and vomiting (Garcia *et al*., 2015).

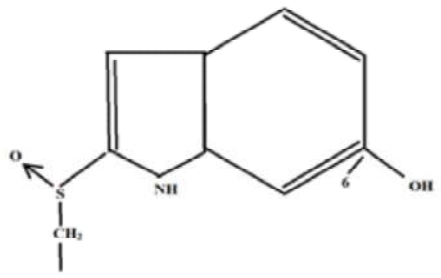

Fig. 7. Chemical structure of alpha Amanitin

CONCLUSION

Mushrooms are nutritious and beneficial fungi that have natural antioxidants in them. Mushrooms are claimed to be rich in protein and fiber, that's why they are used as plant-based supplements for meat products. Mushrooms provide essential minerals like selenium, potassium, iron, copper, and phosphorous as well as one naturally occurring, non-fortified, nutritional supplement that contains vitamin D. These are considered under Kingdom Mycota and not in Kingdom Plantae, because of the presence of chitin in the cell wall. Most of the mushroom fruit bodies have 90% of water in them except *Ganoderma luciderm*. These are spore-bearing reproductive parts of fungi. The mushrooms are categorized into three major types: Edible, poisonous, and medicinal. Edible mushrooms are considered edible because they have ample amounts of nutrition, enzymes, amino acids, etc in them. As an obvious fact, these mushrooms should be treated before consumption by human beings; not necessary for animals as such. *Agaricus bisporous* (Button Mushroom) is the variety that is mostly used. Traditionally, the use of mushrooms for different diseases has been in use. More highly Basidiomycetes fungi also referred to as "pillar mushrooms" or "stand mushrooms" are fungi that have a variety of biologically potent, strong, and weak molecular (such as β-glucans, triterpenes, lectins, and steroids) substances in their fleshy bodies. The mycelium are cultured by the spores, and fluid that may have anti-carcinogenic effects is also cultured. It contains anti-diabetic, and anti-inflammatory characteristics. Mushroom poisoning is a very harmful event if occurs. Most cases of mushroom poisoning occur at the time of Autumn and Spring because of the warm daytime and the cool and pleasant evenings. Proteins and Peptides are the ones that cause cytotoxic effects in mushrooms. There are various toxins that are found in mushrooms such as cytotoxin, amatoxin, phallotoxin, neurotoxin, and myotoxin, etc. Most of the poisoning cases have occurred because of amatoxin content in mushrooms, but the other toxins that are highly poisonous are gyromitrin and orellaline. Gyromitrin is found in the *Gyromitra* spp. Some Gyromitra species can also be eaten if cooked. With the seasonal consideration and brain-like structure, the *Gyromitra* spp. is distinguished from the *Amanita* spp. Gyromitrin could cause gastroenteritis, hemolysis,

headaches, diarrhea, *etc*. if ingested. Orellaline is found in the genus *Cortinarius*. It is nephrotoxic and it mainly affects the Kidney. Orellaline are considered therm-sensitive. Rhabdomyolysis is a disorder that is caused by mycotoxins. Basically, the muscle tissue disintegrates and necrotizes the striated muscles. The necrotic tissues get released into the bloodstream. Clinically, erythromelalgia is identified by looking at symptoms like burning pain in the extremities, pain that is made worse by warmth, pain that is made better by cooling *etc*. Agaritin in mushrooms when combined with different cancer-fighting factors showed therapeutic progress by amplifying the immune system response. The digestive tract, skin, kidney, immunological, and hematopoietic progenitor cell systems are all affected by TCT, which is a harmful intoxicant. The neurological, immunological, haematological, and reproductive effects are among the more susceptible targets, making dairy cattle and pigs more susceptible. Amanitin content is the most hazardous toxin of mushrooms which leads directly to total death. Its initial stage/phase is a gastrointestinal discrepancy, nausea, diarrhea, hematuria, cramps *etc*.

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Research Article

Plant growth promoting, biochemical and antifungal properties of microbes isolated from cow dung

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ABSTRACT

Cow dung is a rich source of microbial diversity and being used for a long time in India for seed treatment, plastering cut ends of vegetatively propagated plant parts, and sprinkling diluted solution on crops. In the present study, microbes from fresh cow dung of indigenous cows were isolated and biochemically characterized. Cow dung enriched with the diversity of microorganisms in different medium *i.e.,* 38 x 10^6 cfu g^{-1} (Nutrient agar), 26.0 x 10^6 cfu g^{-1} (Actinomycetes isolation agar), 36 x 10^6 cfu g^{-1} (Kings B agar), 16 x 10^6 cfu g^{-1} (CV agar), 13 x 10^6 cfu g^{-1} (Methyl red), 15 x 10^6 cfu g^{-1} (Pikovskyas agar), 30 x 10^6 cfu g^{-1} (CRYEMA), Azospirillum 70 x 10^6 cfu g^{-1} (N-free maleate), and 30 x 10^6 cfu g^{-1} (Jenson agar medium). Based on microbial enumeration, a total of 18 bacterial isolates were selected for biochemical, PGPR characterization, and biocontrol activity. All isolates tested positive for phosphate and Zn solubilization. Isolates CD2, CD5, CD13, CD14, and CD19 tested highly positive for zinc, phosphate, K-solubilisation, IAA, and amylase production. Test isolates CD2, CD5, CD6, CD7, CD10, and CD13 were tested highly positive for β-galactosidase activity, ornithine utilization, nitrate reduction, citrate utilization, malonate utilization, esculin hydrolysis, trehalose, glucose, and lactose utilization. All isolated microbes tested for biocontrol activity (antifungal property) against standard pathogen procured from microbial type culture collection (MTCC), *i.e.*, *Colletotrichum gloeosporioides* (MTCC 2190), *Fusarium oxysporum* (MTCC 10247), *C. fimbriota* (MTCC 2281), and *Pythium aphanidermatum* (MTCC 284). CD2, CD34, and CD20 isolates significantly inhibited the growth of *C. gloeosporioides* and *P. aphanidermatum*. The result of the study advocates that cow dung and cow dung-based formulations are cheap sources for soil, plant health, and management of *C. gloeosporioides*, *P. aphanidermatum* and *F. oxysporum* in various crops.

Keywords: Microbial population, Azotobacter, P-solubilising bacteria, Bio-chemical test, plant growth promoting activity and *Ceratocystis fimbriota.*

Application of cow dung in agriculture is reported since ancient times and it is used for various purposes *viz.*, seed treatment, pasting of cut ends in pruned trees, and spraying on crops. Cow dung is digested residue of multifarious bacteria found in a cow's rumen. Feed given to cows passes through the intestine and is mixed with the microbes. Cow dung consists of crude fiber, crude protein, and other materials. Cellulose along with lignin makes up the crude fiber, hemicelluloses and pentosans (poly-saccharides based on pentose sugars) are also present. Bile salts confer a hydrophilic coat to otherwise hydrophobic droplets, thus acting as emulsifying agents and having antiseptic properties (Ram and Pathak, 2017). More than 60 species of bacteria and 100 species of protozoa are found in the rumen of cows (Nene, 2003). Most of the bacteria are cellulose, hemicelluloses, and pectin fermenters. Cow bile consists of salts, acids, and pigments. Cowdung contains microbial diversity, different species of bacteria (*Bacillus* sp., *Corynebacterium* sp. and *Lactobacillus* sp.), protozoa and yeast (Saccharomyces and Candida) (Nene, 1999; Randhawa and Kullar 2011). Sawant *et al*. (2007) isolated various bacteria *viz., Citrobacter koseri*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Kluyvera* spp., *Morganella morganii*, *Pasteurella* sp., *Providencia alcaligenes*, *Providencia stuartii,* and *Pseudomonas* sp. In cow dung. Keeping the above points in view a study was performed to isolate beneficial microbes from fresh cow dung and test them for plant growth-promoting, biochemical and antifungal properties against some selected pathogens.

MATERIALS AND METHODS

Enumeration of microbial populations

Enumeration of different beneficial microbial populations *viz.*, bacteria, fungi, actinomycetes, *Pseudomonas*, gram-positive bacteria, gram-negative bacteria, p-solubilizing bacteria, *Rhizobium*, *Azotobacter* and *Azospirillum* were carried out by using dilution plate count method using selective media *viz.*, Nutrient agar (generalized medium for bacteria), Rose Bengal Chloramphenicol Agar (for fungi), actinomycetes isolation agar (for actinomycetes isolation),

King's B for *Pseudomonas* sp. (King *et al*., 1954), methyl red agar for G^- bacteria (Hagedorn and Holt, 1954), crystal violate agar for G^+ bacteria (Goud, *et al*., 1985), Pikovskaya's agar for P-solubilization (Pikovskaya, 1948), Yeast extract mannitol agar with congo red for rhizobium (CRYEMA, Fred *et al.*, 1932), modified Jenson's agar for Azotobacter (Jensen, 1954; Norris and Chapman, 1968) and N-free malate medium for Azospirillum (Okon *et al*., 1977). Petri dishes were made by pouring each specific solid medium. Then 1.0 g of cow dung samples were diluted to nine ml of sterile water and that was considered to be a 10^{-1} dilution factor. Transfer of 1 ml of 10^{-1} dilution to nine ml sterilized water by sterilized pipettes produced 10^{-2} dilution. Therefore, a series of up to 10^{-8} dilutions were prepared under aseptic conditions. The suspension of 0.1 ml from the required dilution (10^{-8}) was added to the respective agar media on a petri dish and spread with L-spreader with the help of Plate Master (Hi-Media). Then plates were incubated at 28±2°C for 3-5 days. The number of visible colonies was counted. The total count was obtained by multiplying the number of visible colonies on the plate by the dilution factor.

Isolation and characterization of plant growth-promoting bacterial isolates

Based on the enumeration of microbial populations on different selective media, a total of 32 bacteria were isolated from cow dung.

Biochemical characterization

All the isolates were morphologically and phenotypically characterized on the basis of Gram's staining, and differential utilization of different substrates (all from Hi-Media). The reactions tested were indole formation, phosphate solubilization, protease production, citrate and oxidase, acid from glucose, sucrose, lactose, Starch hydrolysis, H2S production *etc*. The results were then interpreted after the addition of the respective reagents where necessary with the help of the colour change.

Plant growth-promoting properties

Test isolates giving encouraging results were selected for plant growth promotory properties like: (a) phosphate solubilization using Pikovaskaya agar (Nautiyal, 1999). (b) Zn solubilization was screened on nutrient agar medium plates supplemented with Zn salts. (c) K-solubilization was screened on modified Aleksandrov agar medium plates. (c) IAA produced by isolates was evaluated by the recommended procedure mentioned by Gordon and Weber (1951) later modified by (Brick *et al*. (1991). (d) Incubated plate of starch agar media pored with iodine. The blue colouration of the media and the clear zone around the colony was observed. Ammonia production using peptone water (e) Hydrogen cyanamide production using King's B media amended with glycine (Bakker and Schipper, 1987). The experiment was laid out in a completely randomized design and data were statistically analyzed using ANOVA and means were compared using Duncan's Multiple Range Test (P=0.05) as per Gomez and Gomez (1984).

Biocontrol properties of isolates

Biocontrol properties (antifungal activity) of bacteria isolates from cow dung were tested by plate assay method against pathogenic fungi like *C. gloeosporioides* (MTCC 2190) *C. fimbriota* (MTCC 2281) on Potato Carrot Agar (PCA), *F. oxysporum* (MTCC 10247) and *P. aphanidermatum* (MTCC 284) were tested on Potato Dextrose Sucrose Agar (PDSA) and Corn Meal Agar (CMA), respectively. Isolates were spot inoculated on a specific medium for pathogens. A five mm mycelial plug was taken from the peripheral edge of five days old cultures of the fungal colony and poured into the center of the plates and inoculated plates were incubated at 30°C for 3 days. Afterwards, the area of inhibition (distance of the fungal mycelium measured in mm) was recorded after 3 days of growth (Zhang *et al.* 2015) and measured the colony size of fungi by the Himedia colony size scale.

RESULTS AND DISCUSSION

Microbial population dynamics in cow dung

Cow dung consists of diverse microbial materials and selected mediums were used to enumerate the microbial population as mentioned in the materials and methods. It was observed that cow dung contained bacteria (38×10^6 cfu g^{-1}), fungus (0×10^6 cfu g^{-1}), actinomycetes (26.0×10^6 cfu g^{-1}), Pseudomonas (36×10^6 cfu g^{-1}), gram-positive bacteria (16×10^6 cfu g^{-1}), gram-negative bacteria (13×10^6 cfu g^{-1}), P-solubilizing bacteria (15×10^6 cfu g^{-1}), Rhizobium (30×10^6 cfu g^{-1}), Azospirillum (70×10^6 cfu g^{-1}) and Azotobacter (30×10^6 cfu g^{-1}) (Fig. 1). Swain *et al*. (2006) isolated thermo tolerant *Bacillus subtilis* isolate in cow dung which has the property of phosphate solubilization. These Bacillus isolates also showed antagonistic activities against plant pathogens along with the production of growth hormones. Cow dung is an active source of beneficial microbes proved by Girija *et al*., 2013). Many biodynamic preparations and bio-enhancers prepared with cow dung contained beneficial microbes (Ram *et al.*, 2018a).

Plant growth promotory properties of isolated microbes

Based on biochemical characterization, 18 bacterial isolates were selected for PGP activities. CD2, CD5, CD13, CD14, and CD 19 were found to be highly positive for zinc, phosphate, K-solubilisation, IAA, and amylase production.

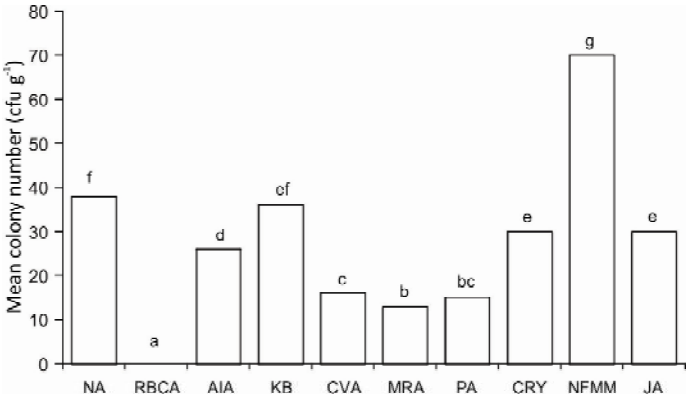

Fig. 1. Enumeration of the microbial population (cfu g^{-1}) in cow dung by using different selective media

[NA- Nutrient agar, RB- Rose Bengal Chloramphenicol Agar, AIA- Actinomycetes isolation agar, KB- King's B, CV- Crystal violate agar, MRA- Methyl red agar, PA- Pikovskaya's agar, C-CRYEMA, NMM- N-free malate medium, JA- Jenson's agar. Bar with the same alphabet are significantly not different as per Duncan's Multiple Range Test (P=0.05)]

All test isolates showed positive results for phosphate and Zn solubilization. Whereas, CD4, CD10, CD11, CD14, CD22, CD24, CD25, CD26, CD31, and CD32 showed negative results for IAA production (Table 1). None of the isolates showed a positive test for HCN production. Ram *et al.* (2018b) have also isolated beneficial microbes from cow pat pits prepared from cow dung. Isolated microbes tested positive for P-solubilisation, IAA, HCN, and ammonia production.

Biochemical characterization of selected microorganisms

Biochemical characterization was performed with different sugar and chemicals for microbial utilization and observed that all the selected bacterial isolates showed effective utilization and positive results for β-galactosidase activity, ornithine utilization, urease activity, phenylalanine deamination, nitrate reduction, H_2S production, citrate utilization, acetoin production, acid production, deamination of tryptophan, malonate utilization, esculin hydrolysis, arabinose utilization, xylose utilization, adonitol utilization, rhamnose utilization, cellobiose utilization, melibiose utilization, saccharose utilization, raffinose utilization, trehalose utilization, glucose utilization, and lactose utilization (Table 2). Test isolates CD2, CD4, CD5, CD6, CD6, CD7, CD9, and CD10 were negative for ornithine utilization, phenylalanine deamination, acetoin production, and arabinose utilization. Test isolates CD2, CD5, CD6, CD7, CD10, and CD13 were tested highly positive for β-galactosidase activity, ornithine utilization, nitrate reduction, citrate utilization, malonate utilization, esculin hydrolysis, trehalose, glucose, and lactose utilization. Perumal *et al.* (2006) have also recorded plant growth hormones such as IAA (28.6 mg/kg), Kinetin (7.6 mg/kg), and Gibberellic acid (23.6 mg/kg) in CPP prepared with cow dung.

Antimicrobial properties of microbes isolated from cow dung

All isolated microbes were tested for antimicrobial

Table 1: Plant growth-promoting properties of selected microbes.

Isolates	Zinc solubilizing	p- solubilising	K- solubilising	IAA production	Amylase production	HCN production
CD2	++++	+++	++	++++	++++	-
CD4	+++	++++	++++	-	++++	-
CD5	++++	+++	+++	++++	+++	-
CD6	++++	++++	+++	++	-	-
CD7	++++	++++	++	++++	-	-
CD9	+++	+++	++	++++	-	-
CD10	++++	+++	++	-	-	-
CD11	+++	+++	+	-	-	-
CD13	++++	++	++	+++	++++	-
CD14	+	+++	+	+	-	-
CD19	+++	++++	++	++++	++++	-
CD20	+++	+++	+++	++	-	-
CD22	-	++	+	-	-	-
CD24	++++	+++	++	+++	-	-
CD25	-	+++	++	-	-	-
CD26	-	++	+++	-	-	-
CD28	-	+++	+	-	+	-
CD31	-	++++	+++	++	-	-
CD32	-	++	++	-	-	-
CD34	+++	+++	++	+++	-	-

Table 2: Biochemical characterization of selected bacterial isolates.

Test	Principle	CD2	CD4	CD5	CD6	CD7	CD9	CD10	CD13	CD19	CD20	CD34
ONPG	Detects β-galactosidase activity	+++	_	++++	++++	++++	++++	+++	++++	++++	++++	++++
Lysine utilization	Detects Lysine decarboxylation	+++	++	++++	++++	+++	++++	++++	++++	++++	++++	++++
Ornithine utilization	Detects Ornithine decarboxylation	_	+	_	_	_	_	_	+++	++++	-	++++
Urease	Detects Urease activity	+	+	++	++++	+	++	++	++++	++++	++++	++++
Phenylalanine Deamination	Detects Phenylalanine deamination activity	_	_	_	_	_	_	_	_	_	_	_
Nitrate reduction	Detects Nitrate reduction	++	++++	+++	++++	++	++++	+++	++++	++++	+	++++
H2S production	Detects H2S production	+++	_	_	_	+	+	+	+	_	+	++
Citrate utilization	Detects capability of organism to utilize citrate as a sole carbon source	++++	_	++++	++++	++++	++++	++++	++++	++++	++++	++++
Voges Proskauer's	Detects acetoin production	_	_	_	_	_	_	_	_	++++	_	_
Methyl red	Detects acid production	+	_	+	_	_	_	+++	+++	_	++	+
Indole	Detects deamination of tryptophan	_	_	+	+	+	+	_	++++	+	_	_
Malonate utilization	Detects capability of organism to utilize sodium malonate as a sole carbon source	+++	+++	+++	++++	+++	++++	+++	++++	+++	++++	++++
Esculin hydrolysis	Esculin hydrolysis	++++	++++	++	++++	++++	+++	++++	++++	++++	++++	++++
Arabinose	Arabinose utilization	_	_	_	_	+	_	_	_	_	_	_
Xylose	Xylose utilization	++++	_	++++	++++	++++	++	++	_	_	+	+++
Adonitol	Adonitol utilization	-	+	-	-	-	-	-	-	-	-	-
Rhamnose	Rhamnose utilization	_	+	++++	++	++++	_	_	_	_	_	_
Cellobiose	Cellobiose utilization	_	+	_	_	_	_	_	_	_	_	_
Melibiose	Melibiose utilization	+	++	++++	+	++	_	_	_	_	_	+
Saccharose	Saccharose utilization	+++	_	++	_	_	_	_	_	+	_	++
Raffinose	Raffinose utilization	_	++	_	_	_	_	+	_	_	_	_
	Trehalose utilization	++++	++	++++	++++	++++	++++	++++	++++	+	++++	++++
Glucose	Glucose utilization	++++	_	++++	++++	++++	++++	++++	++++	++++	+++	+++
Lactose	Lactose utilization	++++	_	++++	++++	++++	++++	++++	++	++++	++++	+++

activities against some selected pathogens. Microbial isolates CD2, CD20, and CD34 showed inhibition against the fungus *C. fimbriota, C. gloeosporioides, F. oxysporum* and *P. aphanidermatum*. CD2, CD20, and CD 34 isolates significantly inhibited the growth of *C. gloeosporioides* by 50.06, 52.34 and 51.39%, respectively. Isolate CD20 inhibited the growth of *C. fimbriota*, *C. gloeosporioides*, *Fusarium oxysporum* and *P. aphanidermatum* by 10.86, 52.24, 40.49, and 32.51 per cent, respectively. Isolate CD 34 also showed inhibition properties against the pathogens and inhibited the growth of *C. fimbriota*, *C. gloeosporioides*, *Fusarium oxysporum* and *P. aphanidermatum* by 7.44, 51.39, 41.67, and 36.04 per cent, respectively (Fig. 2 & 3). Mary *et al.* (1986) reported that cow dung extract is more effective than antibiotics like Penicillin, Paushamycin, and Streptomycin

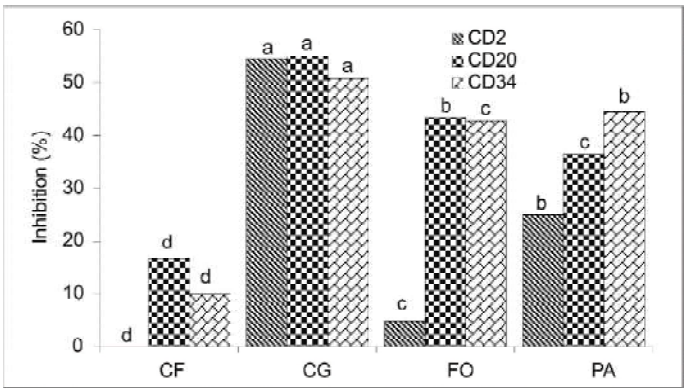

Fig. 2. Antimicrobial properties of microbes isolated from cow dung (CF- *Ceratocystis fimbriota*, CG- *Colletotrichum gloeosporioides*, FO- *Fusarium oxysporum*, PA- *Pythium aphanidermatum*)

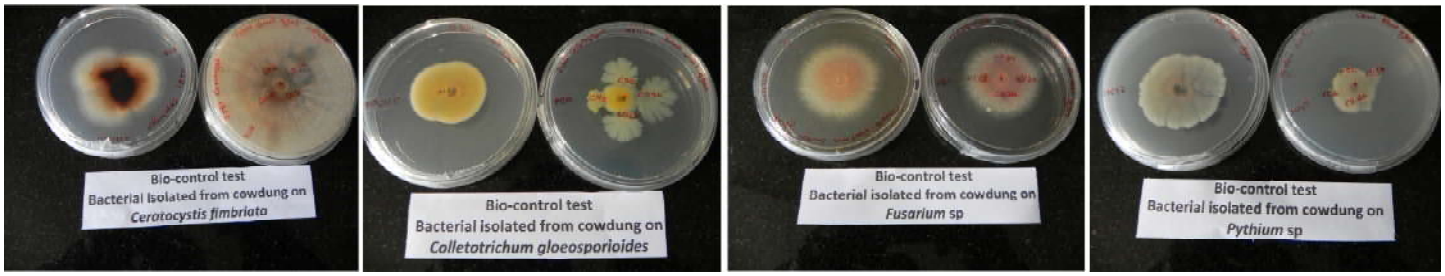

Fig. 3. Antimicrobial properties of microbes isolated from cow dung (CF- *Ceratocystis fimbriota*, CG- *Colletotrichum gloeosporioides*, FO- *Fusarium oxysporum*, PA- *Pythium aphanidermatum*)

in the management of bacterial blight in rice. *B. subtilis* isolates were more predominant from culturable cow dung microbes. Joseph and Sankarganesh (2011) have also reported the antifungal property of microbes isolated from *Panchagavya* prepared with cow dung and other materials. Yangabi *et al.* (2009) have recorded cow dung slurry's effect on the antimicrobial property of medicinal crops. Swain *et al.* (2008) have isolated *B. subtilis* from cow dung which is used as a biocontrol agent in the management of plant pathogens. Waziri and Suleiman (2013) also reported the antimicrobial property of cow dung vapour against pathogens. Proctor (2008) has suggested seed/seedling treatment with cow pat pit (cow dung based) for the management of the soil-borne disease. In another study, Utpal *et al.* (2013) found that the use of Panchagavya showed 86.30 per cent suppression of fungal growth and 95.9% of spore germination of *Curvularia lunata*. They have concluded that Neemazol, Trichoderma, and Jeevamrita (prepared with cow dung) can be used to manage *P. sorghi* instead of fungicides which will be the cheapest and eco-friendly way to manage the common rust of maize. Seed treatment with Panchagavya further improved the seed germination by 99% and vigour index 2822 and 2477 with PB9 and 15. Application of Panchagavya @ 3% was found effective in the management of leaf spot, blight, mildew, and rust diseases of vegetables (Nagaraj and Sreenivasa, 2009).

CONCLUSION

The study concluded that cow dung is a potent source of agriculturally important microbes which pass multifarious PGPR and biochemical activities. These microbes have the potential for various biochemical properties, IAA production, and P, K, Zn solubilization properties. Isolated microbes have shown inhibition properties against pathogenic fungus also. Therefore, cow dung can be used in the production of different bio-enhancers for nutrient, and pest management in organic production.

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Research Article

Soil aggregation and aggregate-associated carbon affected by long-term crop residues incorporation in rice-wheat cropping system

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ABSTRACT

Crop residue management is one of the options for enhancing soil organic carbon stabilization by improving soil aggregation in semi-tropical soil. We studied the influence of different levels of crop residue incorporation on aggregation and carbon stabilization in different soil aggregates after 23 years of continuous rice-wheat cropping system on sandy loam calcareous soil. Residue incorporation (100%) caused a significant increment of 49.09% in total water-stable aggregates (>0.25 mm) in surface soil. Carbon content in both macro- and micro aggregates was increased, but the increment was greater in macroaggregates (33.47%) than in microaggregates (16.23%). These results show that the long-term application of organics in combination with inorganic fertilizers helped in carbon accumulation in macroaggregates, thereby improving soil carbon status.

***Keywords*:** Soil aggregation, aggregate associated carbon, long-term. crop residues.

Soil aggregates are basic units of soil structure and they comprise primary particles and binding agents. The soil aggregates and the pores between them affect water movement and storage, aeration, erosion, biological activity, and therefore, the growth of crops (Verhulst *et al*., 2010). Thus, soil aggregates influence a wide range of physical and biogeochemical processes in the agricultural environment (Amezketa, 1999). Soil-aggregate stability is a crucial soil property affecting soil sustainability, productivity, and crop production. However, soil aggregate formation and stabilization are affected by several factors. The straw residue quality and amount play a very important role in the process of aggregate formation (Lynch and Bragg, 1985).

Soil aggregates physically protect the organic matter. Organic inputs, like crop residues, organic manures *etc*. improve soil aggregation and aggregate stability (Bandopadhyay *et al*., 2010; Karami *et al*., 2012). Organic amendments help in improving the formation of macroaggregates (Mikha and Rice, 2004; Bandyopadhyay *et al*., 2010) with a proportionate decrease in microaggregates. It means that the addition of organics favours the formation of macroaggregates through binding of microaggregates (Haung *et al*., 2010). Soil aggregate formation and stabilization are linked to soil organic carbon (SOC) dynamics in soil. Organic inputs have significant impacts on both the bulk soil and aggregate-associated carbon content (Benbi and Senapati, 2010; Bandyopadhyay *et al*., 2010). The C sequestration in the soil through enhanced aggregation is an important approach of judicious soil management to mitigate the increasing concentration of atmospheric CO_2. Aggregate-associated C is an important reservoir of soil C, protected from mineralization because it is less subjected to physical, microbial, and enzymatic degradation. Carbon inputs from different organics may affect SOC distribution and stabilization in soil-aggregate size fractions and for maintaining the productivity of the rice-wheat cropping system. A fixed-site field experiment was performed for Twenty-three consecutive years to determine the influence of crop residue incorporation on percent water-stable aggregate, mean weight diameter, and aggregate-associated organic carbon preservation under rice-wheat rotation.

MATERIALS AND METHODS

Site description

A long-term field experiment with a rice-wheat cropping system on an Entisol located in the hot, humid

subtropics was established at the University Research Farm, Rajendra Prasad Central Agricultural University, Pusa, Bihar, India (25° 98'N, 85° 66' E, 52.00 above sea level), in *Kharif*, 1994. The experimental site receives an average annual rainfall of approximately 1330 mm and the mean monthly maximum and minimum temperature fluctuate between 28.7 to 35.1 °C and 15.3 to 26.2 °C, respectively. The total rainfall received during 2017-18 was 1135 mm. The soil is sandy loam, with pH 8.4, and contains 558, 308, and 134 g/kg of sand, silt, and clay, respectively. The initial surface soil (0-0.15 m) of the experimental site had 5.07 g/kg oxidizable organic C (Walkley and Black, 1934) and 366 g/kg $CaCO_3$ content.

Treatments and crop management

The experimental design was split-plot with four crop-residue (0, 25, 50 & 100%) levels in main plots and four levels of Zn application (0, 2.5, 5.0 & 10 kg/ha) in sub-plots. subplot treatments were applied only once in the year 1994. Each treatment was replicated three times. Rice and wheat crops are being grown continuously with necessary tillage under the rice-wheat system during *kharif* and *rabi* seasons. The chopped straw of the previous crops treated as crop residues were incorporated as per treatment. The source of N, P, and K was urea, di-ammonium phosphate (DAP), and muriate of potash (MOP), respectively. The dose of fertilizer was 120, 60, and 40 (N, P_2O_5, K_2O).

Soil sampling and analysis

Soil samples (post-harvest soil of 23rd wheat crop) were collected from 0-15 cm depth. The samples, thus obtained were air-dried in shade at room temperature, powdered, and passed through a 0.2-mm sieve. Thereafter, the soil was thoroughly mixed to make it homogeneous and kept in a polythene bag with proper labeling for chemical analysis. The methods used in the analysis for the soil properties of initial and post-harvest soil samples were as follows:

Aggregate stability

Large clods were broken by hand into smaller pieces along natural cleavage having a size greater than 8 mm. The aggregates were separated using the wet-sieving technique (Yoder, 1936). After wet sieving, aggregate from each sieve was transferred with a stream of distilled water into a Buchner funnel with a pre-weighed filter paper. The soils along with filter paper were oven-dried at 65°C until the water evaporated and weighed. The water-stable macroaggregate (>0.25 mm) and mean weight diameter (MWD) were calculated as an index of aggregation (Kemper and Roseneau, 1986) using the following formula:

$$MWD = \sum W_i X_i$$

Where,

X_i : Arithmetic mean diameter of each size fraction (mm)

W_i : Proportion of the total sample weight occurring in the fraction i

Aggregate-associated carbon

Organic carbon content in different aggregate-size fractions (8.0-5.0 mm, 5.0-2.0 mm, 2.0-1.0 mm, 1.0-0.5 mm, 0.5-0.25 mm, 0.25-0.1 mm) obtained by the process of wet sieving technique was determined. The soil samples after oven drying at 65 °C for 48 hours and passing through a 0.2 mm sieve, were used for determination. From this, 1 g soil sample was taken and organic carbon content was determined by the wet digestion method (Walkley and Black, 1934). Macroaggregate-associated carbon was calculated by taking the average value of aggregate-associated carbon of sieves having a diameter more than 0.25 mm (8.0-5.0 mm, 5.0-2.0 mm, 2.0-1.0 mm, 1.0-0.5 mm, 0.5-0.25 mm) and microaggregate-associated carbon was determined from the soil aggregates passed through 0.25 mm sieve and retained in 0.1 mm sieve.

RESULTS AND DISCUSSION

Water-stable macroaggregates (%)

The aggregate-size distribution and stability are important indicators of the soil's physical quality. The management of previous crop residues is the key to soil structural development and stability since organic matter is an important factor in soil aggregation (Verhulst *et al.*, 2011). After 23 years of straw incorporation, the >0.25 mm water-stable macroaggregates increased significantly by 10.85, 29.24, and 49.09% in the treatments receiving 25, 50, 100% crop residues, respectively in comparison to no residue (Table 1). This result might be mainly because fresh straw and other organic materials release organic substances, such as polysaccharides and organic acids, which provide attachment points and nutrients for the growth of fungi and microorganisms and simultaneously promote the cementation of soil particles to form macroaggregates. Similar, result was found by Zhang *et al.* (2014) and Wei *et al.* (2006). No significant decrement or increment pattern in water-stable macroaggregates was found in treatment receiving different residual zinc levels.

Mean weight diameter (MWD)

The effects of straw incorporation on the mean-weight diameter of water-stable aggregates are shown in Table 1.

Table 1: Effect of long-term crop-residue incorporation on water-stable macro-aggregates (>0.25 mm) and mean weight diameter of soil.

Crop residue level (% of straw produced)	Water stable macro-aggregates (%)	Mean weight diameter (mm)	Zn levels (kg ha^{-1})	Water stable macro-aggregates (%)	Mean weight diameter (mm)
0	41.82	1.58	0	49.56	1.95
25	46.36	1.80	2.5	52.76	1.94
50	54.05	2.05	5	49.83	1.93
100	62.35	2.34	10	52.44	1.94
S.Em.±	1.747	0.022	S.Em.±	1.516	0.029
C.D. (P=0.05)	6.16	0.08	C.D. (P=0.05)	NS	NS

These data showed significant improvement in soil aggregation with the addition of crop residue. After 23 years of crop residue incorporation, MWD significantly increased with subsequent increases in crop residue levels.

Residue-derived organic components (e.g., humic acids, polysaccharides *etc.*) and living organisms (*e.g.*, fungi) enmesh and bind inorganic particles into aggregates (Tisdall and Oades, 1982). A similar result was observed by Bandyopadhyay *et al*. (2010). They found that structural indices *viz*., MWD and water-stable aggregates were higher in the soil receiving organic amendments than that in the minerally fertilized soil. No significant increase in MWD was found with increased residual zinc level.

Aggregate associated carbon

Long-term incorporation of crop residue increased aggregate-associated carbon as compared to bulk soil. Macroaggregate-associated carbon increased significantly with subsequent increases in crop residue levels. After 23 years of residue incorporation macroaggregate-associated carbon increased by 11.2, 21.1 and 33.5 per cent in the treatments receiving 25, 50 and 100 per cent residue levels, respectively as compared to that in no crop residue treatment (Table 2 and Fig. 1). Carbon content in microaggregate also increased with increase in crop residue levels, but the increment was less than macroaggregates. These results show that the long-term application of organics in combination with inorganic fertilizers helped in carbon accumulation in macroaggregates, thereby improving soil carbon status.

The concentration of carbon was higher in macroaggregates than in microaggregates. Among the aggregate-size classes, carbon concentration was the highest in 1-2 mm and it decreased as the aggregate size decreased. The incorporation of organic manures induces the decomposition of organic matter where roots, hyphae, and polysaccharides bind mineral particles into microaggregates and then these microaggregates bind to form carbon-rich macroaggregates. This type of carbon is physically protected within macroaggregates. It suggests that SOC concentration increases with increasing aggregate size and a slower macroaggregate turnover, leading to greater stabilization of carbon in microaggregates within macroaggregates (Six *et al*., 2014). Cheng *et al*. (2015) found that the 0.25-20 mm

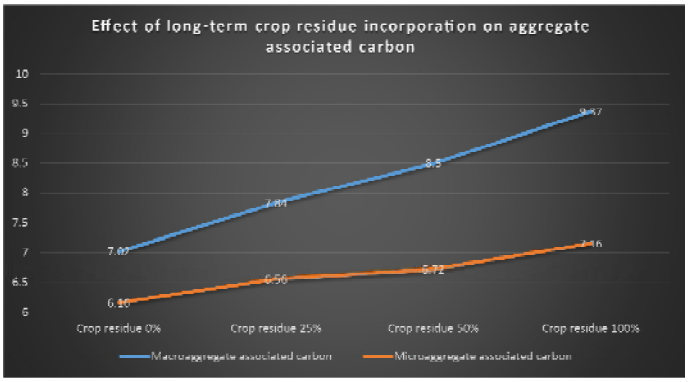

Fig. 1. Effect of long-term crop-residue incorporation on aggregate associated carbon of soil.

Table 2: Effect of long-term crop-residue incorporation and starter zinc on aggregate associated carbon of soil.

Crop residue level (% of straw produced)	Macroaggregate associates C (g/kg)	Microaggregate associated C (g/kg)	Zn levels (kg ha^{-1})	Macroaggregate associates C (g/kg)	Microaggregate associated C (g kg^{-1})
0	7.02	6.16	0	8.08	6.62
25	7.84	6.56	2.5	8.13	6.64
50	8.50	6.72	5	8.28	6.66
100	9.37	7.16	10	8.24	6.68
S.Em.±	0.048	0.063	S.Em.±	0.17	0.081
C.D. (P=0.05)	0.17	0.22	C.D. (P=0.05)	NS	NS

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aggregates had higher soil organic carbon content than the other aggregates, and vegetation restoration generally benefited the formation of these aggregates. Similar results of carbon accumulation in macroaggregates have been reported earlier by several other researchers (Benbi and Senapati, 2010; Das *et al*., 2014).

CONCLUSION

Application of crop residues in combination with inorganic fertilizers significantly improves water-stable aggregates, aggregation indices, and organic carbon protected in aggregates. A higher amount of carbon is present in macro-aggregates than in micro-aggregate. These help in retaining carbon in soil, and thus in reducing atmospheric carbon dioxide levels.

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Research Article

FLD: An effective approach to improve the production potential of groundnut in Guntur district of Andhra Pradesh

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ABSTRACT

Cluster Frontline Demonstration (CFLD) is a unique approach to demonstrate the production potential of newly released technologies on the farmers' fields. The Krishi Vigyan Kendra, LAM, Guntur conducted the Cluster Frontline Demonstration (CFLDs) on oilseeds from 2016 to 2019. The KVK had organized 150 CFLDs on Groundnut (*Arachis hypogaea* L.) in five villages *viz.,* Yazilli, Danduvaaripaalem, Pachala Thaatiparru, Kaskarru and Karlapalem of Guntur district. To get the adequate size of the sample, 150 CFLDs beneficiary farmers were selected as the sample for the present study. The highest average yield of 40.25 q ha^{-1} was recorded in the groundnut variety Kadiri 7 Bold, while the lowest average yield was 22.5 q ha^{-1} (Kadiri 6) with 12.9 and 12.4 per cent increases in yield, respectively. Extension gaps were observed as 3.9, 4.6, and 3.6 in Kadiri 6, Kadiri 7 bold and Kadiri Haritandra. The technology index ranges from 9.75 to 19.6 in demonstrated groundnut varieties in the CFLD programme. There was a significant increase in area from 25 to 230 ha (increased up to 205%), 10 to 110 ha (increased up to 100%), 30 to 190 ha (increased up to 160%) in Kadiri 6, Kadiri 7 bold and Kadiri Haritandra groundnut varieties, respectively. Impact on the adoption (percent change) was prominent in the case of Kadiri 6 (820%), Kadiri Haritandra (533.3%), and Kadiri 7 bold (90.9%) because of their yield potential, crop management practices followed by the farmers suggested by KVK scientists through CFLD. A maximum (48.6%) number of respondents are highly satisfied with the services rendered through CFLD. The Local variety of groundnut variety TAG-24 was replaced by improved varieties developed by ANGRAU, Andhra Pradesh such as Kadiri-6, Kadiri 7 bold, and Kadiri Harithandra'. Thus, it can be inferred that CFLDs is an effective extension intervention to demonstrate the production potential of improved technologies in Groundnut on farmers' field. Therefore, it is recommended that the extension agencies engaged in the transfer and application of agricultural technologies on farmers' fields.

***Keywords*:** CFLD, Groundnut (*Arachis hypogaea* L), Kadiri-6, Kadiri 7 bold and Kadiri Haritandra

Groundnut (*Arachis hypogaea* L.), an annual legume, belongs to the family Fabaceae and originated from South America. It is one of the principal food and oilseed crops of tropical and sub-tropical regions of the world. Moreover, it is also an important traditional cash crop for peasants in poor tropical countries (Shiyam, 2009). Groundnut is one of the major oilseed crops of India accounting for 25 per cent of total oilseed production in the country. India's vegetable oil requirement by 2022 is estimated to be 33.2 million tonnes and currently imports about 70 per cent of the requirements accounting for about 73,000 crores rupees per annum. Further, industrialization and urbanization have led to a decrease in land availability, moreover, farmers primarily focus on the cultivation of cash crops like cotton, maize, *etc*. This along with the pulses shortage has led to malnutrition creating a serious consequence for the growing generation. Groundnut, a major oilseed of India, accounts for 25 per cent of the total oilseed production of the country with a yearly production of 6.73 million tonnes occupies an area of 4.59 m ha, with an average productivity of 1465 kg ha^{-1} (Chauhan *et al*., 2016). Groundnut not only tastes good but is also rich in protein, fat, and various healthy nutrients. The fat content ranges from 44-56 per cent mainly containing mono and poly-unsaturated fatty acids like oleic acid (40-50%) and linoleic acid (24-35%). In addition, it is also fairly rich in calcium, iron, and vitamin B complex like thiamine, riboflavin, niacin, and vitamin A (Baraker *et al*., 2017). The National Development Council (NDC) in its 53rd meeting held on 29th May 2007 adopted a resolution to launch a Food Security Mission comprising rice, wheat, and pulses to increase the annual production of rice by 10 million tonnes, wheat by 8 million tonnes and pulses by 2 million tonnes by the end of the Eleventh Plan (2011-12). Accordingly, a Centrally Sponsored Scheme, 'National Food Security Mission' (NFSM), was launched in October 2007.

The Mission met with overwhelming success and achieved the targeted additional production of rice, wheat, and pulses. The Mission continued during the 12th Five Year

Plan with new targets of additional production of food grains of 25 million tonnes of food grains comprising 10 million tonnes of rice, 8 million tonnes of wheat, 4 million tonnes of pulses, and 3 million tonnes of coarse cereals by the end of 12th Five Year Plan. Considering the experience and feedback received from the state governments, major changes were made in approach, norms of financial assistance, and programme implementation strategy which are reflected in the revised operational guidelines.

Based on the experience and performance of 12th Plan, it has been decided to continue the programme beyond 12th plan *i.e.*, 2017-18 to 2019-20, which is co-terminus with the Fourteenth Finance Commission (FFC) period with new targets to achieve 13 million tonnes of additional food grains production comprising of Rice- 5 million tonnes, Wheat- 3 million tonnes, Pulses- 3 million tonnes and Coarse Cereals- 2 million tonnes by 2019-20. Therefore, the aim of the present study was to determine the impact of Cluster Frontline Demonstrations (CFLDs) on yield, adoption, and varietal replacement of Groundnut and its horizontal spread in Guntur district of Andhra Pradesh state. An in-depth study on the performance of front-line demonstrations in respect of yield and the economic parameter was needed for further strengthening of the programme for effective results. In this context, the present investigation was implemented with the following objectives. 1) To ascertain knowledge and adoption of groundnut production technology and to find out the relationship with profile characteristics, 2) To assess the performance of front-line demonstrations on groundnut with respect to yield and economics, and 3) To study the satisfaction of the beneficiaries regarding services rendered through front-line demonstrations.

MATERIALS AND METHODS

The Krishi Vigyan Kendra, Lam, Guntur conducted the Cluster Frontline Demonstration (CFLDs) on oilseeds from 2016 to 2019. The KVK had organized a total of 150 CFLDs on Groundnut (*Arachis hypogaea*) in 5 villages *viz.*, Yazilli, Danduvaaripaalem, Pachala Thaatiparru, Kaskarru and Karlapalem of Guntur district in sandy loam soils in *Rabi* season. Varietal characteristics of improved varieties introduced in adopted villages against the age-old existing Groundnut variety TAG 24 are presented in Table 1. Farmer's practices were compared with improved management practices taking a sample size of 150 farmers and adoption gaps were identified. The gaps were categorized into three groups: no gap given a score of 1, partial gap given a score of 2, and full gap given a score of 3. The adoption gap index was calculated using the formula given by Dubey *et al.* (1981).

Yield parameters of both demonstrations and checks involving farmer's practices were recorded. Satisfaction of beneficiaries for the present investigation is taken as, reacting positively or negatively towards the services rendered through front-line demonstrations. The satisfaction of services rendered through the organization of front-line demonstrations was measured based on various dimensions like technology demonstrated, training of participants, timeliness of services, provision of inputs, field visits, diagnosis and advisory services to field problems rendered, organization of extension activities, the performance of variety demonstrated and overall impact of front line demonstrations. The selected respondents were interviewed personally with the help of a structured interview schedule on different dimensions of services rendered. The collected data were tabulated and analyzed by using statistical tools like frequency and percentage. To estimate the extension gap, the technology gap and technology index following formulae was considered as suggested by Samui *et al*. (2000), Kadian, *et al.* (2004) and Sagar and Chandra (2004). The client Satisfaction Index (CSI) was calculated by using a formula developed by Kumaran and Vijayragavan (2005). The economics of the demonstrations and checks were recorded. Based on economics additional cost, effective gain, additional returns, and incremental BC ratios were calculated. Paired t-test was applied to know if there exists a significant difference in the economics of demonstration and check.

The adoption gap index is the percent deviation in farmers' practices as compared to the improved practices.

$$\text{Adoption Gap Index} = \frac{(R - A)}{R} \times 100$$

Where,

R = Total number of improved practices

A = Number of improved practices actually adopted by the farmer

Extension Gap (q/ha) = Demonstrated Yield – Yield under Existing Farmers Practice

Technology Gap (q/ha) = Potential Yield – Demonstration Yield

Yield Gap (%) = (Extension Gap/Yield under Existing Farmers Practice) x 100

Technology Index (%) = Technology Gap/Potential Yield x 100

RESULTS AND DISCUSSION

The traits of the selected groundnut varieties implemented in CFLD are given in Table 1. In a survey conducted among 150 respondents, the results revealed that a full gap was identified with the adoption of variety, seed treatment, fertilizer management, and plant protection. Partial gaps were identified with land preparation, seed treatment, and weed management. While no gaps were identified with sowing time, irrigation management, and harvesting presented in Table 2. Findings are in coordination with Subhaiah and Jyothi (2019), in groundnut and sesame crops, Raghava and Rao (2013) in groundnut crop in Guntur district. Ranjan *et al*. (2014) observed knowledge and adoption gaps in relation to the use of farm machinery and emphasized the need for improved variety in groundnut.

The data regarding yield parameters of the demonstration are presented in Table 3. The highest average yield of 40.25 q ha^{-1} was recorded in the groundnut variety Kadiri 7 Bold, while the lowest average yield was 22.5 q ha^{-1} (Kadiri 6) with 12.9 and 12.4 per cent increase in yield, respectively. Whereas Kadiri Haritandra variety recorded 36.1 compared to 32.5 q/ha in check yield with 11.0 per cent increase in yield. Data presented in Table 3 confirms that there was a positive and significant increase in the average crop yield. The increase in the percentage of yield was ranging 11 to 12.9 per cent. Similar trends of yield enhancement in front-line demonstration of pulse crops have been documented by Dwivedi *et al*. (2013). Extension gaps were observed as 3.9, 4.6, and 3.6 in Kadiri 6, Kadiri 7 bold and Kadiri Haritandra. With the adoption of improved practices, the technology gap can be reduced as a result technology index will be reduced. The technology index ranges from 9.75 to 19.6 in demonstrated groundnut varieties in CFLD programme. With the adoption of improved practices, the technology gap can be reduced as a result technology index will be reduced. Similar findings were reported by Sreenivasulu *et al*. (2014); Joshi *et al*. (2014) and Kumar *et al*. (2014).

The data regarding economic parameters of demonstrations *viz*., the total cost of cultivation, gross returns, estimated net returns, and benefit-cost ratio are presented in Table 4. Economic analysis of yield performance of demonstrations revealed that Kadiri 7 bold variety resulted in Rs. 81,650 ha^{-1} with benefit-cost ratio of 2.0:1 compared to farmers practice Rs. 58,200 ha^{-1} with 1.6:1 benefit cost ratio. Whereas Kadiri 6 and Kadiri Haritandra varieties resulted in net returns of Rs. 58470 and 58788 ha^{-1} with benefit-cost ratio of 1.8:1 and 1.7:1, respectively as per the data presented in Table 5. These findings are in accordance with Honnali and Chittapur (2014) in the groundnut crop. Mehriya e*t al*. (2020) also confirm the positive impact of CFLD and training on mustard, also Ganga Devi *et al.* (2017) in black gram crop in Guntur district.

Table 1: Varietal traits of Groundnut crop implemented in CFLD in Guntur district.

Variety	Duration (days)	Potential yield (kg $acre^{-1}$)	Percent Oil content	Seed percentage	Seed dormancy	Characters
Kadiri -6	100	Kharif: 22 Rabi: 42	48.0	72.0	No	Small bunch type plant. The size of the seed is 5% bigger than JL 24
Kadiri -7 bold	Kharif: 120-125 Rabi: 130-135	Kharif: 25 Rabi: 50	49	70	40 days	Bold seeded large bunch type plant. Resistant to Leaf spot and Thrips.
Kadiri Haritandra	105-110	Kharif: 25 Rabi: 40	48	70	No	Resistance to drought, thrips, and leaf spot. High Green haulm content.

Table 2: Identified adoption gaps in Groundnut cultivation in Guntur district of Andhra Pradesh.

Particulars	Improved practices	Farmers practices	Gap
Variety	Kadiri-6, Kadiri-7 bold, Kadiri Haritandra	TAG 24	Full gap
Land preparation	Tractor drawn cultivator	Tractor or bullock-drawn cultivator	Partial gap
Sowing time	*Rabi* (November 15th)	*Rabi* (November 15th)	No gap
Seed rate	75 kg $acre^{-1}$	100 kg $acre^{-1}$	Partial gap
Seed treatment	Carbendazim 50% WP @ 1 g kg^{-1} seed, Imidacloprid 17.8 SL @ 2.0 ml kg^{-1} seed and *Trichoderma viride* 10 g kg^{-1} seed	No Seed treatment	Full gap
Fertilizer management	4 Tonnes of FYM, 27-100-33 NPK and 200 kg gypsum kg $acre^{-1}$	30-46-30 kg ha^{-1}	Full gap
Irrigation management	Irrigation at critical stages	Irrigation at critical stages	No gap
Weed management	Pre emergence Alachlor 50% EC @ 1.0 lit $acre^{-1}$ and harrowing at 25- 40 DAS	Manual weeding and Harrowing at 25-40	Partial gap
Plant protection	IPM practice for lepidopteron pests	IPM not practiced	Full gap
Harvesting	Manual harvesting	Manual harvesting	No gap

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Table 3 : Yield details of groundnut under CFLD.

Year	Variety	Demo Area (ha)	Farmers (No's)	Demo yield (q ha^{-1})			Check yield (q ha^{-1})	Increase (%)
				Max	Min	Avg		
2016-17	Kadiri 6	30	75	36.2	33.8	35.2	31.3	12.4
2017-18	Kadiri 7 Bold	20	50	41.5	38.9	40.2	35.6	12.9
2018-19	Kadiri Haritandra	10	25	37.5	34.8	36.1	32.5	11.0

Table 4 : Gap in yield production of Groundnut.

Year	Variety	Potential yield (q ha^{-1})	Extension gap (q ha^{-1})	Technology gap (q ha^{-1})	Yield gap (%)	Technology index (%)
2016-17	Kadiri 6	42	3.9	6.8	12.4	16.1
2017-18	Kadiri 7 Bold	50	4.6	9.8	12.9	19.6
2018-19	Kadiri Haritandra	40	3.6	3.9	11.07	9.75

Table 5 : Economic parameters of Groundnut under CFLD.

Year	Variety	Economics of Demonstration (Rs ha^{-1})				Economics of Farmers' Practice (Rs ha^{-1})			
		Grass cost	Gross returns	Net returns	BC ratio	Grass cost	Gross returns	Net returns	BC ratio
2016-17	Kadiri 6	68250	126720	58470	1.8:1	72750	112680	39930	1.5:1
2017-18	Kadiri 7 Bold	79540	161200	81650	2.0:1	84200	142400	58200	1.6:1
2019-20	Kadiri Haritandra	81280	140068	58788	1.7:1	85670	126100	40430	1.4:1

Efforts were made to study the impact of CFLDs on the horizontal spread of improved groundnut varieties. It was evident from Table 5 that CFLDs organized on groundnut crops helped to increase the area under improved varieties in selected villages. There was a significant increase in area from 25 to 230 ha (increased up to 205%), 10 to 110 ha (increased up to 100%), 30 to 190 ha (increased up to 160%) in Kadiri 6, Kadiri 7 bold and Kadiri Haritandra groundnut varieties, respectively. Impact on adoption (per cent change) is prominent in the case of Kadiri 6 (820%), Kadiri Haritandra (533.3%), and Kadiri 7 bold (90.9%) because of their yield potential, crop management practices followed by the farmers as per the data in Table 6.

The concept of satisfaction of beneficiaries for the present investigation is measured as reacting positively or negatively towards the services rendered through frontline demonstrations. Questions through a pre-tested structured interview schedule were posed to the beneficiaries and the responses were pooled, and the results are presented in Table 7. A close observation of the figures presented in Table 7 implies that the majority (48.6%) of respondents expressed a medium to high (73) level of satisfaction with the extension services rendered and the performance of technology demonstrated. Relatively few respondents (18.6%) expressed a lower level of satisfaction.

Table 6: Horizontal spread of Improved Groundnut varieties through CFLDs in Guntur district.

Sl. No.	Groundnut variety	Area (ha)		Change in Area (ha)	Impact on Adoption (% change)
		Before demonstration	After demonstration		
1	Kadiri 6	25	230	205	820.0
2	Kadiri 7 Bold	10	110	100	90.9
3	Kadiri Haritandra	30	190	160	533.3

Table 7: Extent of satisfaction of beneficiaries about services rendered through the organization of FLDs (Index).

Sl.No.	Satisfaction of beneficiaries	Beneficiaries (n=150)	
		Frequency (No)	Percentage (%)
1	Low (<85)	28	18.6
2	Medium (86-95)	73	48.6
3	High (>96)	49	32.6

CONCLUSION

It can be concluded that a maximum (48.6%) number of respondents are highly satisfied with the services rendered through CFLD. The results signified the positive response of the beneficiaries towards the services rendered through CFLDs. It also depicts the stronger conviction and active involvement of beneficiaries in laying the demonstrations which intern would lead to an increase in knowledge level and higher adoption. The present findings are in accordance with the reports of Singh *et al*. (2020) in pulse crops and Kulkarni *et al.* (2018) in sunflower crop.

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Research Article

Effect of organic nutrition on growth, yield and economics of knol-khol (*Brassica oleracea* L. var. gongylodes) cv. Pusa Virat

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ABSTRACT

A field experiment was carried out at the Organic Research Farm, Karguan ji, Jhansi, Department of Horticulture, Institute of Agricultural Sciences, Bundelkhand University, Jhansi, Uttar Pradesh, India during *rabi* season 2020-21 to study the "Effect of organic nutrition on growth, yield and economics of knol-khol cv. Pusa Virat". Application of 33 per cent each of mustard cake + PSB + *Azotobacter* (T_6) resulted in significantly maximum growth and yield attributing parameters, consequently yield of knol-khol. The higher plant height (35.21 cm), leaves per plant (28.97), knob length (24.11 cm), knob breadth (6.59 cm), biological yield (181.79 kg ha^{-1}), weight of knob (132.58 g), volume of knob (112.35 cc), yield of knob (148.06 q ha^{-1}), with net return Rs. 131632 ha^{-1} and 2.62 B:C ratio were recorded in the above treatment. The second best treatment was T_7 having 33 per cent each of neem cake + PSB + *Azotobacter* which yielded 143.27 q ha^{-1} knob *i.e.*, 4.79 q ha^{-1} less as compared to T_6 treatment. The knob yield in the control treatment was only 90.64 q ha^{-1} with a net income Rs. 92570 ha^{-1}.

Keywords: Organic nutrition, knol-khol, Pusa Virat

Knol-khol (*Brassica oleracea* L. var. gongylodes) *i.e.*, Ganth Gobhi is a coil season crop belonging to the family *Cruciferae*. It originated from the coastal countries of the Mediterranean region. The knob is generally used as a cooked vegetable. It is also utilized for making salad and pickles. Occasionally young leaves are also cooked as vegetables. As it is gaining popularity in the big cities and small towns as well. Standardization of agro-techniques for its successful cultivation, particularly in semi-arid areas is very essential.

It is well-documented that the growth and quality of plants are greatly influenced by a wide range of applied nutrients (Chaudhary *et al.*, 2017). The use of organic manures to meet the nutritional requirement of crops would be an inevitable practice in the years to come for sustainable agriculture. Because organic manure generally improves the physico-chemical and biological properties of soil along with conserving, its moisture-holding capacity and thus resulting in enhanced crop productivity.

The bio-fertilizers are organic in origin which are absolutely safe. Therefore, it is essential to adopt a strategy of integrated nutrient management using a combination of chemical fertilizers, organic manures, and bio-fertilizers so as to minimize the cost of production and maintain the biological productivity of soils (Kumar *et al.*, 2018). The application of *Azospirillum* and *Azotobacter* inoculants in vegetable crops has been of much significance because they not only fix atmospheric nitrogen but also produce growth-promoting and antifungal substances (Parkey *et al.*, 2017). Since no work has been done so far on knol-khol in the Jhansi region, hence looking to the above facts, the present research was taken up so that proper recommendations may be made for the vegetable growers.

MATERIALS AND METHODS

The field experiment was carried out at the Organic Research farm Karguan ji, Department of Horticulture, Institute of Agricultural Sciences, Bundelkhand University, Jhansi, Uttar Pradesh, India during *Rabi* season of 2020-21. The soil of the experimental field was red-loamy having pH 7.5, EC 0.45 dS/m, OC 0.64 per cent, available N, P_2O_5 and K_2O 214, 18 and 203 kg ha^{-1}, respectively. The rainfall received during the cropping season was 3.0 mm. The experiment was laid out in a randomized block design with three replications. The treatment comprised seven treatments having different combinations of organic manures with a control. The treatments included, T0: 0 per cent control, T1: 100 per cent cow dung (CD), T2: 100 per cent mustard cake (MC), T3: 100 per cent Neem cake (NC), T4: 100 per cent Poultry manure (PM), T5: 33 per cent CD + 33 per cent PSB+33 per cent *Azotobacter,* T6: 33 per cent MC+ 33 per cent PSB+33 per cent *Azotobacter,* T7: 33 per cent NC+ 33 per cent PSB+33

per cent *Azotobacter,* T8: 33 per cent PM+33 per cent PSB+33 per cent *Azotobacter.* Organic manures were applied as a basal dose according to the treatments. The knol-khol var. Pusa Virat was transplanted on 30 November 2020 at a planting density of 45 x 45 cm. The crop was grown as per recommended package of practices. The crop was harvested on 18 March 2021.

The observations on plant height at harvest (cm), number of leaves per plant at 60 days after transplanting, knob length (cm), knob breadth (cm), biological yield (kg ha^{-1}), weight of knob (g), volume of knob (cc) and yield of knob (q ha^{-1}) were recorded as per recommended procedures. The net returns (Rs ha^{-1}) and benefit-cost ratio were also worked out. The data were analyzed statistically (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

Growth parameters

It is apparent from the data in Table 1 that varying nutrition with or without the combination of organic and biofertilizers has influenced the growth parameters significantly. Application of 33 per cent each of Mustard cake + PSB + *Azotobacter* in T_6 treatment recorded the maximum plant height (35.21 cm), leaves count (28.97 $plant^{-1}$), knob length (24.11 cm), and knob breadth (6.59 cm). The second best treatment was T_7 having 33 per cent each of Neem cake + PSB + *Azotobacter*. The increase in growth attributes could be because of certain growth-promoting substances secreted by the microbial inoculants, which in turn might have led to better root development, better transpiration of water, and uptake of nutrients. Similar observations have been reported by Mishra *et al*. (2014), Ramavtar *et al*. (2017), Choudhary *et al*. (2017), Gogoi *et al*. (2018), Gogoi and Phookan (2018) and Yadav *et al.* (2021).

Yield attributes

The application of different sources of organic manures and biofertilizers nutrition significantly increased the biological yield of plants at the final harvest. At the final harvest on application with a supplement dose of 33 per cent each of Mustard cake + PSB + *Azotobacter* in T_6 treatment recorded the maximum biological yield (181.79 kg ha^{-1}). The next best treatment was T_7 having 33 per cent each of Neem cake + PSB + *Azotobacter* producing 178.58 kg ha^{-1} knobs. Similarly, knob weight and knob volume in T_6 treatment were 132.58 g and 112.35 cc, respectively. In the second-best T_7 treatment, the weight and volume were 129.57 g and 99.96 cc, respectively.

The increasing yield attributes may be due to better root proliferation, increased uptake of nutrients and water from the soil, and higher photosynthetic activity due to higher leaf number per plant that provided the higher food accumulation per plant. Similar findings have been reported by Mutum *et al.* (2017), Gogoi *et al*. (2018), Ramavtar *et al.* (2017), Kumar *et al.* (2018), and Gogoi and Phookan (2018).

Gogoi *et al.* (2018), and Gogoi and Phookan (2018) reported that organic manures and the microbial consortium had a positive impact on the yield of knol-khol as well as soil health. Among the organic treatments, the Enriched compost @ 5 t ha^{-1} was found to be best and can be recommended for the plains of Assam after critical evaluations.

Mutum *et al*. (2016) revealed that plants treated with 50 per cent RDF + VC @ 5 t ha^{-1} + biofertilizers @ 2 kg ha^{-1}

Table 1: Effect of different sources of organic manures on growth, yield and economics of knol-khol.

Treatments		Plant height at final harvest (cm)	Leaves $plant^{-1}$ at 60 DAT	Knob length (cm) at harvest	Knob breadth (cm) at harvest	Biological yield (kg ha^{-1})	Average weight of knob (g)	Volume of knob (cc)	Yield of knob (q ha^{-1})	Net return (Rs ha^{-1})	BC ratio
T_0	0% Control	26.793	20.79	17.56	4.03	147.55	88.67	77.02	90.64		
T_1	100% Cow dung (CD)	28.797	21.89	19.33	4.70	150.97	93.45	82.69	93.48	92570	1.585
T_2	100 % Mustard cake (MC)	29.973	22.35	20.26	4.83	153.68	96.68	85.69	95.86	94280	1.587
T_3	100 % Neem cake (NC)	30.240	22.51	21.57	5.04	156.68	99.66	87.92	97.20	92787	1.452
T_4	100 % Poultry manure (PM)	30.900	23.91	22.57	5.07	158.51	110.48	93.60	99.18	96710	1.56
T_5	33% CD + 33% PSB+33% *Azotobacter*	31.310	30.80	22.56	6.77	163.57	113.56	94.93	101.05	115883	2.43
T_6	33% MC+ 33% PSB+33% *Azotobacter*	35.210	28.97	24.11	6.59	181.79	132.58	112.35	148.06	131632	2.62
T_7	33% NC+ 33% PSB+33% *Azotobacter*	33.670	25.68	23.80	4.69	178.58	129.57	99.96	143.27	131556	2.79
T_8	33% PM+ 33% PSB+33% *Azotobacter*	31.790	26.49	22.98	5.46	159.69	122.73	89.69	110.40	112006	2.34
	S.Em.±	0.113	0.005	0.047	0.017	0.072	0.142	0.166	0.216	-	-
	C.D. (P=0.05)	0.341	0.016	0.142	0.052	0.217	0.430	0.503	0.652	-	-

registered maximum growth parameters *viz*., plant height, number of leaves per plant, leaf length, leaf width, root length and number of secondary roots in knol-khol cv. Early White Vienna.

Kumar *et al*. (2014) documented that the application of 100 per cent RDF + *Azosprillum* resulted in the maximum and significant effects on different horticultural traits *viz*., days taken to first knob initiation, days taken to first knob harvest, plant height at harvest, length of knob, the diameter of knob, average weight of the knob and yield per plot as compared to control. The above reports also support the results of the present findings.

Yield and economics

The application of different sources of organic nutrition significantly influenced the yield of knol-khol. The application of 33 per cent each of mustard cake + PSB + *Azotobacter* in T_6 treatment registered the maximum yield (148.06 q ha^{-1}). The next best treatment was T_7 where the yield was 143.273 q ha^{-1}. The minimum yield (90.64 q ha^{-1}) was registered in T_0 (control treatment). The maximum net income of Rs 131556 ha^{-1} with a BC ratio of 2.79 was obtained in treatment T_7. The second best treatment was T_6 which gave the net income up to Rs 131632 ha^{-1} with BC ratio of 2.62. The minimum net income of Rs 92570 ha^{-1} with a BC ratio of 1.58 was obtained from the control (T_0) treatment. Similar findings have also been reported by Mutum *et al*. (2016), Mutum *et al*. (2017), Kumar *et al*. (2018), and Yadav *et al*. (2021).

CONCLUSION

It is concluded that the application of 33 per cent each of mustard cake + PSB + Azotobactor resulted in significant increase in growth and yield attributing parameters as also as highest net return of Rs. 131632 ha^{-1} and B:C ratio of 2.62 in knol-khol cv. Pusa Virat.

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Research Article

Effect of Nano urea on growth and yield of potato in lower Gangetic planes of West Bengal

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ABSTRACT

In today's world, it is the need of the hour to adopt new technology to sustain the production of the future. Nanotechnology is gaining popularity for its efficiency in various applied fields of science. In agriculture, nanomaterials have a huge impact on the efficiency of fertilizers, pesticides, *etc.* As it requires very fewer quantities and has a negligible residual effect and the environment is safe with the innovation of Nano fertilizer. Keeping this view into account an experimental Trial at a farmer's field was carried out at Berui village in the Hooghly district of West Bengal during the winter (*rabi*) season of 2019-20 under the supervision of Berui Cooperative, KVK-Hooghly, BCKV, and IFFCO with nano-Urea, nano-Zn, and nano-Cu using RBD design with 10 treatments and three replications. Experimental results revealed that the highest tuber yield was obtained in T-7 (50% N+100% P & K+ 2 spray of nano-Urea), and that was followed by T-10 (50% N + 100% P & K + 50% Zn + 1 spray each of Nano-Urea, Zn, and Cu) and T-8 (100% N-P-K + 50% Zn + 2 spray of Nano-Zn). The performance of nano fertilizers, Nano- Urea, was quite promising and economically viable as compared to the 100% recommended dose with commercial fertilizers (RDF).

Keywords: Nanotechnology, plant nutrition, nano urea, potato

Potato is the third most important food crop after wheat and rice in India. It is an important crop in West Bengal. In potato production, West Bengal ranks second, only after Uttar Pradesh. Since nanotechnology is becoming recognized as one of the most promising strategies for increasing agricultural output in recent years, it has become vital to apply a variety of techniques in agriculture, including this one. Because potatoes are a heavy feeder crop, they require substantial amounts of fertilizer for growth, development, and production (Nityamanjari, 2018). Thus, nutrient management of Potato is very much essential for soil and environmental health without compromising the yield of the crop. Potato crop requires 180-240 kg N/ha fertilizers to produce a tuber yield of 35-45 t/ha. The use efficiency of nitrogen in potato is only 40-50% and the remaining N is lost in the environment (Trehan and Singh, 2013). Thus, any alternative way to mitigate the loss and increase the use efficiency of the fertilizer is very important in terms of the sustainability of agriculture. Nano urea, which has been authorized and listed in the fertilizer control order (FCO), was introduced by IFFCO in India. They have also concluded its advantages over the traditional techniques after conducting a thorough investigation and trials.

Despite many efforts, the N use efficiency (NUE) in agriculture remains in a range of less than 50 per cent. Reaching targeted crop yields has resulted in N overuse, which is an economic and environmental concern worldwide. The continuous exploration of innovative solutions has led to the synthesis of novel nanomaterials, resulting in a powerful tool for the development of new technological products (Mejías *et al*., 2021). Nitrogen use efficiency is an established metric used to benchmark N management. There are numerous approaches to calculate NUE, but it is difficult to find an authoritative resource that collates the various NUE indices and systematically identifies their assets and shortcomings (Congreves *et al*., 2021). With the all observation, we must say that agriculture will be illuminated by the bright rays of Nano Fertilizer efficiencies in the days to come (Dutta and Bera, 2021).

With these points in view, the field trial was conducted with the objectives 1) To find out the effect of nano fertilizers on the growth and yield of the potato crop, and 2) To observe the feasibility of reducing of dose of commercial prill Urea-fertilizer with the application of nano-N fertilizer.

MATERIALS AND METHODS

The field trial was conducted at Berui of Hooghly district of West Bengal during the winter (*Rabi*) season of 2019-20. The potato cultivar used in the experiment was Kufri Jyoti and medium size tubers (30-35 g) were planted on 12-

12-2019 at a spacing of 60 cm and a depth of 5-7 cm and covered with soil. The soil of the experimental plot was sandy loam. Normal land preparation was done with plowing, followed by harrowing and planking or leveling. The experiment was laid out in RBD with three replications and 10 treatments. The treatments were as follows:

T-1 = Control (No Fertilizer)

T-2 = Control + 2 sprays of tap water

T-3 = 2 sprays of nano-N

T-4 = 2 sprays of nano-Zn

T-5 = 2 sprays of nano-Cu

T-6 = 100% RDF (N, P, K & Zn @ 200- 150-150-15 kg/ha)

T-7 = 50% N+100% P & K + 2 spray of nano-N

T-8 = 100% NPK + 50% Zn + 2 sprays of Nano-Zn

T-9 = 100% NPKZn + 2 sprays of Nano-Cu

T-10 = 50% N + 100% P & K + 50% Zn + 1 spray each of Nano-N, Zn, and Cu.

Nano fertilizers were applied twice, at 30 and 45 days after planting, except in T-10, where the first spray was done 30 days after planting with nano-N, followed by spraying of nano-Zn and nano-Cu at an interval of 15 days, which means the third spray was done at 60 days after planting. Nano fertilizers were sprayed @ 4 ml/liter of water as per the treatment stated above.

RESULTS AND DISCUSSION

It was observed that plant height, root length, and finally, tuber yield were recorded maximum in treatment T-7 (Table 1 & Figure 1) where the crop was fertilized with 2 sprays of Nano-N + a recommended dose of P, K, and 50 per cent of the recommended dose of N and the treatment was found best among all the treatments. A critical examination of data reveals that treatment T-7 with 50 per cent of the full recommended dose of N (through urea) + 2 sprays of nano-N proved significantly superior to T-6 (100% RDF) in terms of tuber yield. The crop yield of treatment in T-8, fertilized with nano-Zn spraying along with 100 per cent NPK + 50 per cent Zn also gave a better yield than T-6 and the difference in yield was statistically significant. The treatment T-10 with 100 per cent P and K, 50 per cent N, 50 per cent Zn and the single spray of each nano - N, nano - Zn, and nano - Cu recorded significantly higher yield than T-6 (100% RDF), however, the yield in T-10 was less than T-7 and T-7 was significantly better than T-10. The benefit-cost ratio (B:C) indicated that T-7 was better than the rest of the treatments. A study by Abd El-Azeim *et al.* (2020) has found to produce more yield and starch content and enhanced nutrient use efficiency. According to a study by DeRosa *et al*. (2020), controlling the nutrient release and providing crops with the exact amounts of nutrients in the right proportions, Nano fertilizers can increase yield while maintaining environmental safety.

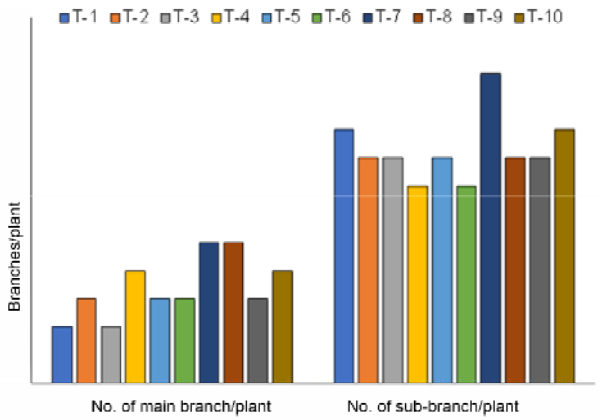

Fig. 1. Effect of Treatments on Growth Parameters of Potato

Table 1: Effect of Nano Fertilizer on growth and Yield of the Potato crop.

Treatment	Plant height (cm)	Root length (cm)	Main stem girth (cm)	Tuber yield (kg ha^{-1})	BC ratio
T-1: Control (No Fertilizer)	40.8	7.0	3.2	8330.0	0.75:1
T-2: Control + 2 sprays of tap water	42.4	6.8	3.4	8540.0	0.75:1
T-3: 2 sprays of nano-N	41.6	6.6	3.1	9187.5	0.80:1
T-4: 2 sprays of nano-Zn	43.2	7.1	3.3	8662.5	0.75:1
T-5: 2 sprays of nano-Cu	44.8	6.7	3.1	8575.0	0.74:1
T-6: 100% RDF (N, P, K & Zn @ 200- 150-150-15 kg/ha)	51.2	7.3	4.3	25795	2.01:1
T-7: 50% N+100% P & K+ 2 spray of nano-N	59.2	8.2	4.1	27580	2.10:1
T-8: 100% NPK + 50% Zn+ 2 sprays of Nano-Zn	56.8	7.9	4.4	26635	2.04:1
T-9: 100% NPKZn + 2 sprays of Nano-Cu	54.4	7.6	4.3	26005	1.95:1
T-10: 50% N + 100% P & K + 50% Zn + 1 spray each of Nano-N, Zn, and Cu.	56.0	7.8	3.8	26705	1.99:1
S.Em.±	1.09	043	0.20	47.58	-
C.D. at 0.05	3.24	1.28	0.59	141.36	-

To assess the efficacy of nano products on the yield of potato, data on some growth parameters were recorded and then analyzed statistically. The data revealed that T-7 produced a greater number of sub-branches per plant (Fig. 1), as a result, crop canopy was also more in T-7, which indicated better photosynthesis in T-7 over other treatments. There was 12.3 per cent more root length and 15.6 per cent taller plant in T-7 which might have played an important role in the highest BC Ratio of 2.1:1 and 6.9 per cent incremental yield of tuber concerning 100 per cent RDF in T-6 treatment.

In crops like potato, root mass has a positive correlation with shoot mass and tuber bulking, and final tuber yield depends a lot on root length and root mass. It has been observed that root length in T-7 was significantly more than T-2, T-3, and T-5 which could have played an important role in better nutrient uptake and consequent 6.9 per cent incremental yield over RDF. Treatment T-10 with 50 per cent of the recommended dose of N & Zn and 100 per cent P & K fertilizers and one spray of nano-N at 30 days after planting, followed by a second spray of nano-Zn at 45 days after planting and a third spray of nano-Cu at 60 days after planting recorded 9.37 per cent more plant height, 6.85 per cent more root length with marginal 3.5 per cent yield increase concerning 100 per cent RDF (T- 6). In treatments, T-7 and T-10, the size of the last (terminal) leaf, and spread of roots have also been found better than that observed in T-6. Further, it may be worth mentioning here that the crop maturity was advanced in T7 by 8 days over T-6. In a study by Raliya *et al*. (2017), it was shown that zinc oxide, Nanoparticles enhanced the activity of the enzyme's acid phosphatase, alkaline phosphatase, and phytase in the rhizosphere of a cluster bean plant.

The 10-point experiment (with 10 treatments) on potatoes, conducted at Berui Village, Hoogly (W.B.), showed that application of nano-N @ 4 ml litre^{-1} with 2 sprays at 30 & 45 days after planting has an important bearing in sustaining the yield of potato with 50 per cent cut in the recommended dose of fertilizer N (urea), and at the most top dressing of urea (*i.e.*, 50% of the recommended dose) could safely be omitted with 2 sprays of nano-N. Nano-Zn spray with a 50% recommended dose of Zn has also been found satisfactory to sustain yield. However, the role of nano-Cu in potato yield is not conspicuous (clear from the one-year experiment) and needs further experiments to reach any decision. Al-Juthery *et al*. (2019) found that the combination treatment with Nanofertilizers has come up with better production with enhanced crude protein, and starch content of the produce.

CONCLUSION

It may be concluded from the present study that a 50 per cent reduction of recommended N fertilizer dose through conventional prill urea, or in other words substitution of recommended commercial N fertilizer (urea), with Nano Urea (N) in liquid form is very much possible which has not only contributed 6.9 per cent yield increase in the study but also at the same time has given additional income to farmers to the tune of Rs. 17800 ha^{-1} (Rs. 7100 acre^{-1}). Local farmers in adjoining villages of Berui Cooperative were very much impressed with the performance of Nano Urea and transmitted new hope and aspirations in potato cultivation. The application of nano-fertilizer may be considered a sustainable management practice since it will help to reduce the cost of cultivation apart from benefitting soil health.

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Research Article

Impact of beneficial microorganisms on the growth, yield and shelf life of African Marigold

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ABSTRACT

The investigation was conducted in the instructional cum research farm, Department of Horticulture, School of Agricultural Sciences and Rural Development, Medziphema, from November 2021 to March 2022. A field experiment was conducted in Randomized Blocks Design with three replications. Bio-formulations were used as a treatment to assess the impact of beneficial microorganisms on the vegetative, flowering, and yield parameters of African Marigold. Treatment consisted of Water soaked (Control), Phosphate solubilizing bacteria (PSB), Jeevamrutha, Microbial consortia, Indigenous effective micro-organisms (IEM), *Pseudomonas fluorescence*, *Trichoderma harzianum*. The results revealed that the highest plant height (71.73 cm), number of primary branches per plant (12.30), plant spread (51.20 cm^2), leaf length (14.3 cm), leaf width (6.50 cm), earliest flower bud initiation (54.43 days), first flower opening (60.86 days), days to 50 per cent flowering (75.46 days), flower diameter (5.86 cm), fresh flower weight (10.70 g), duration of flowering (41.13 days), number of ray florets per flower (193.66), number of flowers per plant (39.40), the weight of flower per plant (421.57 g), weight of flower per plot (6.745 kg), shelf life (4.70 days), and estimated yield ha^{-1} (263.47 q) were recorded with the use of *T. harzianum*. The present investigation suggests further work to understand the impact of beneficial microorganisms that contribute to increasing the growth, yield, and shelf life of African Marigold.

***Keywords*:** African marigold, *Pseudomonas fluorescence, Trichoderma harzianum*, microbial consortia, yield parameters.

African marigold (*Tagetes erecta* L.) belongs to the family Asteraceae comprising about 50 species that originate from Mexico. The chromosome number of African Marigold is 2n = 24. African marigold is the most commonly grown loose flower and is extensively used in religious and social functions. It is gaining popularity on account of its easy culture, wide adaptability, and increasing demand in the world. It can be planted in beds for mass display in mixed borders and can also be grown in pots. Currently marigold is gaining commercial importance as a source of carotenoid pigments. The principal pigment present in the flowers is a xanthophyll, particularly lutein accounts for 80 to 90% in the form of esters of palmitic and myristic acid. The ground blossom meals or the extract usually saponified for better absorption are added to the poultry feed. The impact of bioagents like *Trichoderma harzianum, Pseudomonas fluoresces* and *Bacillus subtilis* combined application proved to be effective in Alternaria disease management in marigold, and *P. fluoresces* and other plant growth-promoting rhizobacteria enhance plant growth by showing antagonisms to potentially deleterious rhizoplane fungal and bacterial pathogens (Kloepper *et al*., 1980). Chang *et al*. (1986) determined the potential of *T. harzianum* to induce the growth of various ornamental and horticultural crops. The soil infested with *T. harzianum* hastened the flowering in periwinkle, increased the number of blooms per plant on chrysanthemum, and increased the height and weight of other plants.

MATERIALS AND METHODS

An experiment was conducted during 2021-22 at the Research cum Instructional farm of the School of Agricultural Sciences and Rural Development, Nagaland University, Medziphema which is located at 25°45′43′′N latitude and 93°53′04′′E longitude at an elevation of 305 m above mean sea level. The climatic condition of the experimental site was typically a humid sub-tropical zone with a mean temperature ranging from 12.53 to 26.24°C and average rainfall is 12.21 mm. The soil of the experimental site was sandy loam having pH 5.5, organic carbon 1.5% available NPK 298 kg, 48.5 kg, and 90.4 kg per hectare, respectively. A field experiment was laid out in a randomized block design with three replications. Treatment consisted of Water soaked (Control), Phosphate solubilizing bacteria (PSB), Jeevamrutha, Microbial consortia,

Indigenous effective micro-organisms (IEM), *P. fluorescence,* and *T. harzianum*. Before transplanting, the seedling of marigold raised in the nursery was treated by preparing a solution of the different treatments mentioned above with water @ 2% for 1 hour. Then marigold seedlings with 3-4 true leaves were transplanted 30 days after sowing. Light irrigation was given just after planting with the help of a rose can. Gap filling was done after two weeks of transplanting in mortality gaps. The observations on plant height, number of primary branches per plant, plant spread, leaf length, leaf width, days to first flower bud initiation, days to first flower opening, days to 50% flowering, flower diameter, fresh flower weight, duration of flowering, number of ray florets per flower, the shelf life of flowers, number of flowers per plant, the weight of flower per plant, weight of flower per plot, and flower yield per ha were recorded. The data recorded were tabulated and subjected to statistical analysis by following the standard ANOVA method with a 5% level of significance described by Gomez and Gomez (2012).

RESULTS AND DISCUSSION

Vegetative parameters

Plant height and Plant spread: The plant height range was recorded between 63.50 cm to 71.73 cm with a mean value of 67.91 cm. Among the treatments, the maximum plant height (71.73 cm) was recorded in *T. harzianum* followed by Microbial consortia (70.16 cm), while the minimum (63.50 cm) was recorded in the control (Table 1). The plant spread range was observed from 44.49 cm^2 to 51.20 cm^2 with an average value of 48.28 cm^2. Among the treatments highest plant spread (51.20 cm^2) was recorded in the *T. harzianum* followed by Microbial consortia (50.33 cm^2), while the lowest plant spread (44.49 cm^2) was recorded in the control (Table 1). The increase in plant height might be due to the progressive mineralization of nutrients and supplementation of balanced nutrition for crop growth due to the quick and greater availability of plant nutrients, providing a better environment for root growth and proliferation. It also creates a more adsorptive surface for the uptake of nutrients, which in turn leads to an increased rate of meristematic activity resulting in better plant height. These results conform with the findings of Shabnam (2017) in China aster, Karishma *et al*. (2011) in Chrysanthemum, and Kumari *et al*. (2013) in Gladiolus.

Number of primary branches per plant: The variation in the number of primary branches per plant in different treatments was significant with a range of 8.50 to 12.30 branches and a mean of 10.40 branches. Among the treatments, a maximum number of primary branches per plant (12.30) was recorded in *T. harzianum* followed by Microbial consortia (12.1), while the treatment control recorded the minimum (8.50) number of primary branches per plant. The increased number of branches due to plant growth regulators like IAA and cytokinins released by *T. harzianum* and *T. asperellum* might have resulted in the breaking of apical dominance and accelerated the higher number of branches. These results are in agreement with the reports of Karishma *et al*. (2011) in chrysanthemum, and Harshavardhan *et al.* (2017) in Carnation.

Length of the leaf and Width of the leaf: The length of the leaf ranged between 10.20 cm to 14.30 cm with a mean recorded of 11.80 cm. Among the treatment of maximum length of the leaf (14.30 cm) was in *T. harzianum* followed by Microbial consortia (13.20 cm), while the minimum length of the leaf (10.20 cm) was noted in control (Table 1). The width of the leaf ranged between 4.30 cm to 6.50 cm with a mean value of 5.20 cm. Among the treatments of the maximum width of the leaf (6.50 cm) was recorded in treatment *T. harzianum* followed by Microbial consortia (5.86 cm), while the minimum leaf width (4.30 cm) was noted in the control (Table 1). This may be due to the use *T. harzianum*, with a possibility of enzymatic degradation of starch to fructose and glucose, providing plants with more energy and stimulating plant leaf length and leaf width (Amit *et al*., 2011). The results conform with the finding of Dubey *et al*. (2008) in gladiolus, chrysanthemum, and dahlia and Rodriguez-Romero *et al.* (2005) in Banana.

Table 1: Impact of beneficial microorganisms on vegetative parameters.

Tr. No.	Plant height (cm)	No. of primary branches $plant^{-1}$	Plant spread (cm^2)	Length of leaf (cm)	Width of leaf (cm)
T_1	63.50	8.50	44.49	10.2	4.30
T_2	65.70	8.90	45.60	10.5	4.50
T_3	67.53	9.20	47.50	10.8	4.60
T_4	70.16	12.06	50.33	13.2	5.86
T_5	68.53	10.50	48.80	11.5	4.86
T_6	68.46	11.50	50.10	12.5	5.63
T_7	71.73	12.30	51.20	14.3	6.50
S.Em.±	0.11	0.05	0.25	0.06	0.12
C.D. (5%)	0.34	0.17	0.78	0.18	0.37

T_1: Water soaked (Control), T_2: Phosphate solubilizing bacteria (PSB), T_3: Jeevamrutha, T_4: Microbial consortia, T_5: Indigenous effective micro-organisms (IEM), T_6: *Pseudomonas fluorescence,* and T_7: *Trichoderma harzianum*

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Floral Parameters

Days taken to first flower bud initiation

The data presented in Table 2 shows that days taken to first flower bud initiation had significant differences among the treatments. The variation in days taken to first flower bud initiation in different treatments was significant with a range of 54.43 to 66.13 days and a mean value of 59.84 days. Among the treatments minimum days taken to first flower bud initiation (54.43 days) was recorded in treatment *T. harzianum*, while treatment control recorded the maximum (66.13 days) days taken to first flower bud initiation. The plants inoculated with *T. harzianum* were earlier to produce the first flower bud, which can be attributed to the early completion of vegetative growth and changing of vegetative primordia to reproductive primordia, probably due to the secretion of growth-promoting substances like auxins, and gibberellins, vitamins and organic acids, which promoted faster vegetative growth and ultimately flower bud formation took place earlier. These results are in agreement with the reports of Shabnam (2017) in China aster, Dharma (2006) in Carnation, Kumari *et al*. (2013), Sisodia and Singh (2015), and Singh *et al*. (2016) in Gladiolus.

Days taken to first flower opening

The variation in days taken to the first flower opening in different treatments was significant with a range of 60.86 to 73.40 days and a mean of (66.48) days. Among the treatments minimum days taken to the first flower opening (60.86 days) was recorded in treatment *T. harzianum*, while control recorded the maximum (73.40 days) days taken to the first flower opening (Table 2). Earliness in flowering due to the number of days the plant took for bud formation may affect the number of days taken for the first flower opening *i.e.* the plant on which the bud formation occurred earlier will have earlier flowering. This might be due to the proper uptake of nutrients and the production of growth-promoting substances. Thereby, the plant completed its vegetative growth soon, resulting in early flowering. These findings conform with the findings of Shabnam (2017) in China aster, Bellubbi *et al.* (2015) in gerbera, and Kumari *et al*. (2013) in Gladiolus.

Days taken to 50 per cent flowering

The data show highly significant differences among the treatments for the days taken to 50% flowering (Table 2). The days taken to 50% flowering ranged between 75.46 to 83.40 days with a mean of 80.27 days. Among the treatments minimum days taken to 50% flowering (75.46 days) was recorded in treatment *T. harzianum*, while treatment control recorded the maximum (83.40 days) days taken to 50% flowering. The earliness in flowering might be attributed to the amplification of nutrients which might have promoted the translocation of phytohormones to the shoots resulting in the early bud and flower initiation thus resulting in early 50 per cent flowering. This was in line with the results of Shabnam (2017) in China aster, Bellubbi *et al.* (2015) in Gerbera, and Kumari *et al*. (2013) in gladiolus.

Flower diameter (cm)

The diameter of the flower among the different treatments has been presented in Table 2. A close view of the data illustrates that there were significant differences among the treatments for this trait. The flower diameter ranged between 3.26 to 5.86 cm with a mean of 4.81 cm. The performance of treatments concerning flower diameter was

Table 2: Impact of beneficial microorganisms on floral parameters.

Tr. No.	Days first flower bud initiation	Days to first flower opening	Days to 50% flowering	Flower diameter (cm)	Fresh flower weight (g)	Duration of flowering (days)	Number of ray florets per flower	Shelf life of flowers (days)
T_1	66.13	73.40	83.40	3.26	6.83	34.43	170.26	2.46
T_2	63.46	71.60	83.66	4.46	7.73	36.60	174.50	3.46
T_3	63.56	70.76	83.73	4.56	7.63	36.46	175.40	3.23
T_4	55.53	61.40	77.63	5.46	10.46	40.53	191.53	4.43
T_5	58.56	64.80	81.53	4.86	9.70	37.60	189.63	3.50
T_6	57.53	62.73	76.73	5.26	9.60	38.50	190.53	3.60
T_7	54.43	60.86	75.46	5.86	10.70	41.13	193.66	4.70
S.Em.±	0.09	0.08	0.09	0.02	0.11	0.25	0.10	0.12
C.D. (5%)	0.29	0.26	0.29	0.05	0.36	0.79	0.32	0.38

T_1: Water soaked (Control), T_2: Phosphate solubilizing bacteria (PSB), T_3: Jeevamrutha, T_4: Microbial consortia, T_5: Indigenous effective micro-organisms (IEM), T_6: *Pseudomonas fluorescence,* and T_7: *Trichoderma harzianum*

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maximum (5.86 cm) in treatment *T. harzianum* followed by Microbial consortia (5.46 cm), while minimum flower diameter was (3.26 cm) recorded in control. The flower head diameter and disc diameter were better with *T. harzianum* treatment. This might be ascribed to the proper uptake of nutrients by plants with inoculation of bioagents and their better translocation to the flowers. The results are in agreement with the findings of Bellubbi *et al.* (2015) in Gerbera, and Dharma (2006) in Carnation.

Fresh flower weight (g)

It is evident from the data that, the treatments varied significantly for the fresh flower weight (Table 2). It ranged between 6.83 to 10.70 g with a mean value of 8.90 g. Among the treatments maximum fresh flower weight of 10.7 0g was recorded in the treatment *T. harzianum* followed by microbial consortia (10.46 g), while the minimum fresh flower weight (6.83 g) was noted in the control.

Duration of flowering (days)

The different treatments under study varied significantly for the duration of flowering (Table 2). The range of the duration of flowering recorded was from 34.43 to 41.13 days with a mean of 37.87 days. Among the treatments maximum duration of flowering (41.13 days) was recorded in the treatment *T. harzianum* followed by Microbial consortia (40.53 days), while the minimum flowering duration was (34.43 days) was observed in the control. Duration of flowering was maximum with *T. harzianum* which could be attributed to the release of growth regulator GA_3, which increases the level of RNA and protein in plant tissues increasing total metabolites in plant cells, which probably plays some role in augmenting flowering characteristics. Similar results were observed by Shabnam (2017) in China aster, Bellubbi *et al.* (2015) in Gerbera, and Kumari *et al.* (2013) in Gladiolus.

Number of ray florets per flower

The number of ray florets per flower among the different treatments has been presented in Table 2. A close view of the data illustrates that there were significant differences among the treatments for this trait. Among the treatments, the number of ray florets per flower ranged between 170.26 to 193.66 with a mean value of 183.66. The performance of treatments for the number of ray florets per flower was maximum (193.66) in treatment *T. harzianum* followed by Microbial consortia (191.53), while the minimum number of ray florets per flower was (170.26) recorded in treatment control.

Shelf life of flowers (days)

The data on the average shelf life of flowers for different treatments are presented in Table 2. The treatments differed significantly in shelf life. The range recorded was between 2.46 to 4.70 days with a mean of 3.60 days. Among the treatments maximum shelf life (4.70 days) was observed in treatment *T. harzianum* followed by Microbial consortia (4.43 days), while the minimum shelf life (2.46 days) was recorded in control. The longer shelf life of flowers might be because of the assimilation of more photosynthates due to proper uptake of nutrients which ultimately resulted in more reserve of food and higher retention of water in the cells of flowers and lower desiccation. These results conform with Karishma *et al.* (2011) and Saini *et al.* (2019) in Chrysanthemum.

Yield parameters

Number of flowers per plant

The data shows highly significant differences among the treatments for the number of flowers per plant (Table 3). The number of flowers per plant ranged between 26.46 to 39.40 with a mean of 33.40. Among the treatments, the maximum number of flowers per plant (39.40) was recorded in treatment *T. harzianum* followed by Microbial consortia (38.40), while the minimum number of flowers per plant was recorded (26.46) in treatment control. The *T. harzianum* had recorded maximum plant height, more number of branches, and plant spread and it was early flowering and resulted in more number of flowers per plant. This might be ascribed to the easy uptake of nutrients and simultaneous transport of

Table 3: Impact of beneficial microorganisms on flower yield and yield parameters.

Tr. No.	Number of flowers per plant	Weight of flower (g/plant)	Number of flowers (kg/plot)	Flower yield (q/ha)
T_1	26.46	180.85	2.872	112.20
T_2	28.70	221.96	3.535	138.08
T_3	28.56	218.03	3.465	136.26
T_4	38.40	401.91	6.389	249.57
T_5	35.26	342.09	5.463	213.39
T_6	37.60	360.93	5.775	225.58
T_7	39.40	421.57	6.745	263.47
S.Em.±	0.10	11.16	0.05	2.23
C.D. (5%)	0.33	3.58	0.17	6.97

T_1: Water soaked (Control), T_2: Phosphate solubilizing bacteria (PSB), T_3: Jeevamrutha, T_4: Microbial consortia, T_5: Indigenous effective micro-organisms (IEM), T_6: *Pseudomonas fluorescence,* and T_7: *Trichoderma harzianum*

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growth-promoting substances like cytokinins to the axillary buds resulting in breakage of apical dominance. Ultimately, they resulted in a better sink for the mobilization of photosynthates and the transformation of plant parts from the vegetative to the reproductive phase. This is in line with Shabnam (2017) in China aster, Bellubi *et al.* (2015) in Gerbera, Kumari *et al*. (2013) and Singh *et al.* (2016) in Gladiolus.

Weight of flower (g plant^{-1})

The data about the weight of flower per plant have been presented in Table 3. The weight of flower per plant ranged between 180.85 to 421.57 g with a mean of 306.76 g. Among the treatments maximum weight of flower per plant was recorded in treatment *T. harzianum* (421.58 g) followed by Microbial consortia (401.91 g) which were significantly higher as compared to the rest of the treatments. Similarly, the minimum weight of flower per plant was recorded in control (180.52 g). The *T. harzianum* had recorded maximum plant height, more number of branches, and plant spread and it was early flowering and resulted in more number of flowers per plant. This might be ascribed to the easy uptake of nutrients and simultaneous transport of growth-promoting substances like cytokinins to the axillary buds resulting in breakage of apical dominance. Ultimately, they resulted in a better sink for the mobilization of photosynthates and the transformation of plant parts from the vegetative to the reproductive phase. This is in line with Shabnam (2017) in China aster, Bellubi *et al.* (2015) in Gerbera, Kumari *et al*. (2013), and Singh *et al.* (2016) in Gladiolus.

Weight of flowers (kg plot^{-1})

The data about the weight of flowers per plot have been presented in Table 3. The weight of flowers per plot ranged between 2.872 to 6.745 kg with a mean of 4.892 kg. Among the treatments maximum weight of flower per plot was recorded in treatment, *T. harzianum* (6.745 kg) followed by Microbial consortia (6.389 kg) which were significantly higher as compared to the rest of the treatments. Similarly, the minimum (2.872 kg) weight of flower per plot was recorded in 'control'.

Flower yield (q ha^{-1})

It is evident from the data that, the treatments varied significantly concerning the flower yield per hectare (Table 3). It ranged between 112.200 to 263.47 q/ha with a mean of 191.22 q/ha. Among the treatments, maximum flower yield per hectare was recorded in treatment *T. harzianum* (263.47 q/ha) followed by Microbial consortia (249.57 q/ha) which were significantly higher as compared to the rest of the treatments. Similarly, the minimum (112.200 q/ha) flower yield per hectare was recorded in control. The *T. harzianum* had recorded maximum plant height, more number of branches, plant spread, early flowering, more number of flowers, and maximum individual flower weight and thus resulted in maximum flower yield. The increased flower yield might be attributed to the production and accumulation of maximum photosynthesis in flowering branches and individual flowers, which ultimately resulting the production of maximum flower yield. Similar findings have been reported by Shabnam (2017) in China aster, Karishma *et al*. (2011) and Kumari *et al*. (2016) in Chrysanthemum, Harshavardhan *et al.* (2017) in Carnation, Bhargava *et al*. (2015) in Antirrhinum.

CONCLUSION

It is concluded that the use of *T. harzianum* in African Marigold could lead to significant increase in its all growth, yield and shelf life parameters.

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Research Article

Influence of plant growth regulators on morphological and flowering behaviour of bottle gourd (*Lagenaria siceraria* (Mol.) Standl.)

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ABSTRACT

A field experiment was conducted for evaluating the influence of three plant growth regulators (PGR) *viz*., gibberellic acid (GA_3), naphthalene acetic acid (NAA) and ethrel on morphological and flowering behaviour of bottle gourd. The foliar application of ethrel @ 300 ppm was found beneficial for days taken for the appearance of the first female flower, number of male flowers, number of female flowers, and sex ratio. Whereas, the maximum length of the vine at 60 and 90 days after sowing, the minimum number of days taken from fruit set to edible maturity was obtained by the foliage application of GA_3 @ 40 ppm however minimum days taken for the appearance of the first male flower was obtained with GA_3 @ 60 ppm. Based on these observations, it could be suggested that a significant increase in flowering and growth characteristics would be obtained by the spraying of ethrel @ 300 ppm at the 2-4 true leaf stage.

***Keywords*:** Ethrel, GA, NAA, PGR, bottle gourd.

Vegetable growing is the most remunerative enterprise as it is adopted on small and marginal holdings with high production in a short duration. Being a source of farm income, it creates an impact on the agricultural development and economy of the country. Vegetables are a cheaper source of minerals, vitamins and with high caloric values. There is an increasing demand for vegetables both for domestic as well as for export, which can be valuable foreign exchanges for India.

Cucurbitaceous vegetables are one of the largest groups in the vegetable kingdom consisting of large edible species. Among gourds, bottle gourd [*Lagenaria siceraria* (Mol.) Standl. 2n = 2x = 22] commonly known as lauki, kadu, ghiya or doodhi is grown extensively in India which might have originated in Tropical Africa. It is a photo-insensitive crop but sensitive to thermoperiodism. The name bottle gourd is due to the bottle like shape of fruit and its use as a container in the past. Fruits at the tender stage are used as cooked vegetables and for the preparation of sweets and pickles. Hard shells of mature fruits are used as water jugs, domestic utensils, floats for fishing nets, musical instruments *etc.* Bottle gourd is a vegetable high in water and is a rich source of vitamins C, K and Calcium. It helps in maintaining a healthy heart and brings down bad cholesterol levels. The juice is also beneficial for diabetic patients as it stabilizes the blood sugar level and maintains blood pressure.

The yield of cucurbits depends to a great extent on sex expression and sex ratio. Early nodes bear male flowers with a higher number, whereas pistillate flowers are found in later nodes. These mechanisms are responsible for delayed harvesting as well as yield reduction. The problem can be overcome by the exogenous application of PGRs. It directly affects male and female flower ratio, fruit set, fruit drop and ultimately yield. Therefore, the use of PGRs like NAA, Ethrel and GA_3 in bottle gourd may become an important tool for yield increment as well as timely harvest (Komal Kumari *et al*., 2019).

MATERIALS AND METHODS

A field experiment was conducted during the summer season of 2020 under field conditions at College Farm, College of Horticulture, Sardarkrushinagar Dantiwada Agricultural University, Jagudan, Dist. Mehsana, Gujarat, India. The present investigation was carried out with a popular and high-yielding variety of bottle gourd cv. "Anand Bottle Gourd 1" released by Anand Agricultural University, Anand during the year of 2005. The fruits of this variety are long, tender, attractive light green. The fruits are

slightly rounded at the stem as well as blossom end with fine lustre. The experiment was laid out in Randomized Block Design with three replications. Three PGRs *viz*., GA_3 (gibberellic acid), NAA (naphthalene acetic acid) and Ethrel (2-chloroethyl phosphoric acid) were used for the study. Sprays of each PGR at 2-4 true leaf stages with the altogether 10 treatments made for the field trial. Ten treatments evaluated in the present study included T_1: Control (Water spray), T_2: NAA @ 50 ppm, T_3: NAA @ 75 ppm, T_4: NAA @ 100 ppm, T_5: GA_3 @ 20 ppm, T_6: GA_3 @ 40 ppm, T_7: GA_3 @ 60 ppm, T_8: Ethrel @ 100 ppm, T_9: Ethrel @ 200 ppm and T_{10}: Ethrel @ 300 ppm.

The plants were trained upward so that the main stem was allowed to climb to the overhead wire. Wires were fixed 1-2 feet above the ground up to the height of 5-6 feet with horizontal wires. Various cultural operations right from the beginning till the end of experimentation have been performed successfully as per recommendations. Randomly selected and labelled plants in each treatment were used for recording observations with respect to morphological and flowering behaviour attributes. The data were analyzed statistically by adopting the standard procedures described by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Morphological characters

Table 1 shows that the maximum length of the main vine at 60 and 90 days after sowing was observed with the application of GA_3 @ 40 ppm (T_6) *i.e.* 167.98 cm and 258.99 cm. The increased vine length is a result of the rapid elongation of internodes by both cell division and cell elongation (Krishnamoorthy and Sandooja, 1981). These findings are in agreement with the results of Ansari and Chaudhary (2018) and Komal Kumari *et al.* (2019) in bottle gourd and Kadi *et al.* (2018) in cucumber. Significantly minimum days for the appearance of the first male flower (36.39) was observed with T_7 (GA_3 @ 60 ppm). These findings are in accordance with the results of Dostogir *et al.* (2006) in bitter gourd and Farahana (2015) in cucumber. The minimum days taken for the appearance of the first female flower were recorded with the application of Ethrel @ 300 ppm (T_{10}) *i.e.* 50.36 days. Exogenous application of Ethrel probably has increased the endogenous auxins level in the plants which hastened the female flowering (Elshokri *et al*., 2022). The results obtained are also in agreement with Ansari and Chowdhary, (2018) and Komal Kumari *et al.* (2019) in bottle gourd. The minimum days taken from fruit set to edible maturity was recorded with treatment T_6 (GA3 @ 40 ppm) *i.e.* 7.73 days. These findings are in accordance with the findings of Ansari and Chaudhary (2018) in bottle gourd and Mehdi *et al.* (2012) in cucumber.

Flowering behaviour

The analysis of Table 2 confers that application of ethrel @ 300 ppm (T_{10}) recorded minimum number of male flowers per vine *i.e.* 118.39. The maximum number of female flowers recorded with the application of ethrel @ 300 ppm (T_{10}) *i.e.* 19.19. The lowest sex ratio (male: female flowers) was found with the application of ethrel @ 300 ppm (T_{10}) *i.e.* 6.18. The application of Ethrel increased the number of female flowers per vine and reduced the number of male flowers per vine and sex ratio in bottle gourd (Patel *et al.*, 2017). Ethrel increase the number of female flowers and lower concentration of GA3 also increase female flowers. These findings are in conformity with the results of Hiyadtullah *et al.* (2012), Ansari

Table 1 : Effect of plant growth regulators on morphological characters of bottle gourd.

Tr. No.	Treatment	Length of main vine (cm)		Days taken for appearance of first male flower	Days taken for appearance of first female flower	Days taken from fruit set to edible maturity
		60 DAS	90 DAS			
T_1	Control (Water spray)	131.89	205.22	43.67	59.82	12.66
T_2	NAA @ 50 ppm	153.36	217.55	40.98	58.13	11.22
T_3	NAA @ 75 ppm	153.70	219.83	41.20	54.78	11.10
T_4	NAA @ 100 ppm	160.04	231.36	41.77	51.63	10.84
T_5	GA_3 @ 20 ppm	154.21	240.14	40.58	60.74	11.53
T_6	GA_3 @ 40 ppm	167.98	258.99	38.19	61.24	7.73
T_7	GA_3 @ 60 ppm	159.52	245.24	36.39	61.85	8.83
T_8	Ethrel @ 100 ppm	166.91	251.35	42.24	56.13	8.96
T_9	Ethrel @ 200 ppm	157.07	243.65	41.24	54.22	9.81
T_{10}	Ethrel @ 300 ppm	154.30	235.70	40.88	50.36	10.23
	S.Em.±	6.14	9.19	1.07	1.83	0.48
	C.D. at 5%	18.25	27.32	3.20	5.45	1.43
	C.V. (%)	6.83	6.78	4.58	5.58	8.12

DAS= Days after sowing

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and Chaudhary (2018) and Komal Kumari *et al.* (2019) in bottle gourd.

Duhan *et al*. (2022) revealed that the application of Ethrel @ 200 ppm significantly increased most of the growth and fruit yield attributes over the control treatment. The spraying of Ethrel @ 200 ppm significantly reduced the length of internodes (11.4 cm), days to the appearance of the first male flower and early fruit harvest (56.4; 62.7 days), number of staminate flowers per plant (102.7), sex ratio (6.0) as well as main vine length at final harvest (353.7 cm), whereas the same dose significantly increases the number of primary branches vine^{-1} (16.8), numbers of pistillate flowers per plant (17.2), longer fruit length and more diameter (31.5; 7.3 cm), number of fruits per vine (7.6), average fruit weight (825.6 g) and fruit yield per plot and per hectare (27.6 kg and 349.2 q), respectively. Whereas, the spray of GA @ 60 ppm significantly reduced the number of primary branches per plant (9.9), number of pistillate flowers per plant (8.7), fruit length and diameter (22.9; 5.8 cm) number of fruit per plant (3.3) and fruit yield per plot and per hectare (18.7 kg, 238.4 q). From the study, it was concluded that bottle gourd cv. GH-22 plants when sprayed with Ethrel @ 200 ppm produced the maximum fruit yield (349.2q/ha). These results also support the present findings.

Kumar *et al*. (2019) reported that the exogenous application of NAA @ 250 ppm recorded maximum vine length (6.70 m), nodes per vine (23.15) and leaf area (264.50 cm^2) in bottle gourd. Further, the maximum net return (Rs. 204440 per hectare) and BC ratio (2.76) were recorded under ethrel @ 400 ppm. Wamiq *et al*. (2020) studied the effect of GA3 and NAA on the yield of bottle gourd (*L. siceraria*) cv (MGH-4) and reported that application of 40 ppm gibberellin at 2, 4 leaf stage was found the most effective in terms of number of female flowers per vine, fruit per plant, fruit yield per plant, fruit yield per hectare. Kumar *et al*. (2020) concluded that foliar application of Ethrel @ 300 ppm sprayed at different stages of growth *i.e*., at two true leaf stages, four true leaf stages and flower initiation stage was beneficial for higher seed yields and better growth of the plant in case of bottle gourd. These reports also support the present findings.

Table 2: Effect of plant growth regulators on flowering behaviour of bottle gourd.

Tr. No.	Treatment	Number of male flowers per vine	Number of female flowers per vine	Sex ratio (male: female)
T_1	Control (Water spray)	142.69	14.74	9.68
T_2	NAA @ 50 ppm	133.75	15.07	8.93
T_3	NAA @ 75 ppm	127.26	15.61	8.21
T_4	NAA @ 100 ppm	121.22	17.13	7.08
T_5	GA_3 @ 20 ppm	131.52	15.01	8.77
T_6	GA_3 @ 40 ppm	134.05	18.50	7.26
T_7	GA_3 @ 60 ppm	140.92	16.39	8.67
T_8	Ethrel @ 100 ppm	124.25	16.80	7.38
T_9	Ethrel @ 200 ppm	122.52	17.97	6.81
T_{10}	Ethrel @ 300 ppm	118.39	19.19	6.18
	S.Em.±	4.70	0.70	0.46
	C.D. at 5%	13.99	2.08	1.38
	C.V. (%)	6.29	7.29	10.18

CONCLUSION

It could be concluded that spraying of etheral @ 300 ppm at 2-4. true leaf stage results in significant increase in flowering and growth of bottle gourd plant.

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Research Article

Screening of chickpea (*Cicer arietinum* L.) germplasms under salt stress

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ABSTRACT

Soil salinity, among other abiotic stresses, is now identified as a significant crop loss factor for chickpea (8-10%). In the current experiment, the response of 20 chickpea accessions was studied under various NaCl solutions in growth chambers and under pot culture in greenhouse conditions in the growing season of 2020. These tests were done in order to categorize the chickpea accessions into distinct salt-tolerant groups and to identify relevant agro-morphological indicators of salinity tolerance. The findings showed that salinity has a considerable negative impact on chickpea growth during its early stages and that there is a statistically significant correlation between salinity and accessions for yield characteristics under various salinity stress levels. In comparison to other accessions, salt-tolerant accessions were less impacted by excessive salinity and may produce a higher seed output. Accessions were grouped into three categories *viz*., highly tolerant, moderately susceptible, and highly susceptible based on the percentage loss of seed yield compared to control under 300 mM saline stress. After taking into account all of the measured parameters, Digbijoy was determined to be the best tolerant line. According to correlation and regression analysis, salinity had the least impact on germination despite severely fracturing the pod and seed yield. In the future breeding program, these desirable parameters might be used as salinity indicators and also introduced into the susceptible lines, while the tolerant accessions might be used as tolerant checks.

Key words: Chickpea, correlation, germplasm, salinity.

The chickpea (*Cicer arietinum* L.), also known as the Bengal gram or Garbanzo bean, is one of the many pulses grown around the world and is well known as a potential crop to satisfy the protein-hunger. It is the third most significant pulse crop behind dry beans and peas (FAO, 2008). It is a great source of energy because it is packed with fibre, vitamins, minerals, and potentially healthy phytonutrients (Ibrikci *et al*., 2003; Jukanti *et al*., 2012). According to FAOSTAT (2019), there were around 13.7 million hectares of chickpeas grown worldwide, producing 14.24 million metric tonnes at a productivity of 1038 kg ha^{-1}. For a number of years, India has been the world's top producer of chickpeas, making up 70 per cent of total production (agricoop.nic.in).

The estimated 3.7 million tonnes of annual output losses worldwide resulting from abiotic stressors represent an average loss of 40-60 per cent. Today, among other abiotic stresses, soil salinity is recognized as a significant crop loss factor (Ashraf *et al*., 2008). However, because the annual loss in chickpea production is estimated to be between 8 and 10 per cent, salinity is considered to be one of its very sensitive factors. The majority of the time, chickpeas are cultivated in semi-arid, irrigated soils where water loss during the dry seasons frequently results in salt deposits in the top layers of the soil. This built-up salt lowers the soil's osmotic potential, causing water stress that brings on nutritional imbalances that harm the plants' metabolism and induce cell death (Hasanuzzaman *et al*., 2013). Chickpeas under salinity stress experience lower seed germination, seedling vigour, root and shoot length, photosynthetic activity, and nitrogen fixation, all of which affect growth and yield (Egamberdieva *et al*., 2015; Khan *et al*., 2017). Abd-Alla *et al*. (2019) observed that salt levels between 25 and 150 Mm severely hindered chickpea growth and yield.

Identification and selection of salt-tolerant genetic resources have attracted a lot of attention over the past few decades due to the detrimental effects that salt stress has on chickpea growth and yield. In order to distinguish between genotypes that are sensitive to or tolerant of salinity stress, a number of agro-morphological traits have been reported. In light of the foregoing description, the main goals of the current study were to identify salt-tolerant chickpea genotypes from a large set of chickpea germplasm accession samples and assess crucial morphological and physiological features under salinity stress.

MATERIALS AND METHODS

Selection of experimental material

Twenty high-yielding desi-type chickpea lines in total (Table 1) were picked up for the current study from the germplasm bank at the Department of Genetics and Plant Breeding, University of Calcutta. Here, three distinct salinity levels (150, 200, and 300 mM) were used in the form of NaCl solution to impose the salinity stress. The control treatment consisted of no salts (0 mM). These lines were sown in a randomized block design with three replications for each treatment on 17.07.2020 and underwent screening for both germination percent in Petri dishes at the early seedling stage and yield characteristics in a pot experiment with mature plants.

Table 1. Description of Chickpea lines under study.

Sl. No.	Germplasm	Seed yield /plant (g)
1	Digbijoy	18.97
2	GCP 105	18.07
3	IC 268971	17.87
4	IC 268900	17.39
5	Virat	16.38
6	WBG 29	15.92
7	ICCV 10	15.82
8	Radhey	15.68
9	Avrodhi	15.13
10	DCP 92-3	15.05
11	Vijoy	14.21
12	G 24	14.14
13	IC 268863	14.07
14	Dahod yellow	13.9
15	B 115	13.84
16	IC 268943	13.16
17	Mahamaya 1	13.01
18	Annigeri	12.99
19	Anuradha 39/2	12.76
20	RSG 888	12.63

Screening at the early seedling stage

Viable, surface-sterilized (1.0% NaOCl solution) seeds of all chickpea lines were placed in Petri dishes with 10 seeds/line/treatment and allowed to sprout on filter paper soaked in 15 ml of saline solutions at various concentrations (0, 150, 200, and 300 mM), in a growth chamber with a mean temperature of 20 °C. Parafilm was used to tightly enclose the Petri dishes in order to reduce evaporative loss and maintain the level of salt. Germination percentage was assessed four days after seeding, and seedling vigour indicators such as root and shoot length were assessed after 7 days.

Screening at different salinity levels for yield parameters in the matured plant (pot experiment)

Viable, surface-sterilized seeds of all the chickpea germplasms were planted on July 17, 2020, in pots filled with sandy loam soil under greenhouse conditions with a mean temperature of 20 °C. Normal irrigation practices were kept up until 15 days after sowing (DAS). After this time, a fertigation technique was used to inflict salt stress using the protocol of Manasa *et al*. (2017). For stressed plants, 500 ml of each salt solution was added in accordance with the soil's field capacity in order to provide the three levels of stress stated above while preventing leaching. Normal water was used to irrigate the control plants. Similarly, stress was applied seven times at the following intervals: 15, 35, 45, 55, 65, 75, and 85 DAS. Normal irrigation was carried out periodically as needed. On five randomly chosen plants/lines/treatments, various agro-morphological parameters, including nodules per plant, days to maturity, number of branches per plant, leaf area index, number of pods per plant, pod length (cm), number of seeds per pod, 100 seed weight (g), and seed yield/plant (g), were recorded at the time of maturity.

Data on different salinity parameters were recorded as follows:

% Reduction of parameters = [(Control – Treatment) / Control] x 100

Grouping of 20 chickpea lines was done on the basis of percent reduction in seed yield parameters under 300 mM salinity treatment.

Statistical analysis

The mean values of each parameter were taken for statistical analysis. All the statistical analyses were done using IBM SPSS version 20.0 software.

RESULTS AND DISCUSSION

Germination percentage and plant growth at different salinity levels at the early seedling stage

Salinity stress decreased the percentage of germination in seeds. The average percentage reduction in seed germination above the control (0 mM) varied from 0-20 per cent in 150 mM, 5-39 per cent in 200 mM, and 20-50 per cent in 300 mM. (Table 2). Therefore, a higher saline level greatly lengthens the germination period and decreases the germination percentage. The likely cause of this phenomenon is an increase in sodium/chloride ions in the soil, which results in an outside osmotic potential and ion toxicity and ultimately prevents the hydrolytic enzymes from being activated, causes turgor loss, prevents apo-plastic

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acidification, and further reduces seed germination and seedling growth (Mohammed, 2007; Murillo-Amador *et al*., 2002; Garg and Chandel, 2011).

Under various salt levels, the seedling growth in all of the studied lines exhibited significant differences. Under 150, 200, and 300 mM salinity stress, it was discovered that seedling length was reduced by 21-84, 34-88, and 48-92 per cent, respectively. The genotypes Virat and WBG 29 showed the highest level of reduction (92%) at 300 mM (Table 2), whereas Digbijoy showed the lowest level of reduction (48%) in seedling growth. This could be attributed to the steady decline in chlorophyll, carotenoid pigment levels, and chlorophyll fluorescence intensity that results from decreased electron transport function and instability of the pigment-protein complex (Promila and Kumar, 2000). In high saline conditions, the total leaf area and stomatal aperture are also reduced (Nandini and Subhendu, 2002).

Table 2. Variation in percent reduction of plant growth at different salinity levels in early seedling stage

	Germination (%)			Seedling Length (mm)		
	150 mM	200 mM	300 mM	150 mM	200 mM	300 mM
Digbijoy	0	5	20	21	34	48
GCP 105	0	10	30	71	76	80
IC 268971	10	15	35	71	73	81
IC 268900	0	10	50	30	38	52
Virat	20	35	45	84	88	92
WBG 29	10	20	30	79	82	92
ICCV 10	20	39	50	78	84	89
Radhey	0	15	35	61	66	72
Avrodhi	10	20	40	74	74	80
DCP 92-3	10	20	30	61	66	72
Vijoy	0	20	40	44	58	66
G 24	10	20	40	70	73	81
IC 268863	0	20	30	73	78	83
Dahod yellow	10	20	30	72	79	85
B 115	10	20	40	82	84	88
IC 268943	10	20	30	77	82	87
Mahamaya 1	0	15	35	69	76	85
Annigeri	10	20	40	53	64	75
Anuradha 39/2	0	30	40	73	79	85
RSG 888	0	20	40	71	77	84
Mean	6.00	20.40	37.20	65.92	72.52	79.61
S.Em.±	1.03	0.89	1.07	2.09	2.18	2.06
Range	0-20	5-39	20-50	21-84	34-88	48-92

Yield parameters at different salinity levels in matured plants

All agro-morphological parameters in the experiment demonstrated decreased performance and a statistically significant variance between genotypes with the increasing salinity level.

At 150 mM, the values of the percent reduction in plant height and branches/plant ranged from 4 to 23 and 1-22 per cent, respectively; at 200 mM, they were 4 to 31 and 1-36 per cent, respectively; and at 300 mM salinity stress, they were 6 to 55 and 1-41 per cent, respectively. At 300 mM salinity stress the maximum reduction in plant height and branch number was recorded in DCP 92-3 (55%) followed by ICCV 10 (40%) and ICCV 10 (41%) followed by Virat (35%) respectively, while minimum reduction over control was reported in Digbijoy (6%) followed by IC 268900 (11%) and Digbijoy (1%) followed by IC 268900 (9%) and Avrodhi (9%), respectively. When it came to the number of nodules and pods per plant as well as pod length, the values of stressed plants likewise showed a substantial decrease among the accessions. Virat reported the greatest reduction in the number of nodules per plant (45% in 150 mM, 68% in 200 mM, and 88% in 300 Mm), while Digbijoy reported the least reduction (5% in 150 mM, 21% in 200 mM, and 34% in 300 Mm). In terms of the average value of pods per plant, pod length, and seeds per pod, Annigeri and Digbijoy recorded the lowest reductions for each of these three parameters, whereas ICCV 10 in terms of pods/plant and pod length, and Virat in terms of seeds per pod, recorded the largest reductions over controls under 300 mM salinity stress. With regard to all the tested lines and tested parameters, higher salinity levels showed greater reductions compared to controls. While Digbijoy experienced the lowest drop in seed yield/plant compared to the control (11.8%), ICCV 10 had the greatest reduction (58.9%) under 300 mM saline stress (Table 3). The differences across germplasms could result from both hereditary variations and specific genetic influences (Uddin *et al*., 2009; Win *et al*., 2011). As salinity stress rose, the percentage of reduction gradually increased [Kaya and Arif (2003), Mahdavi and Modarres Sanavy (2007), Sehrawat *et al*. (2014)] (Fig. 1).

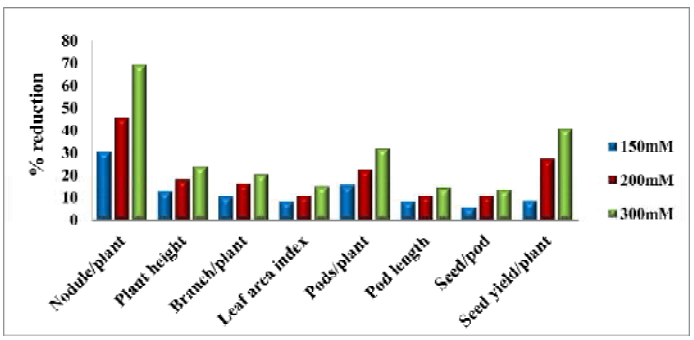

Figure 1. Percent reduction of different agro-morphological parameters under different salinity stress (mM NaCl solution)

Table 3. Variation in % reduction of nodules/plant, plant height, branch/plant, leaf area index, pods/plant, pod length, seeds/pod and seed yield/plant at different salinity level in matured plant.

	Nodule/plant			Plant height			Branch/plant			Leaf area index			Pods/plant			Pod length			Seeds/pod			Seed yield/plant		
	150 mM	200 mM	300 mM	150 mM	200 mM	300 mM	150m M	200 mM	300m M	150m M	200 mM	300 mM	150 mM	200 mM	300 mM	150m M	200m M	300m M	150m M	200m M	300m M	150m M	200m M	300mM
Digbijoy	5	21	34	5	4	6	1	1	1	2	4	3	8	14	26	1	3	6	3.6	2	3	1.5	12	11.8
GCP 105	33	50	67	8	19	12	18	20	29	11	12	13	20	20	34	4	4	7	4	7	11	10.7	26.2	38.5
IC 268971	35	40	65	10	18	26	12	16	28	5	10	27	25	34	40	8	9	12	6.6	16.5	25.3	20.4	43.6	47.5
IC 268900	20	13	40	6	10	11	3	7	9	10	16	21	12	24	35	5	4	6	2.3	6	12	9.6	26.4	20.5
Virat	45	68	88	8	18	35	20	32	35	18	21	29	28	32	39	18	25	32	18.6	24.9	39.5	26.9	41.5	56.3
WBG 29	44	67	78	12	13	19	7	17	22	4	7	11	22	25	31	10	11	12	6.2	16.6	11.2	9.9	22.6	32.9
ICCV 10	46	62	80	23	31	40	22	36	41	20	25	30	29	35	48	19	28	35	19.7	25.3	35.2	23.6	32.9	58.9
Radhey	29	57	71	15	22	25	12	26	30	8	11	25	25	33	35	12	21	32	6.8	15.2	24.6	16.9	30.4	44.7
Avrodhi	0	17	50	4	10	14	4	7	9	3	3	3	17	26	28	2	6	7	1.5	5.5	0	3.2	16.9	32.5
DCP 92-3	29	43	71	23	26	55	9	19	21	15	22	28	19	22	30	4	7	10	0	2.2	7.5	0	32.9	44.9
Vijoy	25	63	88	10	13	16	3	7	10	13	13	13	8	20	30	1	3	7	4.3	5.6	7.3	4.3	42.5	54.6
G 24	33	50	83	16	18	20	4	6	10	1	5	6	14	22	46	17	24	23	5.3	10.9	17.8	3.4	27.8	44.9
IC 268863	50	63	75	19	22	24	11	14	15	0	2	1	8	14	27	12	14	17	2.8	4.9	8.3	3.3	22.4	32.6
Dahod yellow	44	67	78	14	26	29	6	12	18	7	8	9	14	22	33	11	13	14	5.2	5.2	13.3	4.5	15.6	30.8
B 115	40	60	80	10	18	24	21	22	23	12	15	17	16	23	32	13	15	17	4.5	8.6	15.3	1.5	17	35.1
IC 268943	17	17	50	15	20	22	3	6	10	12	13	17	14	20	28	7	5	7	4.4	5.5	3.4	5.1	29.7	46.7
Mahamaya 1	38	42	66	12	15	29	15	21	27	10	17	20	13	21	29	9	10	18	7.2	14.2	25.3	9.2	27.7	45.4
Annigeri	25	38	75	13	19	24	20	28	31	10	13	13	4	11	19	3	4	5	4	13.4	7.8	18.6	31.7	40.1
Anuradha 39/2	17	17	67	15	19	22	18	22	27	3	2	16	13	19	27	8	9	16	3.8	21.9	4	2.2	24.4	49.1
RSG 888	40	60	80	20	24	28	5	10	20	3	2	3	4	15	27	6	7	11	5.1	9.2	1.6	0.2	24	47.5
Mean	30.75	45.75	69.3	12.9	18.25	24.05	10.7	16.45	20.8	8.35	11.05	15.25	15.65	22.6	32.2	8.5	11.1	14.7	5.79	11.03	13.67	8.75	27.41	40.76
S.Em.±	1.8	2.3	1.6	0.7	1.2	1.2	1.3	1.5	2.0	0.6	0.8	1.0	1.3	1.3	1.7	0.8	0.9	1.1	1.2	1.5	1.8	2.1	1.6	1.6
Range	0-50	13-68	34-88	4-23	4-31	6-55	1-22	0-36	1-41	0-20	2-25	1-30	4-29	11-35	19-48	1-19	3-28	5-35	0-19.7	2-25.3	0-39.5	0-26.9	12-43.6	11.8-58.9

The other agro-morphological elements influence seed yield, which is a polygenic trait. The main goal of every breeding programme is to increase seed yield. According to earlier research by several experts, salt-tolerant accessions were identified to be less impacted by higher salinity stress and might yield more seeds than others (Ashraf and Waheed, 1990; Sarkar and Kundagrami, 2016; Uddin and Hossain, 2018). Therefore, all of the examined lines were divided into three main classes: Tolerant 0-35%, Moderately Susceptible 36-50%, and Highly Susceptible 50% based on the percent loss of seed yield characteristics over control under 300 mM saline stress (Table 4). Results revealed that the highest number of accessions *i.e.*, 10 were found in the category moderately susceptible followed by 7 accessions in the tolerant and 3 in highly susceptible. Therefore, comparing the seed yield performance of all the tested germplasms it is found that the germplasms namely IC 268900, Digbijoy, Dahod yellow, Avrodhi, IC 268863, WBG 29, and B115 were categorized as tolerant germplasms, Vijoy, Virat and ICCV 10 were categorized as highly susceptible germplasms and rest were categorized as moderately susceptible germplasms (Table 4). It's interesting to note that when the results of all the testing parameters were added up, Digbijoy recorded a lesser reduction under salinity stress than other accessions, whereas ICCV 10 and Virat, in that order, recorded the highest reduction. Therefore, these germplasms may serve as a useful stock for future breeding initiatives aimed at creating chickpea lines that are tolerant to salinity.

Table 4. Grouping of twenty chickpea lines on the basis of percent reduction in seed yield parameters under 300 mM salinity treatment.

Sl. No.	Salt response	% Reduction	Name of genotypes
I	Tolerant	0-35	Digbijoy, IC 268900, Dahod yellow, Avrodhi, IC 268863, WBG 29, B115
II	Moderately susceptible	36-50	GCP 105, Annigeri, Radhey, G 24, DCP 92-3, Mahamaya 1, IC 268943, RSG 888, IC 268971, Anuradha 39/2
III	Highly susceptible	>50	Vijoy, Virat, ICCV 10

Correlation and regression analysis

Correlation and regression analysis are used to depict the relationships among several agro-morphological factors and salt tolerance under three different salinity loads. The results of a correlation study showed a substantial positive relationship between seed yield and germination percentage, seedling length, plant height, number of branches per plant, number of pods per plant, and pod length for lower salinity stresses (150 and 200 mM), however with the increase in salinity, no such significant association was identified (Table 5). There was no significant relationship between plant height, leaf area index, pod length, or 100 seed weight and the salt tolerance index (Standard Evaluation Score), suggesting that these characteristics may not be a strong predictor of salinity tolerance. However, the number of branches/plant did not significantly correlate with the tolerance index at lower salinities (150 and 200 mM), but a

significant negative correlation (-70) was found with an increase in salinity level (Table 4). This suggests that as the number of branches increases, the tolerance index (Standard Evaluation Score) may gradually decrease, indicating that the germplasms are more tolerant to the environment (Ali *et al*., 2014).

Table 5. Correlation matrix among the agro-morphological parameters at different salinity stress.

		Germination (%)	Seedling Length (cm)	Nodule/ plant	Plant Height (cm)	Branch/plant	Leaf Area Index	Pod/ plant	Pod Length (cm)	Seed/ Pod	100 Seed Weight (g)	Seed Yield/ plant (g)
Seedling Length (cm)	**150**	0.08**										
	200	0.09**										
	300	-0.08										
Nodule/ plant	**150**	-0.03	0.30**									
	200	0.20*	0.09									
	300	0.21	0.26									
Plant Height (cm)	**150**	-0.13	0.01	0.09*								
	200	0.00	-0.23	0.16								
	300	0.00	-0.08	-0.05								
Branch/plant	**150**	0.14	0.32	0.21	-0.40*							
	200	0.17**	0.28	-0.16	-0.52*							
	300	0.20	0.17	0.12	-0.21							
Leaf Area Index	**150**	0.15	0.08	0.35	0.17	0.20						
	200	0.04	0.11	0.22	-0.14	0.27						
	300	-0.02	0.21	0.21	-0.20	0.33						
Pod/plant	**150**	0.26	0.14**	0.14	-0.10	0.30**	0.36					
	200	0.35	0.08	0.22	-0.03	0.23**	0.40					
	300	0.19	0.17	0.20	-0.27	0.13**	0.38					
Pod Length (cm)	**150**	0.19	0.15	0.06	-0.51	0.56*	0.05	0.37				
	200	0.20	0.15	-0.15	-0.37	0.53*	0.08	0.33				
	300	-0.13	0.18	0.23	-0.03	0.26*	0.16	0.10				
Seed/ Pod	**150**	0.00	0.30	-0.03	0.29	0.08	0.12	0.23	-0.06			
	200	0.02	0.34	0.00	-0.09	0.26	0.15	0.20	0.12			
	300	0.13	0.18	0.10	-0.18	0.18	0.20	0.53	0.04			
100 Seed Weight	**150**	-0.14	-0.04	0.17	0.19	0.08	0.23	0.19	0.19	0.00		
	200	-0.11	-0.06	-0.03	0.00	0.08	0.26	0.24	0.17	-0.02		
	300	0.11	0.07	0.22	-0.14	-0.01	0.29	0.18	0.12	0.08		
Seed Yield/ plant	**150**	0.35**	0.13**	0.22	-0.22**	0.37**	0.20	0.39**	0.29*	0.16	0.07	
	200	0.16*	0.05*	-0.05	-0.16*	0.41*	0.01	0.10*	0.27*	0.07	0.12	
	300	-0.16	0.37	0.39	-0.11	0.08	0.06	0.04	0.41	-0.19	0.12	
Tolerance Index	**150**	-0.06*	-0.08*	-0.26*	-0.55	-0.69	-0.03	-0.65**	-0.06	-0.19*	-0.12	-0.19**
	200	-0.09	-0.16*	-0.10	-0.43	0.86	-0.05	-0.58*	-0.06	-0.23*	-0.18	0.23**
	300	-0.06	-0.29	-0.35	-0.69	-0.70*	-0.06	0.30*	-0.08	-0.56	-0.09	0.29**

**indicates 1% level of significance; *indicates 5% level of significance

Table 6. Regression coefficients among agro-morphological parameters at different salinity levels and constant (dependent variable: tolerance index).

Parameter	Tolerance index	Germination (%)	Seedling Length (cm)	Nodule/ plant	Plant Height (cm)	Branch/ plant
150 mM	3.29^{NS}	-0.03^{NS}	0.29^{NS}	-0.11^{NS}	0.59	0.15^{NS}
200 mM	3.86	-0.09^{NS}	0.48^{NS}	-0.21^{NS}	0.82^{NS}	0.29
300 mM	4.19**	-0.12^{NS}	-0.81^{NS}	-0.49^{NS}	0.91	0.41^{NS}
	Leaf Area Index	**Pod/plant**	**Pod Length (cm)**	**Seed/Pod**	**100 Seed Weight (g)**	**Seed Yield /plant (g)**
150 mM	0.12^{NS}	0.18	0.55	0.23^{NS}	0.20	0.46
200 mM	0.24^{NS}	0.23^{NS}	0.76^{NS}	0.54*	0.13	0.59^{NS}
300 mM	0.35	0.56**	0.91**	0.71**	0.14	0.88**

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Furthermore, regression assessment revealed a substantial but unfavourable relationship between salt tolerance and number of pods/plant, pod length, number of seeds/pod, and seed yield/plant, demonstrating that salt-tolerant germplasms may have larger numbers of pods/plant, pod length, seeds/pod, and seed yield/plant (Table 6). These desirable parameters can take part in the future breeding programme for saline soils by getting introduced into the susceptible lines. Additionally, these results were combined with past research by Peng *et al*. (1999), Ali *et al*. (2014), and others. These studies further established the significance of these parameters as valuable selection criteria for screening the salt tolerance in terms of seed yield among different germplasms.

CONCLUSION

From comparing the seed yield performance of all the tested germplasms, it can be concluded that Digbijoy, IC 268900, Dahod yellow, Avrodhi, IC 268863, WBG 29 and B 115 may be used as tolerant germplasms and Vijoy, Virat, and ICCV 10 as highly susceptible germplasms for future breeding programmes. The variations of the agro-morphological parameters among the germplasms were lower at low salinity stress (150 mM) than at high salinity stress (300 mM). In addition to this, as per correlation and regression study, while choosing salt-tolerant genotypes of chickpea in greenhouse conditions, plant breeders may emphasize the number of pods/plant, pod length, number of seeds/pod, and seed yield/plant. These desirable parameters also take part in the future breeding programme for saline soils by getting introduced into the susceptible lines.

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Research Article

Agronomic performance of maize hybrids influenced by sowing dates and plant spacing

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ABSTRACT

A field experiment was conducted during *Kharif*, 2020 at Agronomy Research Farm, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Wadura, Sopore in Split-split plot design with three dates of sowing *viz*; 17th SMW (D_1), 19th SMW (D_2) and 21st SMW (D_3) as main plot treatments, three varied row spacing *viz*; 50 cm × 20 cm (S_1), 60 cm × 20 cm (S_2) and 70 cm × 20 cm (S_3) as sub-plot treatments and two maize hybrids *viz*; Hytech-5801 (H_1) and YSH-1 (H_2) as sub-sub plot treatments. The results of the demonstration showed that growth parameters *viz*., plant height, dry matter production, leaf area index, and functional leaves per plant increased significantly for the crop sown on 17th SMW (D_1) with a concomitant increase in its yield attributing factors, *ie*., cob length, cob girth, number of grains per cob and test weight and yield. The growth, yield, and yield attributing factors also recorded the highest values with a plant spacing of 70 cm × 20 cm (S_3). As far as hybrids are concerned, growth characters showed a significant and consistent increase in hybrid Hytech-5801 compared to hybrid YSH-1 with an associated increase in yield and yield attributing factors.

***Keywords*:** Standard meteorological week, dates of sowing, plant spacing, hybrids, yield.

Maize (*Zea mays* L.) is one of the most versatile food-grain crops (Erenstein *et al*., 2022) grown under diverse soil and climatic conditions and ranks third after wheat and rice worldwide. The three major staples *viz*, rice, wheat, and maize consist of a major component of the human diet, which accounts for the world's 42 per cent of the food (Anonymous, 2021).

Maize is an important cereal crop owing to its highest production potential and wider adaptability under varying agro-climatic conditions and hence known as the "Queen of Cereals" (Choudhari and Channappagouda, 2015). The sowing date is a non-monetary input of agro-techniques that doesn't require extra cost and can be used to increase the efficiency of production by sowing at the right time. Sowing dates have a notable impact on the output of maize (Sab, 2020). Greater the deviation from the optimum date of sowing of each hybrid greater the reduction in yield (Sarvari and Futo, 2000; Berzsenyi and Lap, 2001). One of the key agronomic factors for greater crop output is optimal plant spacing, which is thought to affect how light is intercepted during photosynthesis, the process through which plants' green sections produce energy (Monneveux *et al*., 2005). Proper plant variety has a profound influence on the yield of maize (Azam *et al*., 2007). It has been found that hybrids outyield the local varieties. Also, inadequate spacing influences the photosphere and rhizosphere exploitation and the plants suffer from clustering together. Adjustment of appropriate dates of sowing and proper plant spacing in maize fields is important so as to ensure maximum utilization of solar radiations and to exploit the maximum natural resources such as sunlight, soil moisture, nutrients *etc*. to ensure profitable yield (Kebede, 2019). Hybrid varieties in combination with the optimum date of sowing and proper spacing lead to an increase in the growth and yield of a crop.

MATERIALS AND METHODS

The field experiment was performed at Agronomy Research Farm, Wadura Sopore, Sher-e Kashmir University of Agricultural Sciences and Technology Kashmir in the year 2020 located between 34°21' N latitude and 74°23' E longitude having an altitude of 1590 m above the mean sea level. The experimental site had a level topography and good drainage. The soil in the test field had a silty clay loam texture and was neutral in reactivity to pH (6.8), with medium

availability of available N (306.25 kg ha^{-1}), available P_2O_5 (18 kg ha^{-1}), and available K_2O (213.80 kg ha^{-1}). The experiment consisted of three factors with three sowing dates as main plot treatments *viz*. (17th SMW (23-29 April) (D_1), 19th SMW (7-13 May) (D_2) and 21st SMW (21-27 May) (D_3), three spacings as sub-plot treatments *viz*. 50 cm x 20 cm (S_1), 60 cm × 20 cm (S_2), 70 cm × 20 cm (S_3) and two hybrids as sub-sub-plot treatments *viz*. Hytech-5801 (H_1), and YSH-1 (H_2) laid out in a Split-split plot design replicated thrice. The mean maximum and minimum temperatures during the crop growing season were 34.5 °C and 9.4 °C for the first date of sowing, 32.5 °C and 14.1 °C for the second date of sowing, and 34.5 °C and 10.1 °C for the third date of sowing. The precipitation amounted to 300 mm, 285 mm, and 220 mm for different dates of sowing. The mean maximum and minimum relative humidity were 73.7 per cent and 63.7 per cent for the first date of sowing, 91.4 per cent and 47.4 per cent for the second date of sowing, and 91.9 per cent and 46.9 per cent for the third date of sowing.

RESULTS AND DISCUSSION

Plant height (cm)

Plant height of maize hybrids varied significantly due to dates of sowing and planting geometry (Fig. 1) where maximum plant height (208.09 cm) was noted in Hytech-5801 (H1) sown on 17th SMW (D_1). The early sowing had a significant effect on plant stature due to the presence of favourable weather conditions and longer growth duration leading to a higher accumulation of heat units and profuse vegetative growth (Kharazmshahi *et al*., 2015). Plant height increased significantly with a decrease in plant spacing due to increased intra-plant competition for light, nutrients, *etc*. The tallest plant height of maize (208.09 cm) was recorded in narrow spacing of 50 cm × 20 cm (S_1), whereas, the shortest plant height (195.03 cm) was recorded in wide spacing of 70 cm × 20 cm (S_3). These results are in conformity with Rafiq *et al*. (2010), Sherifi *et al*. (2009), and Mandic *et al*. (2016). Plant height (205.64 cm) was recorded in maize hybrid (Hytech-5801) whereas, a plant height of 195.57 cm was noted in maize hybrid (YSH-1) at all the growth stages. The differential growth with respect to plant height might be attributed to the genotypic variations of the individual hybrids as reported by Azam *et al*. (2007) and Sab *et al*. (2020).

Functional leaves per plant

The functional leaves per plant were statistically significant with respect to dates of sowing at all the growth stages but were statistically at par with respect to plant spacing and hybrids at 20 DAS (Fig. 2). Sowing on the 17th SMW (D_1) recorded the highest functional leaves per plant (9.07) followed by 19th SMW (D_2) (8.76) and 21st SMW (D_3) (8.2). The functional leaves per plant decreased due to delay in sowing because of the reduction of photoperiod with delayed sowing (Sab *et al*., 2020). The better penetration of sunlight into the plant canopy and delayed senescence led 70 cm × 20 cm (S_3) (9.46) to obtain the highest functional leaves per plant compared to 60 cm × 20 cm (S_2) (8.63) and 50 cm × 20 cm (S_1) (7.94). The results are in conformity with (Beiragi *et al*., 2011). The hybrid Hytech-5801 (H_1) recorded the highest functional leaves per plant (9.89) compared to YSH-1 (H_2) (7.46) due to the difference in the genetic constitution between the hybrids.

Leaf area index

Leaf area index (LAI) is the ratio of total leaf area occupied by a plant per unit total area of land (Watson, 1997). It is an important index to be measured to know the rate of plant growth and development status of plants (Stewart and Dwyer, 1999). Significant variations were observed in the

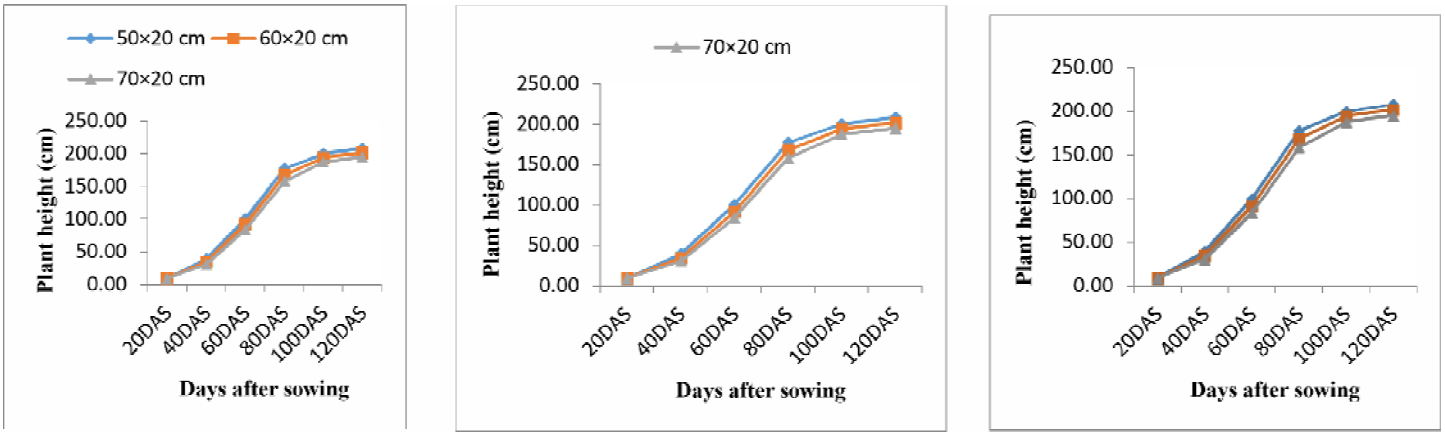

Fig. 1. Effect of dates of sowing and plant spacing on the plant height (cm) of maize hybrids.

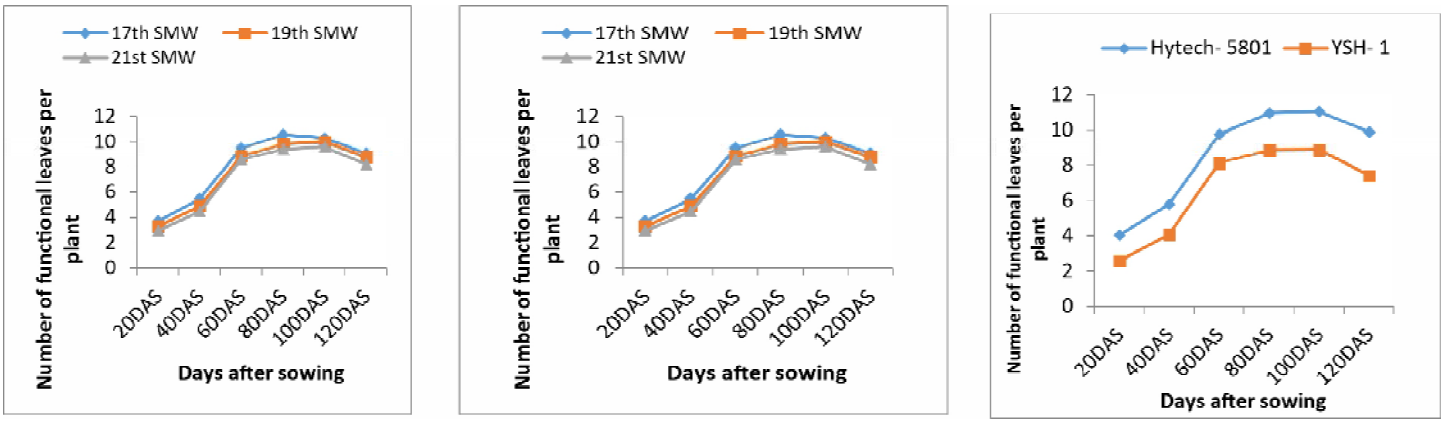

Fig. 2. Effect of dates of sowing and plant spacing on the number of functional leaves per plant of maize hybrids.

leaf area index during the crop growth period with respect to dates of sowing (Fig. 3). The crop sown on the 17th SMW (D_1) obtained a significantly higher leaf area index (3.62), compared to the 19th SMW (D_2) (3.07) and 21st SMW (D_3) (2.86) at all the stages of the crop. The reduction of photoperiod with delayed sowing resulted in decreased LAI with delayed sowing dates (Noferesti, 2006; Moosavi *et al*., 2012). Proper arrangement of leaves and good canopy are required for better sunlight interception promoting photosynthesis and other metabolic processes of the crop (Morrison *et al*., 1992). More number of plants per unit area led to an increased leaf area index in 50 cm × 20 cm (S_1) (3.82) followed by 60 cm × 20 cm (S_2) (3.12) and 70 cm × 20 cm (S_3) (2.60) at all the growth stages except at 20 DAS where leaf area index was statistically at par with each other (Shafi *et al*., 2012). The difference in the leaf area index of the two maize hybrids might be due to the difference in their genetic constitution (Shinde *et al*., 2015) and Hytech-5801 registered a significantly higher value of leaf area index (3.80) over YSH-1 (2.56).

Dry matter accumulation (q ha^{-1})

The dry matter accumulation was found to be highest in earlier sown crop *i.e.*, 17th SMW (D_1) followed by 19th SMW (D_2) and 21st SMW (D_3) (Fig. 4), owing to more favourable growth conditions, more leaf area index and hence higher dry matter accumulation whereas delayed sowing produced less dry matter accumulation at all the growth stages (Sab *et al*., 2020). The dry matter accumulation decreased with increased plant spacing because of more number of plants per unit area and higher leaf area index leading to significantly higher dry matter accumulation in narrow-spaced crop *viz*, 50 cm × 20 cm (S_1) (155.36 q ha^{-1}) at all the growth stages followed by 60 cm × 20 cm (S_2) (144.95) and 70 cm × 20 cm (S_3) (139.00 q ha^{-1}). The results are in association with (Sahoo and Mahapatra, 2007). The difference among the hybrids in terms of dry matter accumulation is due to more plant height, leaf area, and number of functional leaves per plant which is recorded in Hytech-5801 (H_1) (151.43 q ha^{-1}) followed in the hybrid YSH-1 (H_2) (141.44 q ha^{-1}).

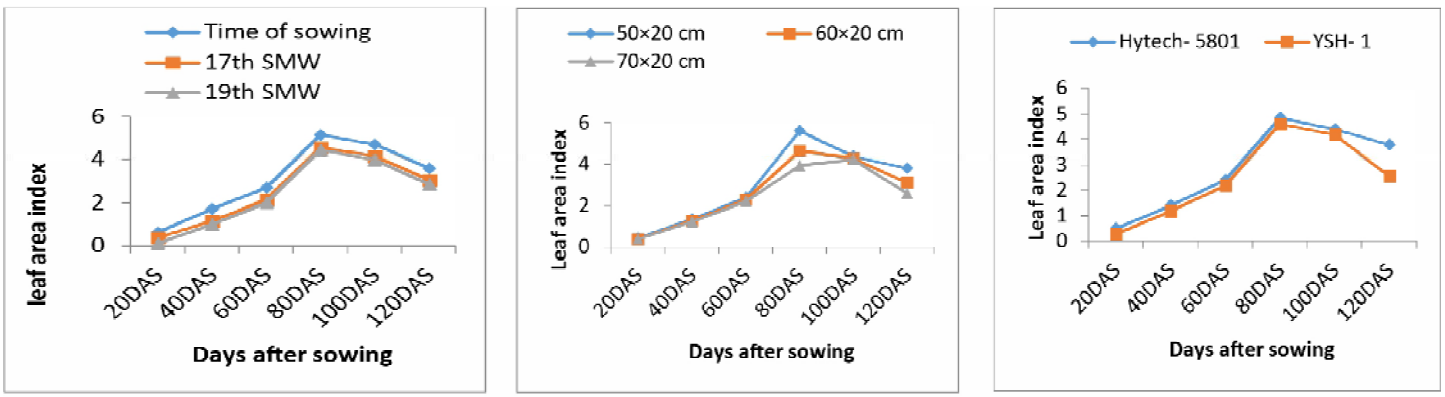

Fig. 3. Effect of dates of sowing and spacing on the leaf area index of maize hybrids.

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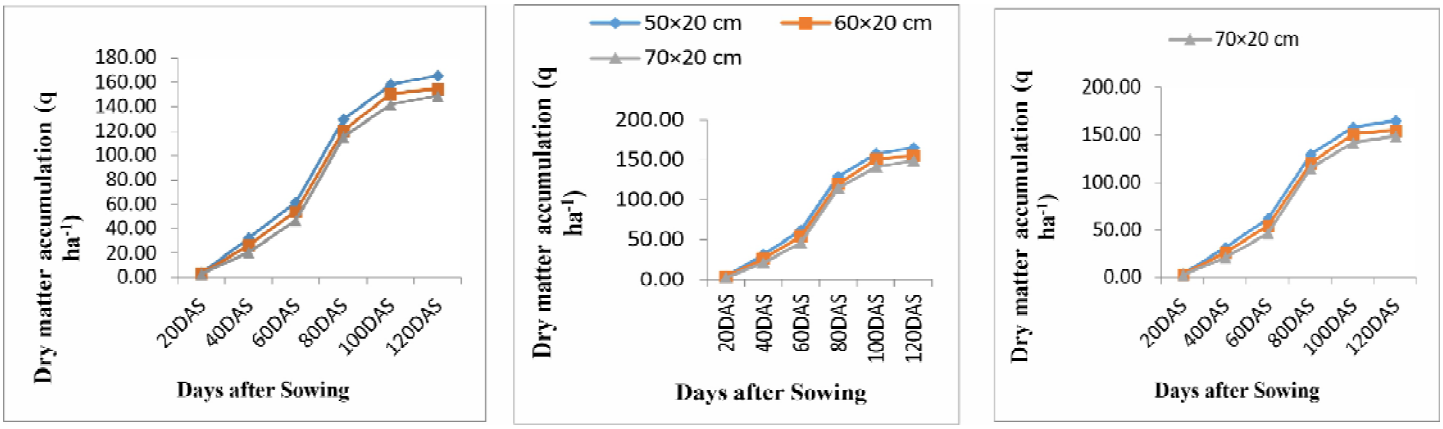

Fig. 4. Effect of dates of sowing and plant spacing on the dry matter accumulation (q ha^{-1}) of maize hybrids.

Yield attributing characters

Data of various yield attributing characters *viz*., cob length (cm), cob girth (cm), number of cobs per plant, number of rows per cob, number of grains per row, and cob weight were statistically computed and the mean values obtained are being presented in Table 1.

Cob length (cm)

Data recorded on the average cob length of maize hybrids are presented in Table 1. Mean comparisons indicated that the highest cob length (20.82 cm) was recorded with the 17th SMW followed by the 19th SMW (19.63 cm) and 21st SMW (18.64 cm). Similar findings were traced by (Hassan, 1998) who found that cob length decreased with a delay in sowing date. The crop sown at 70 cm × 20 cm (S_3) recorded significantly the highest cob length (21.07 cm) followed by 60 cm × 20 cm (19.68 cm) and 50 cm × 20 cm (18.34 cm). The hybrid Hytech-5801 obtained significantly the highest cob length (20.17 cm) followed by hybrid YSH-1 (19.23 cm).

Cob girth (cm)

From the data given in Table 1 a striking variation in cob girth is observed as a result of varying sowing dates, plant spacing, and hybrids. The crop sown on 17th SMW (D_1) showed lengthier cobs for all sowing dates compared to 19th SMW (D_2) and 21st SMW (D_3). There was a significant influence of plant spacing on cob girth. Plant spacing of 70 cm × 20 cm (S_3) recorded significantly the highest cob girth (17.20 cm) over 60 cm × 20 cm (16.07 cm) and 50 cm × 20 cm (15.30 cm). Similar findings were also traced by Namakka *et*

Table 1: Yield attributes of different maize hybrids as influenced by different dates of sowing and plant spacing.

Treatment	Cob length (cm)	Cob girth (cm)	Number of rows cob^{-1}	Cob weight (g)	Number of grains cob^{-1}	Number of cobs $plant^{-1}$	Test weight (g)
Time of sowing							
17th SMW	20.82	16.73	13.72	176.08	388.61	1.18	228.20
19th SMW	19.63	16.17	12.75	162.36	354.05	1.15	220.60
21st SMW	18.64	15.69	12.03	140.16	316.88	1.03	214.55
S.Em.±	0.13	0.09	0.12	3.05	3.04	0.06	0.46
C.D. (p≤0.05)	0.39	0.27	0.36	9.16	9.14	N.S	1.40
Row spacing							
50 cm x 20 cm	18.34	15.30	12.22	147.47	332.05	1.11	211.83
60 cm x 20 cm	19.68	16.07	12.87	158.32	355.61	1.12	222.86
70 cm x 20 cm	21.07	17.20	13.42	172.82	371.88	1.14	228.66
S.Em.±	0.10	0.07	0.11	3.01	3.30	0.02	1.28
C.D. (p≤0.05)	0.31	0.23	0.34	9.04	9.90	N.S	3.86
Varieties							
Hytech-5801	20.17	17.11	13.14	184.72	365.81	1.17	224.03
YSH-1	19.22	15.27	12.52	134.34	340.55	1.08	218.20
S.Em.±	0.12	0.18	0.12	3.08	6.04	0.04	0.16
C.D. (p≤0.05)	0.36	0.55	0.37	9.24	18.13	N.S	0.48

al. (2008) who concluded that the cob girth decreased significantly with a delayed sowing date. With respect to hybrids, Hytech-5801 obtained significantly the highest cob girth (17.12 cm) followed by hybrid YSH-1 which recorded a cob girth of 15.27 cm.

Rows per cob

The significantly highest number of rows per cob (13.72) was recorded with 17th SMW (D_1) followed by 19th SMW (D_2) which was 12.75 and the lowest number of rows per cob (12.03) was recorded with 21st SMW (D_3). Plant spacing of 70 cm × 20 cm (S_3) recorded significantly the highest rows per cob (13.42) followed by 60 cm × 20 cm which recorded 12.87 and the lowest rows per cob was recorded at 50 cm × 20 cm (12.22). With respect to hybrids, Hytech-5801 obtained significantly the highest rows per cob (13.14) followed by hybrid YSH-1 which recorded rows per cob of 12.52.

Cob weight (g)

Significantly highest cob weight (176.08g) was recorded with the 17th SMW (D_1) followed by the 19th SMW (D_2) which was 162.36g and (140.16g) was recorded with the 21st SMW (D_3). Plant spacing of 70 cm × 20 cm (S_3) recorded significantly the highest cob weight (172.8g) followed by 60 cm × 20 cm which recorded 158.32g and the lowest cob weight was recorded at 50 cm × 20 cm (147.47g). With respect to hybrids, Hytech-5801 obtained significantly the highest cob weight (184.72g) followed by hybrid YSH-1 which recorded a cob weight of 134.34 g.

Grain number per cob

A significantly higher number of grains number per cob (388.61) was recorded with 17th SMW (D_1) followed by 19th SMW (D_2) (354.05) and lowest in 21st SMW (D_3) (316.88). Plant spacing of 70 cm × 20 cm (S_3) recorded significantly the highest grain number per cob (371.88) followed by 60 cm × 20 cm which recorded 355.61 and the lowest grain number was recorded in 50 cm × 20 cm (332.05). With respect to hybrids Hytech- 5801 obtained significantly the highest grain number per cob (365.81) followed by hybrid YSH-1 which recorded a number of 340.55.

Number of cobs per plant

The number of cobs per plant was found to be statistically non-significant with respect to dates of sowing, plant spacing, and hybrids as presented in Table 1. Rehman *et al*. (1991) reported that the number of cobs per plant for various maize hybrids was found to be non-significant. The similarity in cobs among maize hybrids was probably due to the same environmental conditions.

Test weight (g)

Test weight of different maize hybrids had a statistically significant effect on dates of sowing, and plant spacing. The results revealed that the highest test weight was recorded in 17th SMW (D_1) (228.20 g), followed by 19th SMW (D_2) (220.60 g) and 21st SMW (D_3) (214.55 g). Crop when sown at a suitable time gave better yield and yield attributes (test wight). Sab *et al*. (2020) reported that good photosynthates accumulate in leaves and transfer to economic parts like grains, ears, etc. Among plant spacing, the statistically significant value of 228.66 g was found in wider spaced crops 70 cm × 20 cm (S_3) than 60 cm × 20 cm (S_2) (222.86 g) and lowest in 50 cm × 20 cm (S_1) (211.83 g). Among hybrids higher test weight was observed in Hytech-5801 (H_1) (224.03 g) followed by YSH-1 (H_2) (218.20 g). The hybrid differences in test weight may be ascribed to the genetic differences among them, which play an important role in exploiting the uptake of the available nutrients and photosynthesis process leading to many noticeable changes in the metabolites accumulated in maize shortage organs.

Yield parameters and harvest index (%)

The data presented in Table 2 shows that the grain yield, stover yield, biological yield, and harvest index were significantly reduced by delaying sowing from the 17th SMW to the 21st SMW (D_3). The late sown crop got less time duration to utilize the available resources which decreases the dry matter production and partitioning of assimilates towards

Table 2: Yield of maize hybrids as affected by different dates of sowing and plant spacing.

Treatment	Grain yield (q ha^{-1})	Stover yield (q ha^{-1})	Biological yield (q ha^{-1})	Harvest index (%)
Time of sowing				
17th SMW (D_1)	70.15	132.81	202.96	34.57
19th SMW (D_2)	65.81	127.23	193.04	34.09
21st SMW (D_3)	61.89	116.75	178.64	34.65
S.Em.±	1.26	0.48	3.03	0.12
C.D. ($p \leq 0.05$)	3.77	1.45	9.09	0.37
Row spacing				
50 cm x 20 cm (S_1)	56.97	136.46	193.43	29.45
60 cm x 20 cm (S_2)	65.49	125.46	190.95	34.30
70 cm x 20 cm (S_3)	75.40	114.86	190.27	39.63
S.Em.±	1.3	3.64	3.16	0.24
C.D. ($p \leq 0.05$)	3.9	10.9	9.48	0.72
Varieties				
Hytech-5801 (H_1)	71.04	132.80	203.83	34.85
YSH-1 (H_2)	60.87	118.39	179.26	33.96
S.Em.±	0.67	3.43	4.47	0.17
C.D. ($p \leq 0.05$)	2.01	10.28	13.41	N.S

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the grain and hence reduction of yield (Sab *et al*., 2020). The maximum grain yield, stover yield, biological yield, and harvest index were registered in 70 cm × 20 cm (S_3) spaced crop compared to the crop spaced at 60 cm × 20 cm (S_2) and 50 cm × 20 cm (S_1). The wider-spaced crop outperformed the narrow-spaced crop, owing to the intra-specific competition (Kebede, 2019). The hybrid Hytech-5801 (H_1) outperformed the yield over YSH-1 (H_2). Andrade (1995) reported that yield varied with varying maturity periods of genotypes.

CONCLUSION

Based on the results, it was concluded that amongst varying dates of sowing, the crop sown on 17th SMW (D_1) recorded significantly higher growth parameters *viz*., plant height, dry matter accumulation, leaf area index, number of functional leaves per plant, yield attributing factors *viz*., cob length, cob girth, number of grains per cob and test weight and yield. The delayed sowing resulted in a decrease in yield parameters and yield. In addition to it the maize hybrid Hytech-5801outyielded YSH-1 when sown on 17th SMW (D_1) and a spacing of 70 cm × 20 cm. Further, the crop sown at a row spacing of 70 cm × 20 cm recorded the highest growth parameters *viz*., plant height, dry matter production, leaf area index, functional leaves per plant, yield attributing factors *viz*., cob length, cob girth, grain number per cob and test weight and yield compared to the crop sown at 60 cm × 20 cm and 50 cm × 20 cm.

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Research Article

Effect of integrated nutrient management on flowering, postharvest life and xanthophyll content of African marigold (*Tagetes erecta* L.) cv. Pusa Basanti Gainda

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ABSTRACT

The present study on the "Effect of integrated nutrient management on flowering, postharvest life and xanthophyll content of African marigold (*Tagetes erecta* L.) cv. Pusa Basanti Gainda" was conducted during 2021-2022 at the College of Agriculture, Central Agricultural University, Imphal with seven treatments and three replications. The minimum first flower appearance and flower longevity on the plant were recorded in the treatment with the application of 75% RDF + vermicompost (1.25 t ha^{-1}) (T_4). The diameter of the flower (cm) and weight of the individual flower (g) was maximum when 75% RDF + FYM (7.5 t ha^{-1}) (T_2) was applied.

***Keywords*:** African Marigold, flowering, xanthophyll, postharvest, shelf life.

Marigold belongs to the family Asteraceae and genus *Tagete*s. The genus *Tagetes* comprises about 33 species of which *Tagetes erecta* (African Marigold) and *Tagetes patula* (French Marigold) are under commercial cultivation in India.

Marigold Flower Extract (MFE) is a natural source of carotenoid having the potential as a natural food colourant and carotenoid extracts from *Tagetes erecta* is applied in poultry feeds as additives to enhance chicken skin and egg yolk colouration (Scott *et al*., 1968). Apart from the poultry industry, marigold dye is also used in textile, pharmaceutical industries, food supplements, cosmetics *etc*. as they offer several advantages over synthetic dyes from a natural point of view, including safety, and being eco-friendly in nature. It is also used to dye fabrics where its ethanol-based flower extracts give different colour to fabrics. Carotenoids are reported to be beneficial for human health such as supporting eyes and skin and decreasing the failure of eyesight due to coronary heart disease, age-related macular degeneration (ADM), and cancer. Commercially, carotenoids extracted from flowers are used in pharmaceutical industries, food supplements, food colorants, and animal feed additives.

The term "integrated nutrient management" describes the process of maximizing the benefits from all potential sources of organic and inorganic components in a coordinated manner in order to maintain the soil fertility and plant nutrient supply at an optimal level for maintaining the desired productivity.

Keeping in view the importance of integrated nutrient management (INM), the present investigation on 'Effect of integrated nutrient management on flowering, postharvest life and xanthophyll content of African marigold (*Tagetes erecta* L.) cv. Pusa Basanti Gainda' was undertaken to evaluate the effect of different combinations of inorganic fertilizers and organic manures on the post-harvest life and xanthophyll content of the marigold cv. Pusa Basanti Gainda.

MATERIALS AND METHODS

The present experiment was carried out at the experimental field of the Department of Horticulture, College of Agriculture, Central Agricultural University, Imphal during 2021-2022. The experiment was carried out in Randomized Block Design (RBD) constituting seven treatments replicated three times with 30 plants per plot spaced at 40 cm × 30 cm. The treatments included 100 per cent Recommended dose of inorganic fertilizer (RDF) (T_1), 75 per cent RDF + FYM (7.5 t ha^{-1}) (T_2), 75 per cent RDF + neem cake (0.63 t ha^{-1}) (T_3), 75 per cent RDF + vermicompost (1.25 t ha^{-1}) (T_4), 50 per cent RDF + FYM (15 t ha^{-1}) (T_5), 50 per cent RDF + neem cake (1.3 t ha^{-1}) (T_6), 50 per cent RDF + vermicompost (2.5 t ha^{-1}) (T_7). Well-decomposed FYM, neem cake, and vermicompost were applied as per the treatment to the experimental plots 15 days before transplanting, after layout and plot preparation of the field. The recommended dose of nitrogen, phosphorus, and potassium for Manipur condition was 250, 100 and 50 kg per hectare (Chokhoni,

2015) was applied in two splits *i.e.*, 50 per cent N and K and a full dose of P at the time of transplanting and remaining 50 per cent N and K was applied 40 days after transplanting as a top dressing in the form of urea, super phosphate and muriate of potash, respectively. During the experiment, various parameters such as flowering parameters, post-harvest parameters like physiological loss in weight (Verma and Jhanji, 2021), sensory quality (colour retention & wilting) of loose flowers (Barrett *et al.*, 2010), spoilage (%) and moisture content (%) (Verma and Jhanji, 2021) and xanthophyll content (Lawrence, 1990).

RESULTS AND DISCUSSIONS

Flowering

Different flowering parameters *viz.*, minimum days to first flower, maximum flower longevity on the plant, the diameter of flower, and weight of individual flower were significantly influenced by different treatments of integrated nutrient management. From Table 1 minimum days to the first flower and maximum flower longevity on the plant were observed with the treatment application of 75 per cent RDF + vermicompost (1.25 t ha^{-1}) [T_4]. Application of 50 per cent RDF + neem cake (1.3 t ha^{-1}) (T_6) took the maximum days to first flower (82.60 days) and treatment T_1 (15.13 days) recorded fewer days of flower longevity on the plant. It may be because nutrients are taken in more readily and growth-promoting agents are sent simultaneously to auxiliary buds, breaking apical dominance. In the end, this may have produced a superior sink for the earlier transition of plant parts out from the vegetative to the reproductive phase, as well as for the quicker mobilization of photosynthates. The application of vermicompost in combination with inorganic fertilizer provides optimum availability of nitrogen and other essential nutrients for longer periods at an optimum level. The results on earliness in first flowering are in agreement with Upadhya *et al*. (2022) in Marigold and Kumar *et al*. (2020) in Chrysanthemum. Positive results on the longevity of flowers in the field experiments were observed by Mohd *et al.* (2006) in the anthurium and Parya (2017) in gerbera.

From Table 1 diameter of the flower (cm) and the weight of the individual flower (g) was maximum when 75 per cent RDF + FYM (7.5 t ha^{-1}) (T_2) was applied and the minimum diameter of the flower (4.50 cm) and weight of individual flower (g) (6.70 g) were noted in treatment T_3 [75 per cent RDF + neem cake (0.63 t ha^{-1})] application. The maximum diameter and weight of the flower by application of FYM might be due to improved nutrient availability, greater photosynthesis, and maintenance of healthy physiological functions of the plant, treatment application of RDF with FYM may have produced more food, which in turn may have been used for improved bud development and increased flower weight. The results are in conformation with the findings of Kumar *et al.* (2009) and Kumura *et al.* (2019) in Marigold. Similar findings on the maximum weight of individual flowers were obtained by Singh *et al*. (2015 in Calendula and Sahu *et al*. (2021) in Dahlia.

Postharvest

The perusal of the data given in the Table 2 shows that significant effect of integrated nutrient management on physiological loss in weight, sensory quality, spoilage (%), moisture content (%), and shelf life of marigold flowers at room condition storage on 1st, 2nd, 3rd, 4th, 5th, 6th and 7th day.

It was found that out of all treatments, minimum physiological loss in weight was obtained in T_7 [50% RDF + vermicompost (2.5 t ha^{-1})] with 22.39 per cent and 53.92 per cent at 4th and 7th day and the maximum weight loss (46.14 and 73.36 per cent, respectively) was observed in T_6 [50% RDF + neem cake (1.3 t ha^{-1})] during the entire postharvest period. A keen observation of the data in Table 3 shows that the treatment, T_7 [50 per cent RDF + vermicompost (2.5 t ha^{-1})] applied flowers obtained the highest score of 8.20 and 5.93, respectively on the 4th and 7th day and the least score

Table 1: Days taken to first flowering, flower longevity on plant (days), diameter of the flower (cm) and fresh weight of individual flower (g) of African Marigold as influenced by integrated nutrient management.

Treatment	Days taken to first flowering	Flower longevity on plant (days)	Diameter of flower (cm)	Weight of individual flower (g)
T_1 : 100 per cent Recommended dose of inorganic fertilizer (RDF)	78.64	15.13	5.26	8.27
T_2 : 75 per cent RDF + FYM (7.5 t ha^{-1})	69.23	17.57	6.35	8.87
T_3 : 75 per cent RDF + neem cake (0.63 t ha^{-1})	82.50	16.27	4.50	6.70
T_4 : 75 per cent RDF + vermicompost (1.25 t ha^{-1})	67.27	17.90	6.04	8.80
T_5 : 50 per cent RDF + FYM (15 t ha^{-1})	74.17	17.23	5.82	8.10
T_6 : 50 per cent RDF + neem cake (1.3 t ha^{-1})	82.60	16.53	4.92	7.20
T_7 : 50 per cent RDF + vermicompost (2.5 t ha^{-1})	75.01	17.43	5.74	8.08
S.Ed.±	1.08	0.67	0.14	0.34
C.D. (p=0.05)	2.35	1.47	0.31	0.75

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Table 2: Physiological loss in weight (%) of African Marigold flower as influenced by integrated nutrient management.

Treatment	Physiological loss in weight (%)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
T_1 : 100 per cent recommended dose of inorganic fertilizer (RDF)	10.65	16.24	29.23	41.58	52.36	61.14	70.22
T_2 : 75 per cent RDF + FYM (7.5 t ha^{-1})	8.34	13.09	24.45	37.21	43.78	55.86	67.67
T_3 : 75 per cent RDF + neem cake (0.63 t ha^{-1})	6.42	12.16	20.56	32.25	41.98	52.77	64.97
T_4 : 75 per cent RDF + vermicompost (1.25 t ha^{-1})	5.10	10.44	17.30	27.59	35.25	47.37	57.30
T_5 : 50 per cent RDF + FYM (15 t ha^{-1})	5.40	11.49	18.68	29.20	39.39	51.13	62.40
T_6 : 50 per cent RDF + neem cake (1.3 t ha^{-1})	12.69	22.47	34.49	46.14	58.58	69.77	73.36
T_7 : 50 per cent RDF + vermicompost (2.5 t ha^{-1})	4.82	9.44	15.35	22.39	36.66	42.14	53.92
S.Ed.±	0.26	0.29	0.33	0.41	0.39	0.62	0.33
C.D. (p=0.05)	0.58	0.62	0.71	0.88	0.86	1.35	0.72

was obtained in the treatment T_6 [50 per cent RDF + neem cake (1.3 t ha^{-1})] with score of 5.80 and 2.00 on 4th and 7th day of postharvest period. A close examination of data given in Table 4 reveals that among all treatments, minimum spoilage of 3.33 and 53.33 per cent on 4th and 7th day was observed with T_7 [50 per cent RDF + vermicompost (2.5 t ha^{-1})] application, however, maximum spoilage of 43.33 and 93.33 per cent on 4th and 7th day of entire postharvest period was observed in T_6 [50 per cent RDF + neem cake (1.3 t ha^{-1})] at room condition storage. The data presented in Table 5 reveal that out of all treatments, T_7[50 per cent RDF + vermicompost (2.5 t ha^{-1})] has the maximum moisture content of 84.07 and 72.98 per cent on 4th and 7th day, the minimum moisture content of 72.12 and 50.34 per cent was obtained in treatment

Table 3: Sensory quality (colour retention & wilting) of loose flower of African marigold as influenced by integrated nutrient management.

Treatment	Sensory quality (colour retention & wilting)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
T_1 : 100 per cent recommended dose of inorganic fertilizer (RDF)	8.70	8.27	7.80	7.07	6.03	5.00	4.80	3.67
T_2 : 75% RDF + FYM (7.5 t ha^{-1})	8.97	8.33	8.00	7.47	6.87	6.00	5.13	3.87
T_3 : 75% RDF + neem cake (0.63 t ha^{-1})	8.77	8.40	8.07	7.73	7.07	6.20	5.33	4.13
T_4 : 75% RDF + vermicompost (1.25 t ha^{-1})	8.90	8.53	8.23	7.93	7.53	6.53	6.13	5.07
T_5 : 50% RDF + FYM (15 t ha^{-1})	8.90	8.60	8.13	7.80	7.40	6.40	5.20	4.80
T_6 : 50% RDF + neem cake (1.3 t ha^{-1})	8.60	8.07	7.33	6.67	5.80	4.70	3.60	2.00
T_7 : 50% RDF + vermicompost (2.5 t ha^{-1})	8.90	8.73	8.40	8.33	8.20	7.53	6.80	5.93
S.Ed.±	0.11	0.10	0.24	0.18	0.32	0.75	0.39	0.30
C.D. (p=0.05)	0.23	0.22	0.53	0.39	0.70	1.64	0.85	0.65

Table 4: Spoilage (%) of African marigold flower as influenced by integrated nutrient management.

Treatment	Spoilage (%)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day6	Day 7
T_1 : 100 per cent recommended dose of inorganic fertilizer (RDF)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	20.00 (4.43)	40.00 (39.15)	53.33 (46.92)	66.67 (54.78)	83.33 (66.14)
T_2 : 75% RDF + FYM (7.5 t ha^{-1})	0.00 (0.71)	0.00 (0.71)	3.33 (1.55)	13.33 (3.67)	33.33 (35.01)	43.33 (41.07)	56.67 (48.85)	80.00 (63.43)
T_3 : 75% RDF + neem cake (0.63 t ha^{-1})	0.00 (0.71)	0.00 (0.71)	6.67 (2.40)	16.67 (4.10)	33.33 (35.01)	40.00 (39.23)	56.67 (48.93)	76.67 (61.22)
T_4 : 75% RDF + vermicompost (1.25 t ha^{-1})	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	3.33 (1.55)	13.33 (17.61)	23.33 (28.08)	46.67 (42.99)	70.00 (57.29)
T_5 : 50% RDF + FYM (15 t ha^{-1})	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	13.33 (3.67)	30.00 (32.71)	36.67 (37.14)	53.33 (47.01)	73.33 (59.00)
T_6 : 50% RDF + neem cake (1.3 t ha^{-1})	0.00 (0.71)	0.00 (0.71)	10.00 (2.31)	40.00 (6.33)	43.33 (41.15)	50.00 (45.00)	73.33 (59.71)	93.33 (81.14)
T_7 : 50% RDF + vermicompost (2.5 t ha^{-1})	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	3.33 (6.94)	13.33 (21.14)	26.67 (30.00)	53.33 (47.01)
S.Ed.±	0.00	0.00	0.96	0.79	8.30	5.47	7.32	6.24
C.D. (p=0.05)	0.00	0.00	2.09	1.72	18.08	11.91	15.95	13.60

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Table 5: Moisture content (%) of African marigold flower as influenced by integrated nutrient management.

Treatment	Moisture content (%)							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
T_1 : 100 per cent recommended dose of inorganic fertilizer (RDF)	91.01	89.03	88.01	83.20	77.65	68.17	62.42	57.31
T_2 : 75% RDF + FYM (7.5 t ha^{-1})	95.23	92.86	91.59	86.52	79.80	73.53	67.54	63.21
T_3 : 75% RDF + neem cake (0.63 t ha^{-1})	93.06	90.87	87.32	84.14	78.70	74.13	69.32	62.08
T_4 : 75% RDF + vermicompost (1.25 t ha^{-1})	93.98	92.47	89.25	86.19	81.16	78.08	72.80	70.20
T_5 : 50% RDF + FYM (15 t ha^{-1})	93.88	91.41	88.94	85.68	80.93	76.67	70.66	68.57
T_6 : 50% RDF + neem cake (1.3 t ha^{-1})	93.80	88.94	81.18	78.44	72.12	65.26	59.39	50.34
T_7 : 50% RDF + vermicompost (2.5 t ha^{-1})	93.33	92.57	90.22	87.20	84.07	80.17	74.58	72.98
S.Ed.±	0.54	0.51	0.61	0.52	0.58	0.45	0.72	0.51
C.D. (p=0.05)	1.18	1.11	1.33	1.13	1.26	0.98	1.57	1.12

T_6 [50 per cent RDF + Neem cake (1.3 t ha^{-1})] in marigold flowers at room condition storage on 4th and 7th day of the postharvest observation period. From the data presented in Table 6, it was found that T_7 (7.32 days) showed maximum shelf life, and the minimum shelf days were observed in treatment T_6 (4.50 days) when flowers are stored at room condition.

Increased postharvest quality parameters may be due to optimum food nutrient availability from different organic and inorganic sources which might have resulted in greater development of water-conducting tissue with a high level of water retention in the cells of flowers thereby lowering the desiccation. Compared to inorganic fertilizer alone, and in combination with other organic sources (FYM, neem cake) the marigold flowers taken from plants that had received both organic (vermicompost) and inorganic fertilizer were of higher quality and lasted longer. This may be because organic manures promoted plant vegetative development, which increased the use of photosynthetic products, leading to better-quality flowers and an increase in their turgidity, which helped them stay longer after harvest. These findings corroborate with that of Mittal *et al.* (2010) along with biofertilizers (azotobacter + azospirillium + PSB) application; Patel *et al*. (2011), Vallabhbhai (2013), Priyadarshini *et al.* (2018), Choudhury *et al*. (2020) and Upadhya *et al*. (2022) in African marigold; Sharukhkhan (2019) in chrysanthemum, and Gopitha *et al*. (2021 in Nerium.

Table 6: Shelf life (days) and xanthophyll (g/kg petal meal) of African Marigold flower as influenced by integrated nutrient management.

Treatment	Shelf life (days)	Xanthophyll (g/kg petal meal)
T_1 : 100 per cent recommended dose of inorganic fertilizer (RDF)	4.73	7.33
T_2 : 75% RDF + FYM (7.5 t ha^{-1})	5.16	7.26
T_3 : 75% RDF + neem cake (0.63 t ha^{-1})	5.33	6.54
T_4 : 75% RDF + vermicompost (1.25 t ha^{-1})	6.67	7.57
T_5 : 50% RDF + FYM (15 t ha^{-1})	6.20	6.63
T_6 : 50% RDF + neem cake (1.3 t ha^{-1})	4.50	5.97
T_7 : 50% RDF + vermicompost (2.5 t ha^{-1})	7.32	7.07
S.Ed.±	0.18	0.24
C.D. (p=0.05)	0.39	0.52

Xanthophyll

From the data presented in Table 6, it was found that all the treatments differed significantly from each other in respect of their response to xanthophyll content in flowers**.** It was observed that treatment T_4 [75 per cent RDF + vermicompost (1.25 t ha^{-1})] has maximum xanthophyll content (7.57 g kg^{-1} petal meal) among all treatments and the minimum xanthophyll content (5.97 g kg^{-1} petal meal) was observed in T_6 [50 per cent RDF + neem cake (1.3 t ha^{-1})]. Vermicompost significantly impacted photosynthetic pigments and gave marigolds their highest level of chlorophyll and carotenoids. The amount of applied nutrients that are translocated to these floral components to increase flower yield and, ultimately, xanthophyll content, determines how much marigold flowering occurred. These results are also in close conformity with the finding of Naik *et al*. (2008) and Singh (2010) in Marigold.

CONCLUSION

It was concluded that the application of 50 per cent RDF + vermicompost (2.5 t ha^{-1}) (T_7) was found to be significantly superior to all the treatments in postharvest parameters *i.e.,* minimum physiological loss in weight (%), highest sensory quality, minimum spoilage (%), maximum moisture content (%) and longest shelf life (days) in room temperature. It was observed that treatment T_4 [75% RDF + vermicompost (1.25 t ha^{-1})] recorded maximum xanthophyll content (7.57 g kg^{-1}) among all treatments.

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Research Article

Evaluation of biopesticides against fall armyworm, *Spodoptera frugiperda* in maize during *kharif* season

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ABSTRACT

With the basic principle of integrated pest management (IPM) and searching for effective and sustainable alternatives for the management of *Spodoptera frugiperda,* different biopesticides were evaluated at Main Agricultural Research Station (MARS), UAS Raichur, Karnataka during *Kharif* 2019 cropping season. The experiment was laid out in a Randomized Block Design with three replications and seven treatments. Biopesticides such as *Metarhizium rileyi* @ 4 g l^{-1}, *Sf*NPV (@ 2 ml l^{-1}) and *Bacillus thuringiensis* var. *kurstaki* (@ 2 ml l^{-1}) were found to be the best treatments in reducing the larval population and percent leaf damage compared to untreated control at five and seven days after two sprays. The yield and cost economics also showed that they were economically viable biopesticides.

***Keywords*:** *Bacillus*, Biopesticides, *Metarhizium*, SfNPV, *Spodoptera.*

In recent years, India has been invaded by a new insect pest, Fall Armyworm, (FAW) *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), that has become a major threat by causing severe damage to maize. In addition to maize, it has also been reported to infest more than 300 other plant species. Among them, the most preferred host plants are maize, rice, sorghum, and sugarcane (Bueno *et al*., 2010). A total of 353 larval host plants of *S. frugiperda* were recorded belonging to 76 plant families, principally Poaceae (106), Asteraceae (31), and Fabaceae (31) (Montezano *et al*., 2019).

The fall armyworm, *S. frugiperda* is a major pest of maize (*Zea mays* L.). The developing larvae eat different parts of the host plant, depending on the stage of the crop. In maize, larger larvae can cut the base of the plant. Blossomed plants suffer more due to larvae as it feeds on reproductive parts. Young larvae skeletonize the leaf lamina in a typical 'window-pane' damage at the early whorl stage. Usually, many young larvae will be present on the same plant, but normally one or two older larvae may be found on a single plant, as others will migrate and feed on neighbouring plants. Later larval instars make larger holes, causing ragged whorl leaves, and produce sawdust-like larval droppings, while fresh feeding produces big lumps. Fall armyworms can also destroy silks and developing tassels, thereby limiting fertilization of the ear. Maize plants may have the cobs attacked by larvae boring through the kernels. Damage to cobs may lead to fungal infection and aflatoxins, and loss of grain quality (Anonymous, 2020).

It was previously restricted only to the Americas (Cruz *et al*., 1999). A severe outbreak of FAW in maize was reported from African countries such as Sao Tome, Nigeria, Benin, and Togo in 2016 (Goergen *et al*., 2016). Subsequently, the pest has spread rapidly to over 44 countries in sub-Saharan Africa causing significant damage to crops (Prasanna *et al*., 2018). Recently this dreaded pest has been reported from Karnataka in India on maize crop (Ganiger *et al*., 2018; Sharanabasappa *et al*., 2018). Similarly, the occurrence of this pest in severe form was noticed in maize-growing areas of Raichur.

The current report of *S. frugiperda* from India is alarming because of its polyphagous nature. Therefore, the recent invasion, ensuing future spread, and the possibility of the emergence of new hybrid races of *S. frugiperda* pose a significant risk to national food security. Thus, curtailing the spread and establishment of this pest in India and also the Indian subcontinent is a need of the hour. As the insect is new to the Indian geography, the goal or ambition of the present investigation was to study the bio-efficacy of biopesticides in the field for its management.

MATERIALS AND METHODS

A field experiment was conducted at Main Agricultural Research Station, Raichur to evaluate the effect of different insecticides to manage fall armyworm during *Kharif* 2019-20. The experiment was laid out in a Randomized Block Design with three replications and seven treatments. The

whole area was divided into individual treatments of 5.4 x 3.3 m^2 and seeds of hybrid maize ('Dhanvantari' during *Kharif*) was sown in the main field with a spacing of 90 × 30 cm. All the management practices except the plant protection measures against fall armyworm were adopted as per the recommended package of practices. The other insect pests encountered during the study were managed using the recommended insecticides. The treatment details are given below in Table 1.

Table 1: Treatment details of different biopesticides used for the management of fall armyworm, *Spodoptera frugiperda*.

Tr. No	Treatment details	Dosage (ml or g l^{-1})	Source of procurement
T1	*Bacillus thuringiensis* var. *kurstaki* (BtG4)	2 ml	ICAR-NBAIR, Bengaluru
T2	*Sf*NPV	2 ml	ICAR-NBAIR, Bengaluru
T3	*Metarhizium rileyi*	4 g	Biocontrol unit, Raichur
T4	*Metarhizium anisopliae* (Ma4)	4 g	ICAR-NBAIR, Bengaluru
T5	*Beauveria bassiana* (Bb5)	4 g	ICAR-NBAIR, Bengaluru
T6	*Metarhizium anisopliae* (Ma4)	2 ml	ICAR-NBAIR, Bengaluru
T7	Untreated control	-	-

Observations on fall armyworm larval population and percent leaf damage were recorded one day before and on 3, 5, 7, and 15 days after the imposition of treatments. Initiation of treatments was taken up on the appearance of fall armyworm using a manually operated knack sack sprayer twice during the experimentation. Observations were made on the number of fall armyworm larvae and percent leaf damage from five randomly tagged plants before treatment imposition. The percent leaf damage caused by larvae of fall armyworm was estimated through visual scoring in 0-9 scale as described by Davis and Williams (1992). The percent leaf damage caused by larvae of fall armyworm was calculated using the formula mentioned below.

$$\% \text{ leaf damage} = \frac{\text{Number of damaged leaves}}{\text{Total number of leaves}} \times 100$$

The data obtained in the experiments in the current investigation for parameters such as the number of fall armyworm larvae were subjected to square root transformation and percent leaf damage was subjected to arcsine transformations. Transformed values were analyzed using ANOVA for a randomized block design. Reduction in pest population and leaf area damage over untreated control was calculated by using the formula mentioned below.

$$\% \text{ reduction over control} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

RESULTS AND DISCUSSION

Number of larvae

A day before the spray, the count on the larval population of *S. frugiperda* was made. The results showed that all the treatments were non-significant and the larval population ranged from 3.07 to 3.60 larvae $plant^{-1}$. On the third day after the first spray, there was a slight increase in the larval population ranging from 3.13 to 3.60 larvae $plant^{-1}$, and found a non-significant difference among the treatments. Five days after the first spray, the population ranged from 1.00 to 3.67 larvae $plant^{-1}$. There was a significant reduction in the number of larvae in *Metarhizium rileyi* @ 4 g l^{-1} (1.00 larva $plant^{-1}$) treated plot followed by *Sf*NPV @ 2 ml l^{-1} (1.27 larvae $plant^{-1}$) and *Bacillus thuringiensis* var. *kurstaki* @ 2 ml l^{-1} (1.33 larvae $plant^{-1}$) which were significantly on par with each other. The next best treatments were *Beauveria bassiana* @ 4 g l^{-1} (1.53 larvae $plant^{-1}$), *M. anisopliae* @ 4 g l^{-1} (1.93 larvae $plant^{-1}$) and *M. anisopliae* @ 2 ml l^{-1} (2.27 larvae $plant^{-1}$). The highest number of 3.67 larvae $plant^{-1}$ was recorded in the untreated control (Table 2).

On the seventh day, there was a slight increase in larval population and the number of larvae ranged from 1.27 to 3.47 $plant^{-1}$. Fifteen days after spray, the same trend was observed as that of seven days after spray where the larvae ranged from 1.33 to 3.40 $plant^{-1}$. A more number of larvae were recorded in the untreated control (3.40 larvae $plant^{-1}$) and found inferior to all other treated plots. Among the treated plots, the maximum number of larvae was recorded in *M. anisopliae* @ 2 ml l^{-1} (2.60 larvae $plant^{-1}$) and was followed by the same insect pathogen *i.e.*, *M. anisopliae* @ 4 g l^{-1} (2.27 larvae $plant^{-1}$). The least number of larvae were recorded in plots sprayed with *M. rileyi* @ 4 g l^{-1}, *SfNPV* @ 2 ml l^{-1} and *B. thuringiensis* var. *kurstaki* @ 2 ml l^{-1} with 1.33, 1.40 and 1.47 larvae $plant^{-1}$, respectively and were statistically on par with each other (Table 2).

The biopesticides spray was repeated at fifteen days intervals. Pre-treatment count on the number of larvae revealed that all the treatments were non-significant and the range was 1.67 to 3.07 larvae $plant^{-1}$. On the third day after the second spray, the per-plant larval population ranged from 1.73 to 3.13. The larval population was minimal in the treatment sprayed with *M. rileyi* @ 4 g l^{-1}. The maximum number of larvae was recorded in untreated control and

found inferior to all other treatments. However, there was no significant difference noticed among all the treatments. Five days after the second spray, there was a significant reduction in a larval population ranging from 0.73 to 3.20 larvae plant^{-1}. The treatments sprayed with *M. rileyi* @ 4 g l^{-1} recorded 0.73 larva plant^{-1} followed by *Sf*NPV @ 2 ml l^{-1} (0.93 larva plant^{-1}) and *B. thuringiensis* var. *kurstaki* @ 2 ml l^{-1} (1.07 larvae plant^{-1}) which were found to be superior over other treatments and were significantly on par with one another. The next best treatments were *B. bassiana* @ 4 g l^{-1} (1.33 larvae plant^{-1}), *M. anisopliae* @ 4 g l^{-1} (1.53 larvae plant^{-1}) and *M. anisopliae* @ 2 ml l^{-1} (1.73 larvae plant^{-1}). However, the highest number of larvae was recorded in the untreated control (3.20 larvae plant^{-1}) (Table 2).

On the seventh day after the second spray, the population ranged from 0.80 to 3.27 larvae plant^{-1} and the same trend was observed as that of fifth day. Finally, on the fifteenth day after the second spray, all the treatments were found non-significant with a population ranging from 0.67 to 1.67 larvae plant^{-1}. The lowest population was recorded in the plot treated with *M. rileyi* @ 4 g l^{-1} (0.67 larva plant^{-1}) followed by *Sf*NPV @ 2 ml l^{-1} (0.80 larva plant^{-1}) and *B. thuringiensis* var *kurstaki* @ 2 ml l^{-1} (1.00 larva plant^{-1}). Apparently, the highest was found in the untreated control (1.67 larvae plant^{-1}) (Table 2).

The mean larval population after two sprays ranged from 1.33 to 3.17 and was lowest in *M. rileyi* @ 4 g l^{-1} (1.33 larvae plant^{-1}), *Sf*NPV @ 2 ml l^{-1} (1.48 larvae plant^{-1}) and *B. thuringiensis* var. *kurstaki* @ 2 ml l^{-1} (1.58 larvae plant^{-1}) which were significantly on par with each other. The highest percent reduction in larval population over control was noticed in treatment sprayed with *M. rileyi* @ 4 g l^{-1} (58.04%), *Sf*NPV @ 2 ml l^{-1} (53.31%) and *B. thuringiensis* var. *kurstaki* (50.16%) @ 2 ml l^{-1} followed by *B. bassiana* @ 4 g l^{-1} (41.32%), *M. anisopliae* @ 4 g l^{-1} (35.02%) and *M. anisopliae* @ 2 ml l^{-1} (25.55%) (Table 2).

The results are in compliance with the findings of Barros *et al*. (2020) who reported 74.50 to 84.00 per cent mortality when treated with different isolates of *M. rileyi*. Firake and Behere (2020) experienced 50 per cent larval mortality throughout the season due to *M. rileyi* and *Spfr*NPV indicating dominant mortality factors.

Percent leaf damage

A day before treatment imposition the leaf damage ranged from 54.64 to 60.71 per cent in different treatments and no significant difference was observed among all the treatments. On the third day after treatment imposition, a gradual increase in the leaf damage was noticed in respective treatments and the damage ranged from 55.84 to 62.96 per cent. However, no remarkable difference was observed among the treatments. Five days after the first spray, there was a significant reduction in the leaf damage which ranged from 32.83 to 65.69 per cent across the treatments. The highest

Table 2: Efficacy of different biopesticides on larval population of *S. frugiperda* in maize during *Kharif* 2019.

Tr. No.	Treatment details	Number of larvae plant^{-1}										Pooled mean	Per cent reduction over control
		First spray					Second spray						
		1 DBS	3 DAS	5 DAS	7 DAS	15 DAS	1 DBS	3 DAS	5 DAS	7 DAS	15 DAS		
T1	*Bacillus thuringiensis* var. *kurstaki* @ 2 ml l^{-1}	3.13 (2.03)	3.27 (2.06)	1.33 (1.52)^{ab}	1.33 (1.53)^{a}	1.47 (1.57)^{a}	1.8 (1.67)	2.00 (1.73)	1.07 (1.44)^{abc}	1.20 (1.48)^{ab}	1.00 (1.41)	1.58 (1.60)^{ab}	50.16
T2	*Sf*NPV @ 2 ml l^{-1}	3.33 (2.08)	3.27 (2.06)	1.27 (1.50)^{ab}	1.33 (1.53)^{a}	1.40 (1.54)^{a}	1.73 (1.65)	1.80 (1.71)	0.93 (1.41)^{ab}	1.00 (1.46)^{ab}	0.80 (1.39)	1.49 (1.58)^{a}	53.00
T3	*Metarhizium rileyi* @ 4 g l^{-1}	3.27 (2.06)	3.13 (2.03)	1.00 (1.48)^{a}	1.27 (1.51)^{a}	1.33 (1.53)^{a}	1.67 (1.63)	1.73 (1.69)	0.73 (1.36)^{a}	0.80 (1.41)^{a}	0.67 (1.34)	1.34 (1.55)^{a}	57.73
T4	*Metarhizium anisopliae* @ 4 g l^{-1}	3.07 (2.01)	3.13 (2.03)	1.93 (1.71)^{bc}	2.13 (1.77)^{bc}	2.27 (1.81)^{b}	2.53 (1.88)	2.60 (1.90)	1.53 (1.59)^{cd}	1.67 (1.63)^{c}	1.33 (1.53)	2.08 (1.75)^{cd}	34.38
T5	*Beauveria bassiana* @ 4 g l^{-1}	3.60 (2.14)	3.60 (2.14)	1.53 (1.59)^{abc}	1.67 (1.63)^{ab}	1.80 (1.67)^{ab}	2.13 (1.76)	2.20 (1.78)	1.33 (1.53)^{bcd}	1.53 (1.59)^{bc}	1.20 (1.48)	1.86 (1.68)^{bc}	41.32
T6	*Metarhizium anisopliae* @ 2 ml l^{-1}	3.40 (2.09)	3.47 (2.10)	2.27 (1.80)^{c}	2.47 (1.86)^{c}	2.60 (1.90)^{bc}	2.93 (1.98)	2.93 (1.98)	1.73 (1.65)^{d}	1.87 (1.69)^{c}	1.53 (1.59)	2.36 (1.83)^{d}	25.55
T7	Untreated control	3.60 (2.14)	3.53 (2.13)	3.67 (2.16)^{d}	3.47 (2.11)^{d}	3.40 (2.09)^{c}	3.07 (2.01)	3.13 (2.03)	3.20 (2.05)^{e}	3.27 (2.06)^{d}	1.67 (1.62)	3.17 (2.04)^{e}	0.00
	S.Em.±	0.08	0.10	0.07	0.05	0.08	0.09	0.08	0.05	0.05	0.05	0.04	-
	C.D. at 5%	NS	NS	0.22	0.17	0.24	NS	NS	0.16	0.16	NS	0.12	-

Values in parentheses are $\sqrt{x+0.5}$ transformations; DAS– Days After Spray; DBS– Days Before Spray; Means followed by the same alphabet in columns did not differ significantly (p=0.05) by DMRT

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efficacy was obtained with *M. rileyi* @ 4 g l^{-1} (32.83%), *Sf*NPV @ 2 ml l^{-1} (33.94%) and *B. thuringiensis* var. *kurstaki* @ 2 ml l^{-1} (34.64%) with non-significant difference among them followed by *B. bassiana* @ 4 g l^{-1} (42.60%), *M. anisopliae* @ 4 g l^{-1} (50.55%) and *M. anisopliae* @ 2 ml l^{-1} (54.97%). The untreated plot recorded the highest leaf damage of 65.69 per cent (Table 3).

On the seventh day, the same trend was followed as that of five days after the first spray except for the control. The leaf damage ranged from 34.57 to 70.65 per cent. At the end of the observation period *i.e.*, on the fifteenth day after the first spray 36.59 to 76.17 per cent leaf damage was noticed. The leading treatments with minimal leaf damage were *M. rileyi* @ 4 g l^{-1}, *Sf*NPV @ 2 ml l^{-1} and *B. thuringiensis* var. *kurstaki* @ 2 ml l^{-1} that experienced 36.59, 37.06 and 38.02 per cent leaf damage, respectively. The treatment with the highest leaf damage was *M. anisopliae* @ 2 ml l^{-1} (60.23%) followed by *M. anisopliae* @ 4 g l^{-1} (56.36%) and *B. bassiana* (48.44%) and were significantly superior over untreated control (76.17%) (Table 3).

The biopesticides spray was repeated at fifteen days intervals. The pre-treatment count was done before taking the second spray, the leaf damage ranged from 46.72 to 78.63 per cent and no significant difference was noticed among the treatments. Even after the third day of treatment imposition, there was a non-significant difference was observed among the treatments where the leaf damage ranged from 45.53 to 80.38 per cent. However, minimal leaf damage was recorded in the plot sprayed with *M. rileyi* @ 4 g l^{-1} (45.53%) and the maximum was in the untreated control (80.38%). At five days after the second spray, the per cent leaf damage was ranged from 36.18 to 80.44. The treatments sprayed with *M. rileyi* @ 4 g l^{-1} (36.18%) experienced significantly minimum leaf damage followed by *Sf*NPV @ 2 ml l^{-1} (38.27%) and *B. thuringiensis* var. *kurstaki* @ 2 ml l^{-1} (39.55%) which were at par with each other. The plots treated with *B. bassiana* @ 4 g l^{-1} (46.30%) and *M. anisopliae* @ 4 g l^{-1} (53.69%) were found to be better treatments whereas the *M. anisopliae* @ 2 ml l^{-1} was the least effective treatment with 60.82 per cent leaf damage. All the treatments were significantly superior over untreated control which experienced leaf damage up to 80.44 per cent (Table 3).

The same trend was leaning even on the seventh day after spray with leaf damage ranging from 39.25 to 83.78 per cent. At the end of the fifteenth day after the second spray, there was a significant reduction owing to leaf damage experienced in all the treatments with a range of 19.25 to 64.68 per cent. The treatment *M. rileyi* @ 4 g l^{-1} (19.25%) recorded the least per cent leaf damage followed by *Sf*NPV @ 2 ml l^{-1} (32.93%) and *B. thuringiensis* var. *kurstaki* @ 2 ml l^{-1} (33.59%) which were significantly on par with each other. The treatment plots sprayed with *B. bassiana* @ 4 g l^{-1}, *M. anisopliae* @ 4 g l^{-1} and *M. anisopliae* @ 2 ml l^{-1} recorded 39.08, 46.62 and 54.87 per cent leaf damage, respectively. The

Table 3: Efficacy of different biopesticides on per cent leaf damage of *S. frugiperda* in maize during *Kharif* 2019.

Tr. No	Treatment details	Per cent leaf damage plant^{-1}										Pooled mean	Per cent reduction over control
		First spray					Second spray						
		1 DBS	3 DAS	5 DAS	7 DAS	15 DAS	1 DBS	3 DAS	5 DAS	7 DAS	15 DAS		
T1	*Bacillus thuringiensis* var. *kurstaki* @ 2 ml l^{-1}	54.64 (47.74)	55.84 (48.39)	34.64 (36.01)^{a}	37.03 (37.43)^{a}	38.02 (38.04)^{a}	49.14 (44.1)	51.29 (45.72)	39.55 (38.93)^{a}	43.59 (41.30)^{a}	33.59 (35.29)^{ab}	41.70 (40.17)^{a}	42.76
T2	*Sf*NPV @ 2 ml l^{-1}	55.93 (48.47)	57.25 (49.23)	33.94 (35.48)^{a}	35.36 (36.45)^{a}	37.06 (37.75)^{a}	48.23 (43.96)	50.89 (45.50)	38.27 (38.19)^{a}	42.93 (40.89)^{a}	32.93 (34.92)^{ab}	41.15 (39.84)^{a}	43.51
T3	*Metarhizium rileyi* @ 4 g l^{-1}	58.24 (49.76)	58.53 (49.94)	32.83 (34.89)^{a}	34.57 (35.95)^{a}	36.59 (37.17)^{a}	46.72 (43.67)	45.53 (43.13)	36.18 (36.94)^{a}	39.25 (38.71)^{a}	19.25 (30.72)^{a}	37.47 (38.84)^{a}	48.57
T4	*Metarhizium anisopliae* @ 4 g l^{-1}	59.23 (50.40)	61.69 (51.76)	50.55 (45.30)^{b}	53.78 (47.17)^{b}	56.36 (48.64)^{b}	60.25 (45.2)	61.45 (51.66)	53.69 (47.11)^{ab}	56.62 (48.88)^{ab}	46.62 (43.03)^{bc}	55.10 (47.92)^{c}	24.37
T5	*Beauveria bassiana* @ 4 g l^{-1}	60.71 (51.32)	62.96 (52.54)	42.60 (40.72)^{ab}	46.20 (42.79)^{ab}	48.44 (44.08)^{ab}	53.74 (44.45)	55.72 (48.28)	46.30 (42.81)^{ab}	49.08 (44.44)^{ab}	39.08 (38.59)^{abc}	48.80 (44.30)^{b}	33.01
T6	*Metarhizium anisopliae* @ 2 ml l^{-1}	60.55 (51.10)	62.77 (52.50)	54.97 (47.84)^{bc}	57.27 (49.18)^{b}	60.23 (50.91)^{b}	65.2 (43.37)	66.86 (54.89)	60.82 (51.31)^{b}	63.20 (52.76)^{b}	54.87 (47.86)^{cd}	60.12 (50.83)^{c}	17.47
T7	Untreated control	58.31 (49.87)	61.04 (51.38)	65.69 (54.36)^{c}	70.65 (57.39)^{c}	76.17 (61.21)^{c}	78.63 (58.02)	80.38 (63.21)	80.44 (65.10)^{c}	83.78 (67.29)^{c}	64.68 (53.58)^{d}	72.85 (58.60)^{d}	0.00
	S.Em.±	4.29	2.80	2.81	2.46	2.48	3.57	3.85	3.60	3.70	3.29	1.14	-
	C.D. at 5%	NS	NS	8.65	7.58	7.63	NS	NS	11.08	11.40	10.13	3.50	-

Values in parentheses are arcsine transformations; DAS– Days After Spray; DBS– Days Before Spray; Means followed by the same alphabet in columns do not differ significantly (p=0.05) by DMRT

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Table 4: Cost economics of *S. frugiperda* management through biopesticides in maize during *Kharif* 2019.

Tr. No	Treatment details	Yield (q ha^{-1})	Percent yield grain over control	Crop production cost (₹ ha^{-1})	Crop protection cost (₹ ha^{-1})	Total cost of cultivation (₹ ha^{-1})	Gross returns (₹ ha^{-1})	Net returns (₹ ha^{-1})	BC Ratio
T1	*Bacillus thuringiensis* var. *kurstaki* @ 2 ml l^{-1}	35.64^{abc}	63.26	33043.50	5750.00	38793.50	71816.38	33022.88	1.85
T2	*Sf*NPV @ 2 ml l^{-1}	38.75^{ab}	77.51	33043.50	4750.00	37793.50	78083.19	40289.69	2.07
T3	*Metarhizium rileyi* @ 4 g l^{-1}	39.74^{a}	82.04	33043.50	1350.00	34393.50	80078.09	45684.59	2.33
T4	*Metarhizium anisopliae* @ 4 g l^{-1}	29.57^{cd}	35.46	33043.50	1350.00	34393.50	59585.03	25191.53	1.73
T5	*Beauveria bassiana* @ 4 g l^{-1}	32.76^{bc}	50.07	33043.50	1350.00	34393.50	66013.04	31619.54	1.92
T6	*Metarhizium anisopliae* @ 2 ml l^{-1}	27.83^{cd}	27.49	33043.50	1050.00	34093.50	56078.84	21985.34	1.64
T7	Untreated control	21.83^{d}	0.00	33043.50	-	33043.50	43988.54	10945.04	1.33
	S.Em.±	2.90	-	-	-	-	-	-	-
	C.D. at 5%	8.70	-	-	-	-	-	-	-

Means followed by the same alphabet in columns do not differ significantly (p=0.05) by DMRT

untreated control plot recorded 64.68 per cent leaf damage which was significantly higher compared to the remaining treatments except for *M. anisopliae* @ 2 ml l^{-1} (Table 3).

The mean of two sprays on leaf damage ranged from 37.84 to 72.85 per cent. The results indicated that *M. rileyi* @ 4 g l^{-1} (37.84%), *Sf*NPV @ 2 ml l^{-1} (41.08%) and *B. thuringiensis* var. *kurstaki* @ 2 ml l^{-1} (41.69%) were the most significant treatments over untreated control and were statistically on par with each other. The per cent reduction in leaf damage over control was determined and the results revealed that per cent reduction was tremendously high in *M. rileyi* @ 4 g l^{-1} (48.06%), *Sf*NPV @ 2 ml l^{-1} (43.61%) and *B. thuringiensis* var. *kurstaki* @ 2 ml l^{-1} (42.77%) and were significantly on par with each other. The per cent reduction in leaf damage was moderate in treatments *viz*., *B. bassiana* @ 4 g l^{-1} (33.01%), *M. anisopliae* @ 4 g l^{-1} (24.37%) and *M. anisopliae* @ 2 ml l^{-1} (17.47%) (Table 3). The present findings are supported by Mallapur *et al*. (2018) who evaluated *M. rileyi* on a large scale and achieved a 66.84 to 73.05 per cent reduction in leaf injury. Similarly, Dhobi *et al*. (2020) reported the minimum per cent plant damage in treatment sprayed with *M. rileyi* and *B. thuringiensis* var. *kurstaki*.

Grain yield

The yield ranged from 21.83 to 39.74 q ha^{-1}, the highest was recorded in *M. rileyi* @ 4 g l^{-1} (39.74 q ha^{-1}) succeeded by *Sf*NPV @ 2 ml l^{-1} (38.75 q ha^{-1}) and *B. thuringiensis* var *kurstaki* @ 2 ml l^{-1} (35.64 q ha^{-1}). The yield was moderate in treatments *viz*., *B. bassiana* @ 4 g l^{-1} (32.76 q ha^{-1}), *M. anisopliae* @ 4 g l^{-1} (29.57 q ha^{-1}) and *M. anisopliae* @ 2 ml l^{-1} (27.83 q ha^{-1}). The lowest was in the untreated control that recorded 21.83 q ha^{-1} grain yield. The per cent yield gain over control was more in *M. rileyi* @ 4 g l^{-1} (82.04%), *Sf*NPV @ 2 ml l^{-1} (77.51%) and *B. thuringiensis* var *kurstaki* @ 2 ml l^{-1} (63.26%) followed by *B. bassiana* @ 4 g l^{-1} (50.07%), *M. anisopliae* @ 4 g l^{-1} (35.46%) and *M. anisopliae* @ 2 ml l^{-1} (27.49%) (Table 4).

The results on the cost and returns of fall armyworm management revealed that the highest net returns were recorded in the plot sprayed with *M. rileyi* @ 4 g l^{-1} (₹ 45684.59 ha^{-1}), *Sf*NPV @ 2 ml l^{-1} (₹ 40289.69 ha^{-1}) and *B. thuringiensis* var *kurstaki* @ 2 ml l^{-1} (₹ 33022.88 ha^{-1}). *B. bassiana* @ 4 g l^{-1}, *M. anisopliae* @ 4 g l^{-1} and *M. anisopliae* @ 2 ml l^{-1} recorded ₹ 31619.54, 25191.54 and 21985.34 ha^{-1}, respectively. The net returns were least in the untreated control (₹ 10945.04 ha^{-1}). The benefit-cost ratio was highest in *M. rileyi* @ 4 g l^{-1} (2.33), *Sf*NPV @ 2 ml l^{-1} (2.07) and *B. thuringiensis* var *kurstaki* @ 2 ml l^{-1} (1.85). *B. bassiana* @ 4.00 g l^{-1}, *M. anisopliae* @ 4 g l^{-1} and *M. anisopliae* @ 2 ml l^{-1} recorded 1.92, 1.73 and 1.64, respectively. The untreated control recorded the least benefit-cost ratio of 1.33 (Table 4). The present findings are in line with the previous investigator who recorded the highest grain yield of 29.57 and 29.32 q ha^{-1} in treatments sprayed with *Metarhizium rileyi* and *Bacillus thuringiensis* var. *kurstaki*, respectively (Dhobi *et al*., 2020).

CONCLUSION

It is concluded that the use of *Metarrhizium releyi* (@ 4 g l^{-1}), *Sf*NPV (@ 2 ml l^{-1}) and *Bacillus thuringiensis* var. *kurstaki* (@ 2 ml l^{-1}) are the best treatments in radueing the percent larval population per cat leaf damage and also the best in yield and cost economics.

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Research Article

Evaluation of efficacy of various plant powders against *Sitotroga cerealella* (Olivier) on stored wheat

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ABSTRACT

The investigation was conducted to evaluate the efficacy of different plant powders against *Sitotroga cerealella* (Olivier) on stored wheat in the laboratory of the Department of Entomology, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India, during 2021-22 at 30.25±0.25°C temperature and 75.00±5.06 per cent relative humidity. All the powder treatments of *Azadirachta indica* A. Juss leaves, *Pongamia pinnata* L. leaves, *Ocimum basilicum* L. leaves, *Piper nigrum* L. seeds, *A. indica* kernel, *Allium sativum* L. cloves, *Allium cepa* L. bulbs and *Curcuma longa* L. rhizome, were superior to the untreated check but the efficacy level varied. The *A. indica* kernel powder @ 5 g kg^{-1} (91.67%) was significantly superior over the rest of the treatments with the highest mortality which was at par with *A. indica* kernel powder @ 2.5 g kg^{-1} (88.30%). Whereas, the significantly lowest mortality was recorded in treatment *O. basilicum* powder @ 5 g kg^{-1} (58.33%) and *O. basilicum* powder @ 10 g kg^{-1} (40.00%).

***Keywords*:** Efficacy, plant powders, *S. cerealella*, stored wheat

Wheat, *Triticum aestivum* L. is one of the most important cereal crops widely cultivated in India. During 2020-21, wheat production reached 108.75 million tonnes with an average national productivity of 3424 kg ha^{-1} (Anonymous, 2021). Food grain losses due to insects alone contributed to a $ 300 million monetary loss (Ghizdavu and Diec, 1994; Mohan and Kavitharaghavan, 2008). It is assessed that 5-10 per cent loss occur during storage of the wheat in the world due to insect pest (Pimentel, 1991). Among the different insect pests attacking wheat grains, *Sitotroga cerealella* **(**Olivier) is the most destructive internal pest of wheat. It also affects paddy, maize, sorghum, oat, and barley (Champ and Dyte, 1977; Bhardwaj *et al*., 1977; Dakshinamurthy and Regupathy, 1988; Ramashrit and Mishra, 1989; Dhaliwal *et al.*, 1989; Visalakshi *et al*., 2005). The infestation of *S. cerealella* in wheat starts from the field as females lay their eggs singly or in groups on grains (Ghizdavu and Deac, 1994). A field infestation of 0.26 per cent at harvest could lead to a total loss in storage (Simmons and Ellington, 1927). Nowadays, this pest is managed by the use of various kinds of pesticides (Rai and Croal, 1973; Bitran *et al.*, 1976; Kao and Tzeng, 1992; Weaving, 1981; Hashem *et al*., 2012; Hung *et al*., 1990; Fields and White, 2002). However, frequent use of pesticides has led to harmful consequences for the environment and human beings (Tavares *et al*., 2013). Therefore, it is necessary to develop effective, safe, and economical alternative means for controlling this pest under storage conditions. Among the available options, some plant powders have been found to have the potential for replacing the chemicals in short and medium-small-scale storage (Ashamo and Akinnawonu, 2012; Amin, 2013; Ileke, 2014; Patil, 2014; Akter and Amin, 2017). Therefore, the present investigation was carried out to study the efficacy of different plant powders against *S.cerealella*.

MATERIALS AND METHODS

The investigation was carried out in the laboratory of the Department of Entomology, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India, during 2021-22 at room temperature, 30.25±0.25°C and relative humidity, 75.00±5.06 per cent.

Insect Culture

Sitotroga cerealella adults used for this study were collected from the infested stored wheat. The moths were then reared on clean, uninfested wheat grains in 2-L jars capped with a piece of muslin cloth which allowed ventilation but prevented the entry or exit of moths, other insects, as well as foreign materials. The jars were kept in insect cages, and the culture was maintained by replacing the infested grains with fresh, uninfested grains. The environment for cultured insects and experimentation was

maintained at 30.25±0.25°C temperature and 75.00±5.06 per cent relative humidity following the method developed by Ashamo and Akinnawonu (2012).

Collection and preparation of plant powders

The leaves of *A. indica*, *P. pinnata* and *O. basilicum* free from any other pesticides application were collected from the College farm and rhizomes of *C. longa*, seeds of *P. nigrum* and *A. indica*, bulbs of *A. cepa* and cloves of *A. sativum*, were purchased from the local market. The plant materials were washed thoroughly with tap water and cut into small pieces and dried for a week under shade. Then, they were ground into fine powder with the help of an electric mixer grinder. The treatments used are shown in Table 1.

The wheat seeds used for the experiments were disinfected from insects by putting them in a deep freezer at –10 °C for 72 hrs and later air-dried to prevent mouldiness. The moisture content was equilibrated to 13.0 per cent.

Table 1: Description of treatments and their parts used against *Sitotroga cereallela* on stored wheat grains.

Treatments	Common name	Plant part used	Dosage/kg of seed
Azadirachta indica A. Juss	Neem	Leaves (50 mesh sieved)	5 and 10 g
Pongamia pinnata L.	Karanj	Leaves (50 mesh sieved)	5 and 10 g
Ocimum basilicum L.	Tulsi	Leaves (50 mesh sieved	5 and 10 g
Piper nigrum L.	Black pepper	Seed (50 mesh sieved)	2 and 3 g
Azadirachta indica	Neem	Kernel (50 mesh sieved)	2.5 and 5 g
Allium sativum L.	Garlic	Clove (50 mesh sieved)	2 and 4 g
Allium cepa L.	Onion	Bulb (50 mesh sieved)	2 and 4 g
Curcuma longa L.	Turmeric	Rhizome (50 mesh sieved	2 and 4 g
-	Control (Untreated)	-	-

Effect of plant powders on mortality and adult emergence of *Sitotroga cerealella*

Fifty-gram sterilized grains of wheat were treated by mixing with the plant powders thoroughly at the above-mentioned dosage with 5 ml of acetone in such a way as to get a uniform coating and placed in transparent plastic boxes (7 cm × 6 cm). Ten pairs of 0-24 hours adults were randomly picked and released into each container. Then, the containers were tightly closed with the perforated lid and stored on a raised platform in the laboratory at room temperature under hygienic conditions. The experiment was conducted in a completely randomized design (CRD) with three repetitions. The corrected mortality, reduction in adult emergence, and weight loss were calculated as follows:

Corrected mortality

The mortality counts were recorded at 24 hrs intervals up to 3 days. Besides the dead, the moribund insect was also considered as dead. The percentage mortality of adults was calculated by using a formula suggested by Abbott (1925),

$$P = \frac{P' - C}{100 - C} \times 100$$

Where,

P = Percent corrected mortality

P′ = Percent mortality in the treatment

C = Percent mortality in the control

The zero and cent percent values were replaced by using the formula (1/4 n) x 100 and (1 -1/4n) x 100, respectively, suggested by Bartlett (1947), Where, n = number of adults per treatment.

Adult emergence

The mean number of adults that emerged from each replication was recorded after 60 days of treatment.

Weight loss

Emerged adults were removed from the sample after 60 days and the damaged and healthy grains were weighed separately with electric balance. The weight loss was calculated as per the methodology suggested by Adams and Schulten (1978),

$$\text{Weight loss} = \frac{(UNd) - (DNu)}{U\,(Nd + Nu)} \times 100$$

Where,

U = Weight of undamaged grains

N_u = Number of undamaged grains

D = Weight of damaged grains

N_d = number of damaged grains

RESULTS AND DISCUSSION

Adult mortality (%)

After 24 hrs of the treatment, *A. indica* kernel powder @ 5 g kg^{-1} was found to be significantly superior over the rest of the treatments by recording 68.33 per cent adult mortality of *S. cerealella* adults, which was at par with the *A. indica* kernel powder @ 2.5 g and *A. indica* leaves powder @ 10 g kg^{-1} with 65.00 and 63.33 per cent mortality, respectively. The next

effective treatment was *A. indica* leaves powder @ 5 g kg^{-1} which recorded 61.67 per cent mortality. Further, the treatment of *O. basilicum* powder @ 5 g kg^{-1} was the least effective with 40.00 per cent mortality. A similar trend was observed after 48 hrs of the treatment with the highest 78.33 per cent and the lowest 46.67 per cent mortality of adults observed in *A. indica* kernel powder @ 5 g kg^{-1} and *O. basilicum* powder @ 5g kg^{-1} of grain, respectively. The treatments of *A. indica* kernel powder @ 2.5 g kg^{-1} and *A. indica* leaves powder @ 10 g kg^{-1} were statistically at par with the *A. indica* kernel powder @ 5 g kg^{-1} having 76.67 and 73.33 per cent adult mortality. After 72 hrs of treatment, the lowest (58.33%) mortality of adults of *S. cerealella* was found in *O. basilicum* powder @ 5 g kg^{-1} whereas, significantly the highest *i.e.*, 91.67 per cent adult mortality was recorded in *A. indica* kernel powder @ 5g kg^{-1}. The treatment of *A. indica* kernel powder @ 2.5 g kg^{-1} was statistically at par with the *A. indica* kernel powder @ 5 g kg^{-1} having 88.30 per cent adult mortality. The treatment of *A. indica* leaves powder @10 g kg^{-1} was found to be the next effective treatment having 86.67 per cent mortality. Further, the least effective treatment was *O. basilicum* powder @ 5 g kg^{-1} with 58.33 per cent mortality. The descending chronological order of effectiveness of treatments based on per cent mortality of adults of *S. cerealella* was *A. indica* kernel powder @ 5 g kg^{-1} (91.67 % mortality) > *A. indica* kernel powder @ 2.5 g kg^{-1} (88.30%) > *A. indica* leaves powder @ 10 g kg^{-1} (86.67 %) > *A. indica* leaves powder @ 5 g kg^{-1} (85.00%) > *A. sativum* bulb powder @ 4 g kg^{-1} (81.67%) > *A. sativum* bulb powder @ 2 g kg^{-1} (78.33%) > *P. pinnata* leaves powder @ 10 g kg^{-1} (76.67%) > *P. pinnata* leaves powder @ 5 g kg^{-1} (75.00%) > *A. cepa* powder @ 4 g kg^{-1} (73. 33 %) > *A. cepa* powder @ 2 g kg^{-1} (71.67%) > *P. nigrum* powder @ 3 g kg^{-1} (70.00%) > *P. nigrum* @ 2 g kg^{-1} (68 .33%) > *C. longa* powder @ 4 g kg^{-1} (62.10%) > *C. longa* powder @ 2 g kg^{-1} > *O. basilicum* powder @ 10 g kg^{-1} (60.00%) > *O. basilicum* powder @ 5 g kg^{-1} (58.33%) (Table 2).

Ashamo and Akinnawonu (2012) observed 100 per cent mortality rate within seven days when *Aristolochia ringens* was applied @ 0.4 and 0.6 g 20 g^{-1} of paddy grains. Ashamo and Akinnawonu (2012) obtained cent percent mortality within seven days when *A. ringens* were applied at 0.4 and 0.6 g 20 g^{-1} of padd y grains. Gemechu *et al.* (2013) reported that among all botanicals *viz., A. indica, Cymbopogon citrates* D.C, *Tagetes erecta* L., *A. sativum, Brassica carinata* A. Barun, *Gossypium hirsutum* L., *Chenopodium ambrosioides* L. and *Maesa lanceolata* Forssk. against *S. cerealella,* the maximum mortality recorded was found 61. 10 per cent by *A. indica* bark powder and *C. ambrosioides* leaves in maize grain. Amin (2013) observed that tobacco leaves dust @7 g kg^{-1} seed and *A. indica* oil 1.0 ml tissue $paper^{-1}$ was more effective than all other treatments against the mortality of *S. cerealella*. Ileke (2014) found that the powder of *Capsicum frutescens* L. had the highest mortality rate (100%) after two days of

Table 2: Efficacy of plant powders against *Sitotroga cerealella* on stored wheat under laboratory conditions.

Treatments	Dosage (g kg^{-1} $grain^{-1}$)	Corrected adult mortality of *Sitotroga cerealella* at different periods of storage interval (%)		
		24 hr	48 hr	72 hr
Azadirachta indica Leaves	5 g	61.67 (51.73) ^{bcd}	71.67 (57.89) ^{bcd}	85.00 (67.19) ^{bc}
	10 g	63.33 (52.72) ^{abc}	73.33 (58.91) ^{abc}	86.67 (68.64) ^{b}
Pongamia pinnata Leaves	5 g	55.00 (47.86) $^{e-h}$	65.00 (53.74) ^{efg}	75.00 (60.05) ^{efg}
	10 g	56.67 (48.82) $^{d-g}$	66.67 (54.73) ^{def}	76.67 (61.12) $^{e-h}$
Ocimum basilicum Leaves	5 g	40.00 (39.20) ^{m}	46.67 (43.06) ^{j}	58.33 (49.78) ^{k}
	10 g	41.67 (40.18) ^{lm}	48.33 (44.03) ^{j}	60.00 (50.77) ^{k}
Piper nigrum Seeds	2 g	44.33 (44.03) ^{ijk}	56.67 (48.82) ^{hi}	68.33 (55.80) ^{hij}
	3 g	50.00 (44.98) ^{hij}	58.33 (49.78) ^{ghi}	70.0 (56.77) ^{ghi}
Azadirachta indica kernel	2.5 g	65.00 (53.74) ^{ab}	76.67 (61.12) ^{ab}	88.30 (70.09) ^{ab}
	5 g	68.33 (55.75) ^{a}	78.33 (62.26) ^{a}	91.67 (73.37) ^{a}
Allium sativum Clove	2 g	58.33 (49.78) $^{c-f}$	68.33 (55.83) $^{c-f}$	78.33 (62.26) ^{de}
	4 g	60.00 (50.77) $^{b-e}$	70.00 (56.82) ^{cde}	81.67 (64.67) ^{cd}
Allium cepa Bulb	2 g	51.67 (45.94) $^{g-j}$	61.67 (51.73) ^{fgh}	71.67 (57.84) $^{f-i}$
	4 g	53.33 (46.89) $^{f-i}$	63.33 (52.72) $^{e-h}$	73.33 (58.91) $^{e-h}$
Curcuma longa Rhizome	2 g	43.33 (41.14) ^{klm}	51.67 (45.94) ^{ij}	63.33 (52.72) ^{jk}
	4 g	46.67 (43.07) ^{jkl}	53.33 (46.89) ^{ij}	66.67 (54.73) ^{ij}
S.Em.±		1.31	1.44	1.32
C.D. at 5%		3.77	4.14	3.80
C.V. (%)		4.79	4.72	3.80

*Figures in parentheses are arc sine transformed values while those outsides are original values; In vertical columns, means followed by the same letter do not differ significantly by DMRT (P=0.05)

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application at all tested concentrations and there was no adult emergence in samples treated with *C. frutescens* and *Anacardium occidentale* L. Patil (2014) reported that the treatment of *A. indica* @ 5 g 100 g grains^{-1} was superior against *T. castaneum* among all treatment recording the highest mortality per cent. Akter and Amin (2017) revealed that among all powders *viz.,* Biskataly, *P. pinnata*, tobacco, and arjun, tobacco leaf powder showed the highest toxic effect against *S. cerealella* in case of the per cent mortality, followed by *A. indica* leaf powder, whereas Biskataly and *P. pinnata* leaf powder showed the lowest effect.

Adult emergence

Data recorded on the number of adults that emerged from wheat grains treated with different plant powder after two months of storage are presented in Table 3, which reveals that the treatment of *A. indica* kernel powder @ 5 g kg^{-1} was significantly superior with the lowest number of adult emergence (14.67) which was statistically at par with *A. indica* leaves powder @ 10 g kg^{-1} (17. 33). The next above treatments were *A. indica* kernel powder @ 2.5 g kg^{-1} (19.33) and *A. indica* leaves powder @ 5 g kg^{-1} (23.00). The highest adult emergence was noticed in control (99.33) followed by *O. basilicum* powder @ 5 g kg^{-1} (71.00) which was at par with *O. basilicum* powder @ 10 g kg^{-1} (66.33). The order of effectiveness of treatments on the basis of the number of adults emerged was *A. indica* kernel powder @ 5 g kg^{-1} (14.67) > *A. indica* leaves powder @ 10 g kg^{-1} (17.33) > *A. indica* kernel powder @ 2.5 g kg^{-1} (19.33) > *A. indica* leaves powder @ 5 g kg^{-1} (23.00) > *A. sativum* bulb powder @ 4 g kg^{-1} (25) > *P. pinnata* leaves powder @ 10 g kg^{-1} (29.67) > *A. sativum* bulb powder @ 2 g kg^{-1} (30.33) > *P. pinnata* leaves powder @ 5 g kg^{-1} (35.33) > *A. cepa* powder @ 4 g kg^{-1} (40.67) > *A. cepa* powder @ 2 g kg^{-1} (46.00) > *P. nigrum* powder @ 3 g kg^{-1} (47.67) > *P. nigrum* powder @ 2 g kg^{-1} (52.33) > *C. longa* powder @ 4 g kg^{-1} (57.00) > *C. longa* powder @ 2 g kg^{-1} (61.67) > *O. basilicum* powder @ 10 g kg^{-1} (66.33) > *O. basilicum* powder @ 5 g kg^{-1} (71.00).

Earlier, Patel (1993) observed that the plant powders *viz*., bel, custard apple, nirghundi, and neem were found effective against *S. cerealella* and the number of adults emerged from different treatments was significantly lower than the control. Prakash and Rao (2006) reported that leaf powders of *Hyptis suaveolens* L. and *Mikania cordata* Kunth @ 0.5 and 1.0 per cent w/w showed a signiûcant reduction in population level for 180 days, also the population of *S. cerealella* was controlled cent percent with the use of dried fruit of Raja mircha and Eucalyptus leaf powders. Amin (2013) observed that tobacco leaves dust @ 7 g kg^{-1} seed and *A. indica* oil 1.0 ml tissue paper^{-1} were more effective than all other treatments against the emergence of *S. cerealella* adult. Patil (2014) reported that the treatment of *A. indica* @ 5 g 100 g^{-1} grains was superior against *T. castaneum* among all treatments recording the lowest number of adults emerged. Akter and Amin (2017) revealed that among all powders *viz.,* Biskataly, *P. pinnata*, tobacco, and arjun, tobacco leaf powder showed the highest toxic effect against *S. cerealella* in the case of the adult emergence followed by *A. indica* leaf powder, whereas Biskataly and *P. pinnata* leaf powder showed the lowest effect.

Weight loss (%)

The data on percent weight loss are presented in Table 3. It is very clear from the data that the *A. indica* kernel powder @ 5 g kg^{-1} was significantly superior over all the treatments with the lowest weight loss (22.24%) which was statistically at par with *A. indica* kernel powder @ 2.5 g kg^{-1} (24.60%). The next effective treatment was *A. indica* leaves powder @ 10 g kg^{-1} (26.77%) which was at par with *A. indica* leaves powder @ 5 g kg^{-1} (29.22%). The highest per cent weight loss was recorded in control (60.52%) followed by *O. basilicum* powder @ 5 g kg^{-1} (49.53%). All the treatments were found to be

Table 3. Effect of plant powders on adult emergence of *Sitotroga cerealella* and weight loss (%) due to it.

Treatments	Dosage (g kg grains^{-1})	*Adult emergence after 2 months of storage	**Average weight loss after 2 months of storage (%)
Azadirachta indica	5 g	23.00 (4.84) ^{cd}	29.22 (32.71) ^{bc}
Leaves	10 g	17.33 (4.21) ^{ab}	26.77 (31.15) ^{b}
Pongamia pinnata	5 g	35.33) (5.98) ^{gh}	36.20 (36.97) ^{d}
Leaves	10 g	29.67) (5.49) ^{ef}	34.73 (36.09) ^{cd}
Ocimum basilicum	5 g	71.00) (8.46) ^{no}	49.53 (44.71) ^{l}
Leaves	10 g	66.33) (8.17) ^{mn}	47.76 (43.70) ^{k}
Piper nigrum Seeds	2 g	52.33) (7.26) ^{jk}	41.99 (40.37) ^{h}
	3 g	47.67) (6.94) ^{jk}	41.16 (39.89) ^{g}
Azadirachta indica	2.5 g	19.33) (4.45) ^{bc}	24.60 (29.72) ^{a}
kernel	5 g	14.67) (3.89) ^{a}	22.24 (28.13) ^{a}
Allium sativum Clove	2 g	30.33) (5.55) ^{fg}	32.95 (35.01) ^{c}
	4 g	25.00) (5.05) ^{de}	31.72 (34.26) ^{c}
Allium cepa Bulb	2 g	46.00) (6.82) ^{ij}	39.63 (39.00) ^{f}
	4 g	40.67) (6.41) ^{hi}	38.59 (38.39) ^{e}
Curcuma longa Rhizome	2 g	61.67) (7.88) ^{lm}	45.82 (42.58) ^{j}
	4 g	57.00) (7.58) ^{kl}	44.65 (41.91) ^{i}
Control	-	93.33) (9.69) ^{p}	60.52 (51.08) ^{m}
S.Em.±		0.15	0.69
C.D. at 5%		0.45	1.98
C.V. (%)		4.26	3.14

*Figures in parentheses are square root transformed values while those outsides are original values; **Figures in parentheses are arc sine transformed values while those outside are original values; In vertical columns, means followed by the same letter do not differ significantly by DMRT (P=0.05).

effective as compared to control. The order of effectiveness of different treatments on the basis of the number of adults emerged was *A. indica* kernel powder @ 5 g kg^{-1} (22.24%) > *A. indica* kernel powder @ 2.5 g kg^{-1} (24.60%)>*A. indica* leaves powder @10 g kg^{-1} (26.77%) > *A. indica* leaves powder @ 5 g kg^{-1} (29.22%) > *A. sativum* bulb powder @ 4 g kg^{-1} (31.72%) > *A. sativum* bulb powder @ 2 g kg^{-1} (32.95%) > *P. pinnata* leaves powder @10 g kg^{-1} (34.73%)> *P. pinnata* leaves powder @ 5 g kg^{-1} (36.20%) > *A. cepa* powder @ 4 g kg^{-1} (38.59%) > *A. cepa* powder @ 2 g kg^{-1} (3.63%)> *P. nigrum* powder @ 3 g kg^{-1} (41.16%) > *P. nigrum* @ 2 g kg^{-1} (41.99%) > *C. longa* powder @ 4 g kg^{-1} (44.65%) > *C. longa* powder @ 2 g kg^{-1} (45.82) > *O. basilicum* powder @ 10 g kg^{-1} (47.76%) > *O. basilicum* powder @ 5 g kg^{-1} (49.53%). Ali *et al*. (2009) revealed that *A. indica* leaf powder and sand had some efficacy in protecting the wheat against insect pests but their effect was not satisfactory. Patil (2014) reported that the treatment of *A. indica* @ 5 g 100 g^{-1} grains was superior against *T. castaneum* among all treatments recording the lowest weight loss.

CONCLUSION

Among the plant powders to be tested against *S. cerealella, O. basilicum* powder @ 5 g kg^{-1} was the least effective causing the least mortality of adults, the highest adult emergence, and the highest weight loss whereas, *A. indica* kernel powder @ 5 g kg^{-1} was the most effective causing the highest mortality of adults, least adult emergence and the least weight loss.

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Research Article

Enhancement of bio-efficacy of insecticides on spray tank mixing with Wetcit surfactant on chilli crop

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ABSTRACT

The compatibility and effectiveness of Wetcit surfactant used as a spray tank mix with insecticide formulations against mites [*Polyphagotarsonemus latus* (Banks)] on the Chilli crop was evaluated. The treatments were taken as propargite 57 per cent EC @ 1500 ml ha^{-1}, emamectin benzoate 5 per cent SG @ 200 g ha^{-1} and buprofezin 25 per cent SC @ 300 ml ha^{-1} alone, and as a tank mix with wetcit @ 500 ml ha^{-1} and also with their lower dose rate 1350 ml ha^{-1}, 180 g ha^{-1} and 270 ml ha^{-1}, respectively. Wetcit alone @ 500 ml ha^{-1} and untreated control (water alone) treatments were also taken for comparison. The mite population recorded at 5, 10 and 15 days after each spray showed that Wetcit used with insecticidal formulations resulted in low mite population than sole insecticidal treatments (without mixing with surfactant). Thus, wetcit @ 500 ml ha^{-1} used with propargite 57 per cent EC @ 1350 and 1500 ml ha^{-1}, emamectin benzoate 5 per cent SG @ 180 and 200 g ha^{-1} and buprofezin 25 per cent SC @ 270 and 300 ml ha^{-1} resulted to be effective with low mite population at each observation time and increased chilli fruit yield. No phytotoxicity to chilli crop was observed at each observation time. Thus, Wetcit surfactant was compatible with tested insecticide formulations and enhanced their effectiveness against mites in chilli crop.

Keywords: Wetcit, insecticides, chilli, surfactant, mite

India is one of the world's largest producers and exporters of chilli (*Capsicum annuum* L.). The contribution of our country to global chilli production is high but the productivity of chilli is only 1500 kg ha^{-1} which may increase to a benchmark level of 5000 kg ha^{-1} to compete in the international market (Reddy, 2010). The production and productivity of this crop are hampered due to many constraints *viz*., the high cost of hybrid seeds and labour wages, pest incidence, heavy price fluctuation for the produce, *etc*. Amongst the insect pests that infest chilli at different crop developmental phases are sucking insects like mites [*Polyphagotarsonemus latus* (Banks)], thrips (*Scirtothrips dorsalis* Hood) and aphids [*Myzuspersicae* (Sulzer) and *Aphis gossypii* Glover] which suck the cell sap and results into poor plant growth and development. The yield loss due to chilli thrips and mites has been estimated to the tune of 50 per cent (Ahmed *et al*., 1987; Kandasamy *et al*., 1990). Due to heavy crop damage and to avoid further economic loss, farmers have to resort to repeated applications of pesticide formulations. The number of insecticidal sprays has increased gradually over the years, to cope with the yield losses. Hence, it is making the cultivation of chilli crop highly risky and non-profitable to the growers. In view of curtailing the multiple sprays of chemical pesticides for public concern, agrochemical industries are fairly working to generate new products and formulations for better efficacy, environment friendly and cost-effective to the users.

Under the present scenario, the emphasis is being laid on application technique improvement to make a formulated product more effective. According to Holden (1992) the formulations that are cleaner and safer for the users, have minimal impact on the environment and can be applied at the lowest dose rate. The active ingredient transport limiting barrier restricts the performance of foliar-applied agrochemical molecules such as pesticides and plant growth regulators (Petracek *et al*., 1998; Schreiber, 2006). As such insecticides applied on the target surface often show poor efficacy due to low absorption of the product by the leaves through the cuticle. Due to the availability of low active ingredients to the target insect pests the chances of resistance development also increase (Tominack, 2000). In most cases, the active ingredient is formulated through complex processes that facilitate mixing, dilution, application and stability of the product. To enhance the efficacy of herbicides, the addition of surfactants within the formulation or to use as a tank mixed preparations are very common. The use of surfactants as an adjuvant in insecticide formulations increases cuticular penetration of active ingredient and as a result, enhance the biological performance of the product.

The use of surfactant as a spray tank mix with insecticidal formulations has not been widely explored. The present field experiment was carried out to study the bio-efficacy and phytotoxicity of Wetcit, a surfactant when tank mixed with different insecticides to use on Chilli crop.

MATERIALS AND METHODS

The experiment was conducted in a simple randomized block design with three replications using Chilli var. PusaJwala in a plot of 5 m x 6 m size at the IPFT Experimental Research Farm, Gurugram during *Rabi* 2021-22. The chilli seedlings (one-month-old) were transplanted in the third week of December 2021. All the recommended agronomical practices were followed to raise a good crop. The treatments Propargite 57 per cent EC @ 1350 and 1500 ml ha^{-1}, Emamectin benzoate 5 per cent SG @ 180 and 200 g ha^{-1} and Buprofezin 25 per cent SC@ 270 and 300 ml ha^{-1} on spray tank mixing with Wetcit surfactant @ 500 ml ha^{-1} were taken for the bio-efficacy and phytotoxicity evaluation against mites (*P. latus*) in chilli crop. The treatments Propargite 57 per cent EC @ 1500 ml ha^{-1}, Emamectin benzoate 5 per cent SG @ 200 g ha^{-1} and Buprofezin 25 per cent SC @ 300 ml ha^{-1} without spray tank mixing of surfactant and Wetcit surfactant alone @ 500 ml ha^{-1} as standard treatments were also taken for comparison. As per protocol provided by M/s Dhanuka Agritech Limited, Gurugram the spraying of treatments started with the appearance of mite infestation on the crop. Repeat spray was done 15 days after the first spray for the effective control of mite infestation. The observations on the mite population were recorded with the help of 10 X hand lens before the first spray and at 5, 10 and 15 days after each spray on five upper leaves of 10 random plants per plot. Based on the pest population, percent reduction in mite population over control was calculated. The plot-wise yield (kg $plot^{-1}$) was recorded at each harvest and pooled yield was expressed as q ha^{-1}. The pest population and mean yield data recorded in different treatment plots were subjected to statistical analysis.

The phytotoxicity evaluation of all the treatments applied to chilli crop was also conducted. The observations were recorded on the phytotoxicity symptoms *viz*. Leaf Injury on tips/ surface, wilting, vein clearing, necrosis, epinasty and hyponasty on randomly selected 5 plants per plot after 1, 3, 5, 7, 10 and 14 days of each spray. Phytotoxicity symptoms were observed and graded on the visual rating based on a 0-10 scale, where 0=0%, 1=1-10, 2= 11-20, 3= 21-30, 4= 31-40, 5= 41-50, 6= 51-60, 7= 61-70, 8= 71-80, 9= 81-90,10= 91-100. The activities of natural enemies in chilli crop ecosystem were also observed periodically during the experimental period.

RESULTS AND DISCUSSION

The mite population recorded before the first spray and at 5, 10 and 15 days after each spray has been presented in Table 1. The results show that the mite population before the first spray in different plots ranged from 25.83 to 29.97 per plant and there was no significant difference in population. The use of Wetcit with insecticide formulations resulted in a low mite population as compared to insecticide treatments alone. Thus, wetcit @ 500 ml ha^{-1} used with emamectin benzoate 5 per cent SG @ 200 g ha^{-1} resulted to be most effective with 4.20 and 2.87 mites per plant 10 days after the first and second spray and it was followed by wetcit @ 500 ml ha^{-1} used with emamectin benzoate 5 per cent SG @ 180 g ha^{-1} with 4.73 and 3.37 mites $plant^{-1}$. Wetcit @ 500 ml ha^{-1} with propargite 57 per cent EC @ 1500 ml ha^{-1} was the next best treatment with 5.60 and 3.73 mites $plant^{-1}$, whereas the treatment wetcit @ 500 ml ha^{-1} used with buprofezin 25 per cent SC @ 300 ml ha^{-1} was comparatively less effective with 8.50 and 6.70 mites $plant^{-1}$. The treatments wetcit @ 500 ml ha^{-1} used with emamectin benzoate 5 per cent SG @ 200 g ha^{-1} and with emamectin benzoate 5 per cent SG @ 180 g ha^{-1} were significantly at par at each observation time. Similarly, wetcit @ 500 ml ha^{-1} used with propergite 57 per cent EC @ 1500 ml ha^{-1} and with propargite 57 per cent EC @ 1350 ml ha^{-1} were significantly at par.

The mite control in emamectin benzoate 5 per cent SG @ 200 g ha^{-1}, propargite 57 per cent EC @ 1500 ml ha^{-1} and buprofezin 25 per cent SC @ 300 ml ha^{-1} without using wetcit were comparatively low. Wetcit treatment alone was not effective to control mites in chilli crop. The results thus showed that Wetcit was compatible with tested insecticide formulations to enhance their effectiveness. The per cent reduction in mite population over control (Fig. 1) also showed that treatments wetcit @ 500 ml ha^{-1} with emamectin benzoate 5 per cent SG @ 200 g ha^{-1} and wetcit 500 ml ha^{-1} with propargite 57 per cent EC @ 1500 ml ha^{-1} effectively controlled mites.

The chilli yield was recorded at different time intervals and the cumulative yield in terms of q ha^{-1} has been presented in Table 1. The data show that the highest yield was recorded in the treatments of wetcit @ 500 ml ha^{-1} used with emamectin benzoate 5 per cent SG @ 200 g ha^{-1} (16.04 q ha^{-1}) followed by the treatment wetcit @ 500 ml ha^{-1} used with propargite 57 per cent EC @ 1500 ml ha^{-1} (15.79 q ha^{-1}). The yield in emamectin benzoate 5 per cent SG @ 200 g ha^{-1}, propargite 57 per cent EC @ 1500 ml ha^{-1} and buprofezin 25 per cent SC @ 300 ml ha^{-1} alone were 13.76, 13.27 and 10.92 q ha^{-1}, respectively. The yield in wetcit @ 500 ml ha^{-1} treatment alone was marginally higher at 9.54 q ha^{-1} as compared to the

Table 1: Effectiveness of Wetcit mixed with different insecticides for mite control in Chilli crop.

Tr. No.	Treatment	Dose (g or ml ha^{-1})	Mean mite population plant^{-1}						Yield (q ha^{-1})
			Before spray	Days after first spray			Days after second spray		
				5	10	15	5	10	15	
T1	Propargite 57% EC	1500	28.23 (5.30)	10.80 (3.28)	7.43 (2.72)	12.70 (3.56)	7.37 (2.70)	5.57 (2.35)	3.90 (1.97)	13.27
T2	Wetcit + Propargite 57% EC	500+1500	25.97 (5.09)	7.37 (2.71)	5.60 (2.36)	8.03 (2.83)	5.40 (2.31)	3.73 (1.93)	2.63 (1.62)	15.79
T3	Wetcit + Propargite 57% EC	500+1350	27.70 (5.25)	8.63 (2.93)	6.07 (2.45)	9.17 (3.02)	5.97 (2.44)	4.57 (2.13)	3.43 (1.85)	14.36
T4	Emamectin Benzoate 5% SG	200	29.07 (5.39)	7.63 (2.76)	6.43 (2.53)	8.37 (2.89)	6.43 (2.53)	4.73 (2.17)	3.07 (1.74)	13.76
T5	Wetcit + Emamectin Benzoate 5% SG	500 + 200	28.60 (5.34)	5.37 (2.31)	4.20 (2.05)	6.07 (2.46)	3.77 (1.94)	2.87 (1.69)	1.40 (1.18)	16.04
T6	Wetcit + Emamectin Benzoate 5% SG	500+180	26.40 (5.13)	6.17 (2.48)	4.73 (2.16)	7.27 (2.69)	4.27 (2.05)	3.37 (1.83)	1.97 (1.40)	15.16
T7	Buprofezin 25% SC	300	27.83 (5.27)	13.30 (3.65)	10.73 (3.27)	14.87 (3.85)	10.73 (3.27)	8.60 (2.93)	6.03 (2.45)	10.92
T8	Wetcit + Buprofezin 25% SC	500+300	29.97 (5.46)	10.93 (3.30)	8.50 (2.90)	12.77 (3.57)	8.03 (2.83)	6.03 (2.45)	4.57 (2.14)	12.86
T9	Wetcit + Buprofezin 25% SC	500+270	25.83 (5.08)	12.73 (3.57)	9.30 (3.04)	12.23 (3.50)	9.80 (3.12)	7.40 (2.72)	5.50 (2.34)	11.73
T10	Wetcit	500	29.40 (5.41)	29.87 (5.46)	31.73 (5.63)	33.27 (5.77)	28.90 (5.37)	26.40 (5.14)	22.43 (4.74)	9.54
T11	Untreated Control	-	27.67 (5.25)	32.87 (5.73)	34.17 (5.84)	34.47 (5.87)	32.67 (5.72)	31.23 (5.59)	24.67 (4.97)	8.96
	S.Em.±		0.21	0.10	0.14	0.09	0.12	0.10	0.09	0.35
	C.D. at 5%		NS	0.30	0.42	0.26	0.36	0.28	0.26	1.05

NS-Non-significant; Figures in parentheses are square root transformed values

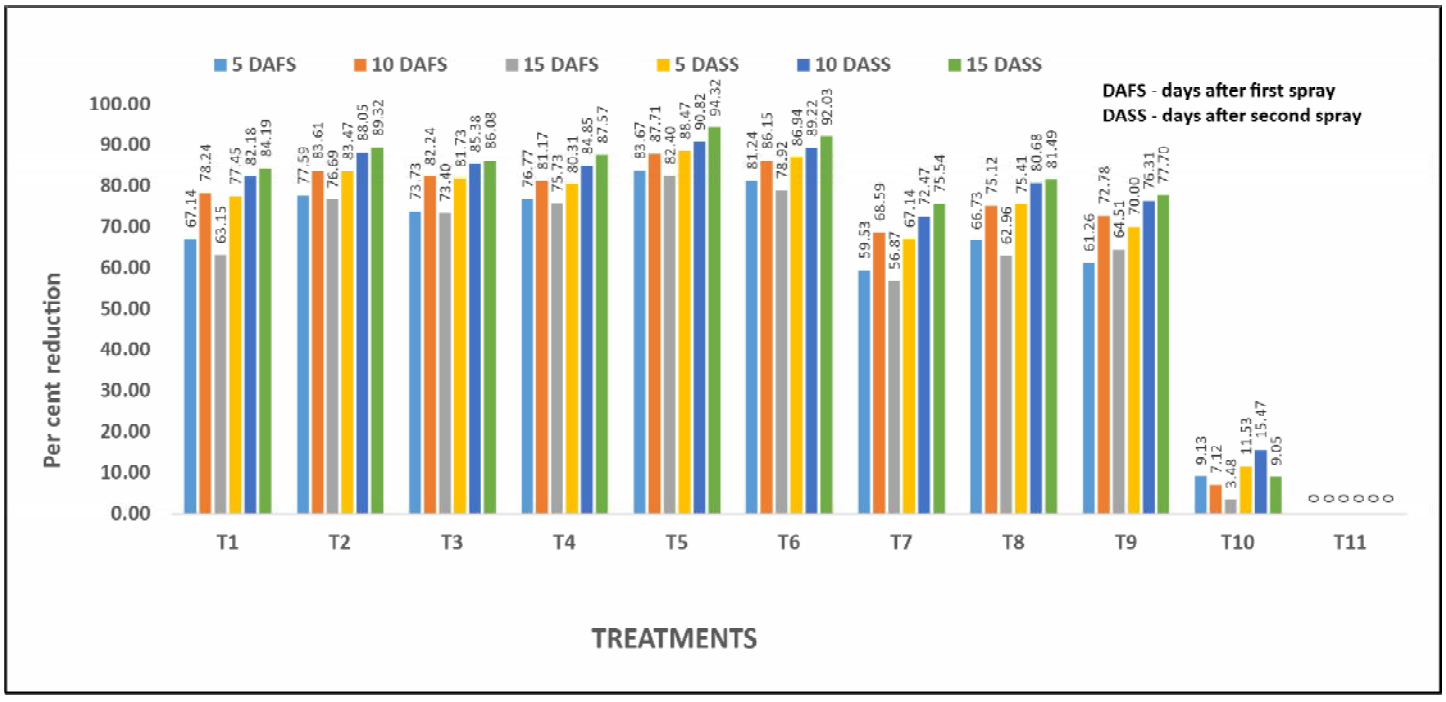

Fig. 1. Percent reduction in mite population over control in different treatments.

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untreated control at 8.96 q ha^{-1}. It showed that the use of wetcit with insecticide formulations increased the yield as compared to without using wetcit. The activities of prevailing natural enemies (coccinellids and spiders) in all the experimental plots were normal. Hence, there was no adverse impact of the treatments applied on the chilli crop on the natural enemies.

The observation for the phytotoxicity symptoms *viz*, leaf injury on tips/ surface, wilting, vein clearing, necrosis, epinasty and hyponasty recorded visually at different time intervals after each spray showed no phytotoxicity to chilli crop due to the application of wetcit @ 500 ml ha^{-1} and 1000 ml ha^{-1} alone and @ 500 ml ha^{-1} in combination with insecticidal formulations. Hence, all the treatments were non-phytotoxic to the chilli crop.

The use of surfactants with herbicide formulations as spray tank mix is in practice to control target weeds with enhanced effectiveness. The commonly used herbicides like chlorimuron ethyl, imazethapyr, metsulfuron methyl, sulfosulfuron, tembotrione, *etc*. have been approved under the Insecticides Act of 1968 (Anonymous, 2022). However, the efficiency evaluation of surfactants with insecticide formulations as spray tank mix has rarely been reported. The present investigation extends the possibilities of a compatible handshake of wetcit surfactant with insecticidal formulations as a spray tank mix.

CONCLUSION

The study conclude that the use of Wetcit surfactant with insecticidal formulations *viz*., proparigite 57 per cent EC @ 1350 and 1500 ml ha^{-1}, emamectin benzoate 5 per cent SG @ 180 and 200 g ha^{-1} and buprofezin 25 per cent SC @ 270 and 300 ml k^{-1} do protect chilli crop from mites infertation besides leaving no phytotoxicity to the crop could effectively managemite infestation as als as brogh in an appreciate increase in chith fruit field.

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Research Article

Bio-efficacy of biopesticides and novel insecticides against *Leucinodes orbonalis* Guenee on brinjal under mid-hill conditions of Himachal Pradesh

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ABSTRACT

Studies on the bio-efficacy of biopesticides and novel insecticides against shoot and fruit borer, *Leucinodes orbonalis* Guenee were undertaken at CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur during 2015 and 2016. The results on bioefficacy showed that chlorantraniliprole resulted in minimum shoot infestation followed by cyantraniliprole, lambda-cyhalothrin, thiacloprid, indoxacarb, azadirachtin, melia and eupatorium during 2015, whereas, during 2016 the shoot infestation was minimum in cyantraniliprole followed by chlorantraniliprole, thiacloprid, lambda-cyhalothrin, indoxacarb, azadirachtin, melia and eupatorium. Fruit infestation was found minimum in chlorantraniliprole followed by cyantraniliprole, thiacloprid, lambda-cyhalothrin, indoxacarb, azadirachtin, melia and eupatorium during both years. Among different treatments highest fruit yield was recorded in chlorantraniliprole (75.25 q ha^{-1}). Lambda-cyhalothrin resulted in the highest IOIR (15.87) in the present study as it is a cheaper insecticide as compared to others. Hence, it is concluded that all the insecticides evaluated were effective *against L. orbonalis* and can be recommended for its control.

***Keywords*:** Bioefficacy, biopesticides, brinjal, incremental output-input ratio (IOIR), *Leucinodes orbonalis.*

Brinjal, *Solanum melongena* Linnaeus is an important and commonly grown vegetable crop, which has high nutritive value. It is native to India (Zeven and Zhukousky, 1975) and is grown throughout the country (Hazra *et al*., 2012). In India, it occupies an area of 0.76 million hectares with an annual production of 12.69 million tonnes (Anonymous, 2022a). It is an important cash crop of Himachal Pradesh especially in low and mid-hills and covers an area of about 1.01 thousand hectares with an annual production of 20.92 thousand tonnes (Anonymous, 2022b).

Among the major constraints in the economic cultivation of brinjal, pest infestation causes heavy losses. Brinjal is attacked by a number of insect pests starting from seedling stage to senescence, the major ones include shoot and fruit borer (*Leucinodes orbonalis* Guenee), jassid (*Amrasca biguttula biguttula* Ishida), aphid (*Aphis gossypii* (Glover)) and white fly (*Bemisia tabaci* (Gennadius)) (Tamoghna *et al*., 2014). Among all *L. orbonalis* is an internal feeder and is the major problem in the cultivation of brinjal. The damage starts soon after the transplanting of seedlings and continues till the last harvest of the fruits. In India, it has been categorized as the most destructive and serious pest causing huge losses in brinjal (Patil, 1990). In Himachal Pradesh, 27 different insect species (belonging to 8 orders) and one mite species (*Tetranychus* sp.; Acarina: Tetranychidae), have been reported to be associated with brinjal crop, among which *L. orbonalis* was found major pest (Patial and Mehta, 2008). Yield losses, reaching as high as 85-90 per cent have been reported by various workers (Misra, 2008 and Chakraborti and Sarkar, 2011).

For the management of this notorious pest, farmers have been using conventional insecticides. The indiscriminate use of these insecticides has led to the development of resistance, resurgence, and environmental contamination (Rahman and Rahman, 2009; Tripura *et al*., 2017). There is an urgent need to look for alternate and safer insecticides such as biopesticides and novel insecticides. Biopesticides are environment-friendly and easily biodegradable, whereas novel insecticides are target specific. Hence, in the present studies biopesticides and novel insecticides were evaluated for the management of *L. orbonalis*.

MATERIALS AND METHODS

The present investigation was conducted at CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India

(latitude 32.6°N, longitude 76.3°E, altitude 1290.8 m above sea level). The trials were laid out during the years 2015 and 2016 in a randomized block design having a plot size of 10.5 m^2 at the experimental farm of the Department of Entomology. The seedlings were transplanted on 24th April 2015 and on 11th April 2016. Brinjal variety, Arka Keshav was raised as per recommended package of practices (Anonymous, 2013), except insect-pest management practices. Bio-efficacy of three biopesticides- azadirachtin (neem baan 1500 ppm) @ 0.00045 per cent, eupatorium 10 EC @ 0.03 per cent, Melia 10 EC @ 0.03 per cent, and five insecticides- chlorantraniliprole (Coragen 18.5% SC) @ 0.007 per cent, cyantraniliprole (cyzapyr 10.26% OD) @ 0.012 per cent, thiacloprid (Alanto 21.7% SC) @ 0.03 per cent, indoxacarb (King Doxa 14.5% SC) @ 0.01 per cent, lambda-cyhalothrin (Far-Rata 5% EC) @ 0.004 per cent was determined during both the years and each treatment was replicated thrice. In an untreated check, water was sprayed. Two sprays of each insecticide were applied with the help of a knapsack sprayer at fortnightly intervals starting from the appearance of the pest. Observations on the percent shoot infestation from five randomly selected plants and percent fruit infestation by *L. orbonalis* on a weight basis and number basis per plot were recorded one day before spray and 7 and 14 days after each spray.

$$\text{Percent shoot/fruitin festation} = \frac{\text{Number of infested shoots/fruits}}{\text{Total number of shoots/fruits}} \times 100$$

$$\text{Percent fruit damage (weight basis)} = \frac{\text{Weight of damaged fruits}}{\text{Total weight of fruits}} \times 100$$

Data obtained were subjected to analysis of variance (ANOVA) after the transformation of data through CPCS-I software.

RESULTS AND DISCUSSION

Shoot infestation

Data presented in Table 1 reveals that during 2015, one day before spray, shoot infestation in different treatments varied from 1.22 to 3.67 per cent, the variation being non-significant. After two consecutive spray applications at an interval of 15 days, shoot infestation varied from 0.00 to 6.92 per cent at different observations. It was evident that insecticidal treatments and the number of spray applications influenced the shoot infestation significantly. All the treatments evaluated resulted in significantly lower infestation as compared to the untreated check. Among different treatments, chlorantraniliprole 18.5 per cent SC @ 0.007 per cent resulted in significantly least mean shoot infestation being at par with cyantraniliprole 10.26 per cent OD @ 0.012 per cent which was in turn at par with lambda-cyhalothrin 5 per cent EC @ 0.004 per cent. It was followed by thiacloprid 21.7 per cent SC @ 0.03 per cent and indoxacarb 14.5 per cent SC @ 0.01 per cent and both were found to be at par with each other. The biopesticide formulations resulted in significantly higher infestation levels as compared to insecticidal treatments. Azadirachtin 1500 ppm @ 0.00045 per cent proved significantly superior to Melia 10 EC @ 0.03 per cent, and Eupatorium 10 EC @ 0.03 per cent. Observations recorded during 2016 revealed that the shoot infestation on one day before spray varied from 2.39 to 4.57 per cent (Table 2) but the variation was found to be non-significant. After the application of different treatments, the shoot infestation varied from 0.00 to 6.45 per cent at different observation intervals. All the treatments were found to be significantly superior over the untreated check. The lowest percent mean shoot infestation among different

Table 1: Effect of different biopesticides and novel insecticides on shoot infestation by *Leucinodes orbonalis* during 2015.

Treatment	Conc. (%)	Percent shoot infestation at indicated days					Mean
		1DBS*	First spray		Second spray		
			7DAS	14DAS	7DAS	14DAS	
Azadirachtin 1500 ppm	0.00045	2.53	3.36 (2.08)	4.66 (2.37)	1.74 (1.65)	1.00 (1.40)	2.69 (1.87)
Chlorantraniliprole 18.5% SC	0.007	2.78	0.00 (1.02)	0.85 (1.35)	0.00 (1.02)	0.39 (1.18)	0.31 (1.14)
Cyantraniliprole 10.26% OD	0.012	2.89	0.61 (1.24)	0.99 (1.39)	0.42 (1.18)	0.00 (1.02)	0.50 (1.21)
Eupatorium 10% EC	0.03	1.56	4.19 (2.27)	6.32 (2.70)	3.52 (2.12)	2.68 (1.91)	4.18 (2.25)
Indoxacarb 14.5% EC	0.01	1.22	2.24 (1.71)	3.08 (2.02)	1.28 (1.47)	1.62 (1.62)	2.05 (1.71)
Melia 10% EC	0.03	2.68	4.02 (2.21)	5.52 (2.55)	2.79 (1.95)	2.36 (1.80)	3.67 (2.13)
Thiacloprid 21.7% SC	0.03	3.67	2.07 (1.75)	2.62 (1.90)	0.56 (1.23)	0.44 (1.19)	1.42 (1.52)
Lambda-cyhalothrin 5% EC	0.004	2.38	1.78 (1.61)	1.03 (1.41)	1.22 (1.46)	0.61 (1.24)	1.16 (1.43)
Untreated check	-	2.18	6.38 (2.72)	6.92 (2.81)	4.44 (2.33)	4.51 (2.34)	5.56 (2.55)
Mean			2.74 (1.95)	3.55 (2.01)	1.77 (1.72)	1.51 (1.79)	

Figures in parentheses are square root transformed values, 1DBS=One day before spray; * Non-significant; DAS=Days after spray
CD (P= 0.05); Treatments (A) = 0.23, No. of applications (B) = 0.11, Days after spray (C) = NS
Bio-efficacy of three biopesticides- azadirachtin 1500 ppm @ 0.00045%, Eupatorium 10 EC @ 0.03%, Melia 10 EC @ 0.03%, and five insecticides- chlorantraniliprole 18.5% SC @ 0.007%, cyantraniliprole 10.26% OD @ 0.012%, thiacloprid 21.7% SC @ 0.03%, indoxacarb 14.5% SC @ 0.01%, lambda-cyhalothrin 5% EC @ 0.004% was determined during both the years and each treatment was replicated thrice.

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treatments evaluated was found in cyantraniliprole 10.26 per cent OD @ 0.012 per cent which was followed by chlorantraniliprole 18.5 per cent SC @ 0.007 per cent both being statistically at par with each other in reducing shoot infestation. Further, it was followed by thiacloprid 21.7 per cent SC @ 0.03 per cent, lambda-cyhalothrin 5 per cent EC @ 0.004 per cent, and indoxacarb 14.5 per cent SC @ 0.01 per cent. The highest mean shoot infestation was recorded in the Eupatorium 10 EC @ 0.03 per cent which was at par with Melia 10 EC @ 0.03 per cent. Azadirachtin 1500 ppm @ 0.00045 per cent was found to be superior among biopesticides.

Fruit infestation (Number basis)

The perusal of data presented in Table 3 reveals that one day before spray percent fruit infestation during 2015 varied from 3.35 to 5.97 per cent and the variation was non-significant. All the treatments were superior in reducing fruit infestation over the untreated check. After spray application, fruit infestation varied from 5.33 to 62.68 per cent at different observations. It was evident that insecticidal treatments and the number of spray applications influenced fruit infestation significantly. The variation in infestation levels recorded at different intervals after foliar applications were also significant. The interaction effect between treatments,

Table 2: Effect of different biopesticides and novel insecticides on shoot infestation by *Leucinodes orbonalis* during 2016.

Treatment	Conc. (%)	Per cent shoot infestation at indicated days					Mean
		1DBS*	First spray		Second spray		
			7DAS	14DAS	7DAS	14DAS	
Azadirachtin 1500 ppm	0.00045	4.57	3.48 (2.09)	3.60 (2.12)	2.74 (1.92)	3.45 (2.10)	3.32 (2.05)
Chlorantraniliprole 18.5% SC	0.007	3.92	0.61 (1.24)	1.50 (1.54)	1.12 (1.43)	0.47 (1.20)	0.93 (1.35)
Cyantraniliprole 10.26% OD	0.012	2.95	0.74 (1.28)	0.56 (1.23)	0.48 (1.20)	0.00 (1.02)	0.45 (1.18)
Eupatorium 10% EC	0.03	3.73	4.47 (2.33)	4.00 (2.23)	3.25 (2.03)	3.13 (2.03)	3.71 (2.16)
Indoxacarb 14.5% EC	0.01	2.73	3.12 (2.02)	3.50 (2.11)	2.46 (1.85)	2.78 (1.85)	2.97 (1.96)
Melia 10% EC	0.03	2.39	4.00 (2.23)	5.31 (2.50)	2.07 (1.73)	2.67 (1.90)	3.51 (2.09)
Thiacloprid 21.7% SC	0.03	3.23	2.55 (1.88)	2.45 (1.86)	1.05 (1.41)	1.43 (1.52)	1.87 (1.66)
Lambda-cyhalothrin 5% EC	0.004	2.90	1.72 (1.59)	3.21 (2.05)	2.01 (1.74)	3.21 (2.04)	2.54 (1.86)
Untreated check	-	2.78	5.11 (2.45)	6.45 (2.72)	5.49 (2.54)	5.82 (2.58)	5.72 (2.57)
Mean			2.87 (1.90)	3.40 (1.97)	2.30 (1.82)	2.55 (1.93)	

Figures in parentheses are square root transformed values, 1DBS=One day before spray; *Non-significant; DAS=Days after spray
CD (P= 0.05); Treatments (A) = 0.27, No. of applications (B) = 0.13, Days after spray (C) = NS

Table 3: Effect of different biopesticides and novel insecticides on fruit infestation (number basis) by *Leucinodes orbonalis* during 2015.

Treatment	Conc. (%)	Per cent fruit infestation at indicated days					Mean
		1DBS*	First spray		Second spray		
			7DAS	14DAS	7DAS	14DAS	
Azadirachtin 1500 ppm	0.00045	4.70	15.47 (23.13)	30.34 (33.41)	22.69 (28.43)	34.67 (36.05)	25.79 (30.26)
Chlorantraniliprole 18.5% SC	0.007	3.40	5.33 (13.31)	18.96 (25.78)	6.56 (14.83)	15.69 (23.31)	11.67 (19.31)
Cyantraniliprole 10.26% OD	0.012	5.23	6.26 (14.47)	19.83 (26.43)	6.64 (14.91)	16.17 (23.66)	12.21 (19.87)
Eupatorium 10% EC	0.03	5.97	17.52 (24.72)	34.45 (35.92)	29.59 (32.93)	39.38 (38.86)	30.24 (33.11)
Indoxacarb 14.5% EC	0.01	5.68	11.16 (19.50)	24.47 (29.64)	13.46 (21.51)	22.75 (28.47)	17.96 (24.78)
Melia 10% EC	0.03	4.43	16.80 (24.16)	32.38 (34.67)	27.06 (31.33)	36.53 (37.17)	28.19 (31.83)
Thiacloprid 21.7% SC	0.03	4.62	9.41 (17.82)	21.41 (27.55)	12.68 (20.84)	19.98 (26.53)	15.87 (23.19)
Lambda-cyhalothrin 5% EC	0.004	4.42	10.83 (19.20)	22.13 (28.04)	13.17 (21.25)	20.30 (26.75)	16.61 (23.81)
Untreated check	-	3.35	23.93 (29.27)	45.37 (42.32)	54.74 (47.70)	62.68 (52.33)	46.68 (42.90)
Mean			12.97 (21.11)	27.71 (29.27)	20.73 (23.30)	29.80 (32.05)	

Figures in parentheses are arc sine transformed values, 1DBS=One day before spray; *Non-significant; DAS=Days after spray
CD (P= 0.05)
Treatments (A) = 0.82; No. of applications (B) = 0.39 ; Days after spray (C) = 0.39; Treatments x No. of applications (A x B) = 1.17
Treatments x Days after spray (A x C) = 1.17
No. of applications x Days after spray (B x C) = 0.55
Treatments x No. of applications x Days after spray (A x B x C) = 1.65

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number of spray applications, and days after spray was significant. During 2016, the fruit infestation recorded one day before spray was found to vary from 4.32 to 6.06 per cent (Table 4). The variation before the spray was non-significant. After the spray application, fruit infestation was found to vary significantly from 5.90 to 56.13 per cent at different observation intervals. All the treatments were significantly superior in reducing fruit infestation over the untreated check. The interaction effect between different treatments and the number of spray applications on fruit infestation was found significant. Significant difference was also observed between both the spray applications and observation periods. During both years, fruit infestation was found minimum in chlorantraniliprole 18.5 per cent SC @ 0.007 per cent followed by cyantraniliprole 10.26 per cent OD @ 0.012 per cent, thiacloprid 21.7 per cent SC @ 0.03 per cent, lambda-cyhalothrin 5 per cent EC @ 0.004 per cent, indoxacarb 14.5 per cent SC @ 0.01 per cent, azadirachtin 1500 ppm @ 0.00045 per cent, melia 10 EC @ 0.03 per cent and eupatorium 10 EC @ 0.03 per cent.

Fruit infestation (Weight basis)

The perusal of data presented in Table 5 reveals that during 2015 the fruit infestation on one day before spray varied from 3.38 to 5.48 per cent and the variation was non-significant. The fruit infestation on a weight basis in different treatments, after the spray application varied from 5.88 to 54.93 per cent at different observation intervals. The evaluated treatments indicated a significantly low percent infestation over the untreated check. Among different treatments, chlorantraniliprole 18.5 per cent SC @ 0.007 per cent was found most effective being at par to cyantraniliprole 10.26 per cent OD @ 0.012 per cent. They were followed by thiacloprid 21.7 per cent SC @ 0.03 per cent and lambda-cyhalothrin 5 per cent EC @ 0.004 per cent both being at par with each other. Biopesticides were found less effective as compared to insecticides. Azadirachtin 1500 ppm @ 0.00045 per cent was found to be statistically superior over melia 10 EC @ 0.03 per cent and eupatorium 10 EC @ 0.03 per cent resulting in minimum fruit infestation. It was evident that treatments and the number of sprays significantly influenced the infestation level. The variation in infestation levels recorded at different intervals after foliar applications were also significant. However, the interaction effect between treatments, number of spray applications, and days after the spray was non-significant. During 2016, the fruit infestation recorded one day before the spray varied from 3.84 to 5.36 per cent (Table 6) and the variation was non-significant. The percent fruit infestation after two consecutive spray applications varied from 4.20 to 57.80 per cent at different observation intervals. All the treatments indicated a significantly low percent of infestation over the untreated check. The interaction effect between different treatments, number of spray applications, and observation periods (days after spray) were non-significant. However, treatments and the number of spray applications significantly influenced

Table 4: Effect of different biopesticides and novel insecticides on fruit infestation (number basis) by *Leucinodes orbonalis* during 2016.

Treatment	Conc. (%)	Per cent fruit infestation at indicated days					Mean
		1DBS*	First spray		Second spray		
			7DAS	14DAS	7DAS	14DAS	
Azadirachtin 1500 ppm	0.00045	4.54	15.82 (23.41)	28.55 (32.28)	24.46 (29.63)	30.33 (33.40)	24.79 (29.68)
Chlorantraniliprole 18.5% SC	0.007	4.89	5.90 (13.95)	14.43 (22.30)	7.60 (15.98)	13.43 (21.45)	10.34 (18.42)
Cyantraniliprole 10.26% OD	0.012	4.53	6.04 (14.21)	15.42 (23.09)	7.72 (16.12)	13.65 (21.66)	10.71 (18.77)
Eupatorium 10% EC	0.03	6.06	19.71 (26.34)	31.72 (34.25)	28.30 (32.12)	34.68 (36.05)	28.60 (32.19)
Indoxacarb 14.5% EC	0.01	5.50	12.67 (20.84)	22.18 (28.07)	15.87 (23.46)	19.27 (26.02)	17.50 (24.60)
Melia 10% EC	0.03	5.12	18.11 (25.17)	30.11 (33.26)	27.03 (31.31)	33.65 (35.43)	27.22 (31.29)
Thiacloprid 21.7% SC	0.03	4.49	11.28 (19.61)	20.78 (27.09)	13.24 (21.29)	18.27 (25.26)	15.89 (23.31)
Lambda-cyhalothrin 5% EC	0.004	4.32	11.61 (19.91)	21.62 (27.68)	13.53 (21.56)	18.52 (25.47)	16.32 (23.66)
Untreated check	-	5.95	24.75 (29.83)	44.71 (41.94)	53.34 (46.90)	56.13 (48.50)	44.73 (41.79)
Mean			13.99 (21.96)	25.50 (28.42)	21.23 (23.86)	26.44 (30.18)	

Figures in parentheses are arc sine transformed values, 1DBS=One day before spray; *Non-significant; DAS=Days after spray
CD (P= 0.05)
Treatments (A) = 0.95; No. of applications (B) = 0.45; Days after spray (C) = 0.45
Treatments x No. of applications (A x B) = 1.34
Treatments x Days after spray (A x C) = NS
No. of applications x Days after spray (B x C) = 0.63
Treatments x No. of applications x Days after spray (A x B x C) = 1.90

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Table 5: Effect of different biopesticides and novel insecticides on fruit infestation (weight basis) by *Leucinodes orbonalis* during 2015.

Treatment	Conc. (%)	Percent fruit infestation at indicated days					Mean
		1DBS*	First spray		Second spray		
			7DAS	14DAS	7DAS	14DAS	
Azadirachtin 1500 ppm	0.00045	4.34	14.22 (22.14)	26.45 (30.94)	22.50 (28.31)	27.33 (31.49)	22.63 (28.22)
Chlorantraniliprole 18.5% SC	0.007	5.48	5.88 (14.00)	14.90 (22.67)	7.45 (15.83)	12.23 (20.43)	10.12 (18.23)
Cyantraniliprole 10.26% OD	0.012	3.38	6.10 (14.29)	15.08 (22.82)	7.97 (16.37)	12.27 (20.49)	10.36 (18.49)
Eupatorium 10% EC	0.03	4.46	16.78 (24.17)	31.54 (34.13)	28.54 (32.27)	33.74 (35.48)	27.65 (31.52)
Indoxacarb 14.5% EC	0.01	3.76	11.44 (19.76)	20.18 (26.68)	14.89 (22.68)	20.64 (27.00)	16.79 (24.03)
Melia 10% EC	0.03	4.15	16.20 (23.72)	29.51 (32.88)	25.11 (30.06)	31.64 (34.21)	25.62 (30.22)
Thiacloprid 21.7% SC	0.03	5.29	10.47 (18.86)	19.05 (25.86)	13.95 (21.92)	18.38 (25.33)	15.46 (22.99)
Lambda-cyhalothrin 5% EC	0.004	4.46	10.66 (19.05)	19.25 (26.01)	13.64 (21.65)	18.71 (25.62)	15.57 (23.08)
Untreated check	-	3.69	23.89 (29.25)	41.01 (39.81)	49.52 (44.71)	54.93 (47.81)	42.34 (40.39)
Mean			12.85 (21.01)	24.11 (27.87)	20.40 (23.28)	25.54 (29.43)	

Figures in parentheses are arc sine transformed values, 1DBS=One day before spray; *Non-significant; DAS=Days after spray
CD (P= 0.05)
Treatments (A) = 0.82; No. of applications (B) = 0.39; Days after spray (C) = 0.39
Treatments x No. of applications (A x B) = 1.16
Treatments x Days after spray (A x C) = NS
No. of applications x Days after spray (B x C) = 0.54
Treatments x No. of applications x Days after spray (A x B x C) = NS

Table 6: Effect of different biopesticides and novel insecticides on fruit infestation (weight basis) by *Leucinodes orbonalis* during 2016.

Treatment	Conc. (%)	Percent fruit infestation at indicated days					Mean
		1DBS*	First spray		Second spray		
			7DAS	14DAS	7DAS	14DAS	
Azadirachtin 1500 ppm	0.00045	4.24	16.03 (23.59)	26.03 (30.66)	25.16 (30.08)	27.80 (31.81)	23.76 (29.04)
Chlorantraniliprole 18.5% SC	0.007	4.17	4.20 (11.81)	12.25 (20.39)	7.89 (16.24)	11.11 (19.45)	8.86 (16.97)
Cyantraniliprole 10.26% OD	0.012	4.42	5.36 (13.38)	12.77 (20.87)	8.11 (16.54)	12.32 (26.57)	9.64 (17.83)
Eupatorium 10% EC	0.03	5.24	18.82 (25.69)	30.29 (33.35)	30.13 (33.25)	35.73 (36.69)	28.74 (32.25)
Indoxacarb 14.5% EC	0.01	5.36	13.71 (21.72)	21.82 (27.82)	14.07 (22.01)	20.03 (26.57)	17.41 (24.53)
Melia 10% EC	0.03	4.69	18.15 (25.20)	28.80 (32.41)	27.18 (31.41)	33.13 (35.10)	26.82 (31.03)
Thiacloprid 21.7% SC	0.03	4.81	10.63 (19.00)	19.97 (26.51)	12.44 (20.63)	18.97 (25.78)	15.50 (22.98)
Lambda-cyhalothrin 5% EC	0.004	3.84	11.23 (19.57)	20.84 (27.15)	13.83 (21.81)	18.93 (25.78)	16.21 (23.58)
Untreated check	-	5.30	24.18 (29.44)	42.16 (40.47)	51.34 (45.75)	57.80 (25.77)	43.87 (41.28)
Mean		4.67	13.59 (21.63)	23.88 (28.27)	21.13 (23.73)	26.20 (29.49)	-

Figures in parentheses are arc sine transformed values, 1DBS=One day before spray; *Non-significant; DAS=Days after spray
CD (P= 0.05)
Treatments (A) = 1.10; No. of applications (B) = 0.52; Days after spray (C) = 0.52
Treatments x No. of applications (A x B) = 1.56
Treatments x Days after spray (A x C) = NS
No. of applications x Days after spray (B x C) = 0.73
Treatments x No. of applications x Days after spray (A x B x C) = NS

the infestation level. The variation in infestation levels recorded at different intervals after foliar applications was also significant.

The present observations on the effectiveness of chlorantraniliprole 18.5 per cent SC @ 0.007 per cent in terms of reduction in percent shoot and fruit infestation by *L. orbonalis* are in close conformity with those of Ghosal *et al*. (2013) who reported that chlorantraniliprole recorded lowest shoot (2.65 %) and fruit infestation (14.07 %) in brinjal. They also confirmed the effectiveness of indoxacarb (3.96 % and 17.18 %) against *L. orbonalis*. Moreover, the results obtained in the present study on the effectiveness of chlorantraniliprole

Table 7: Incremental output-input ratio (IOIR) for the management of *Leucinodes orbonalis* during *Kharif* 2015 and 2016.

Treatments	Market rates (Rs)	Dose (ml ha^{-1})	Yield (q ha^{-1})	Increase in yield over control (q ha^{-1})	Additional input (Rs)	Gross returns (Rs)	Additional returns (Rs)	IOIR
Azadirachtin 1500 ppm	135/250 ml	2250	41.59	12.51	2715	62385	18758	6.91
Chlorantraniliprole 18.5% SC	526/30 ml	300	75.25	46.17	6760	112875	69248	10.24
Cyantraniliprole 10.26% OD	1966/180 ml	900	71.13	42.04	11330	106688	63060	5.57
Eupatorium 10% EC	60/100 ml	2250	35.90	6.82	2850	53850	10223	3.59
Indoxacarb 14.5% EC	230/200 ml	525	51.24	22.15	2104	76853	33225	15.79
Lambda-cyhalothrin 5% EC	62/100 ml	600	48.89	19.81	1872	73335	29708	15.87
Melia 10% EC	60/100 ml	2250	37.88	8.79	2850	56813	13185	4.63
Thiacloprid 21.7% SC	270/100 ml	975	55.68	26.60	4133	83520	39893	9.65
Untreated check	-		29.09	-	-	29085	-	-

Labour charges: Rs. 250/day (2 sprays @ 3 mandays/spray/ha); Cost of brinjal fruits: Rs 1500/q-

18.5 per cent SC @ 0.007 per cent are also supported by the work of Kameshwaran and Kumar (2015), and Sarnabati and Ray (2017). Sajjan and Rafee (2015) and Kodandaram *et al*. (2015) reported cyantraniliprole as the most effective treatment against *L. orbonalis* resulting in a maximum reduction in shoot and fruit infestation. In the present study, no doubt chlorantraniliprole 18.5 per cent SC @ 0.007 per cent was found effective over cyantraniliprole 10.26 per cent OD @ 0.012 per cent but both were at par in their effect.

Natikar *et al*. (2022) reported that among the insecticides evaluated, cyantraniliprole 10 OD @ 0.20 ml l^{-1} proved its excellence against shoot borer and defoliator by recording 65.27 and 72.69 per cent protection, respectively which was followed by spinosad 45 SC @ 0.20 ml l^{-1} (51.96 and 62.40%), respectively. Natikar and Balikai (2022) reported that among the insecticides tested, the treatments *viz*., cyantraniliprole 10 OD @ 0.20 ml l^{-1}, spinosad 45 SC @ 0.20 ml l^{-1}, emamectin benzoate 5 SG @ 0.25 g l^{-1}, diafenthiuron 70 WP @ 1.00 g l^{-1} and chlorfenapyr 10 SC @ 3.00 ml l^{-1} were effective in suppressing the insect pests of potato and resulted in higher tuber yield and economic returns. These reports also support the present findings.

Fruit yield and incremental output-input ratio

Data presented in the Table 7 reveals that among different treatments highest yield (75.25 q ha^{-1}) was recorded in chlorantraniliprole 18.5 per cent SC @ 0.007 per cent followed by cyantraniliprole 10.26 per cent OD @ 0.012 per cent (71.13 q), thiacloprid 21.7 per cent SC @ 0.03 per cent (55.68 q), indoxacarb 14.5 per cent SC @ 0.01 per cent (51.24 q), lambda-cyhalothrin 5 per cent EC @ 0.004 per cent (48.89 q), Azadirachtin 1500 ppm @ 0.00045 per cent (41.59 q), melia 10 EC @ 0.03 per cent (37.88 q) and Eupatorium 10 EC @ 0.03 per cent (35.90 q). The lowest yield was observed in the untreated check (29.09 q ha^{-1}). An increase in yield (q ha^{-1}) and additional returns (Rs) also followed a similar trend. In the present study, the incremental output-input ratio (IOIR) was found to be maximum in the case of lambda-cyhalothrin 5 per cent EC @ 0.004 per cent treated plots (1: 15.87), as it is a cheaper insecticide compared to others. Results obtained with respect to yield are supported by the findings of Kameshwaran and Kumar (2015), Sarnabati and Ray (2017), and Tripura *et al*. (2017) who also reported the highest yield with chlorantraniliprole.

CONCLUSION

From the findings of present investigations, it is concluded that all the insecticides evaluated are effective against *L. orbonalis* and can be recommended as an alternative to the present recommendations for the control of the pest. Because of the increasing public awareness and their demand for pesticide-free food, three biopesticides were included, but their efficacy was found to be far less as compared to the insecticides evaluated in the present investigation. However, the incremental output-input ratio was found to be favourable in all of these treatments.

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Research Article

Effect of different solid media and liquid media on growth, sporulation and biomass of *Hirsutella thompsonii* Fisher

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ABSTRACT

The experiment on the effect of different solid and liquid media on the growth, sporulation, and biomass of *Hirsutella thompsonii* was conducted at the Bio-control research laboratory, Department of Entomology, Navsari Agricultural University, Navasari, Gujarat, India during the year 2020-21. The result revealed that among the five solid media, Sabouraud dextrose agar + yeast medium supported higher radial growth, sporulation, and biomass (wet and dry) of *H. thompsonii* followed by Potato dextrose agar medium. Among the five liquid media tested for sporulation and biomass of *H. thompsonii*, the significantly highest sporulation, as well as wet and dry mycelial biomass, was observed in Sabaurad dextrose broth, whereas significantly lowest spore production and biomass (mycelial wet and dry weight) was recorded in Czapeks dox dextrose broth.

***Keywords*:** Biomass, growth, *Hirsutella thompsonii*, liquid media, solid media, sporulation.

Over-reliance on chemical acaricides with their indiscriminate use created many complications *viz*., food contamination through the accumulation of unwanted residues (Kumar *et al*., 2005; Inobeme, *et al*., 2020), development of resistance against many pesticides, the resurgence of minor pests, outbreak of secondary pests (Hardin *et al*., 1995; Hajek, 2004), and the increasing cost of pest management, *etc*. They are also known for their harmful effect on human health. During long-term exposure, the target insects develop resistant forms to the repeatedly applied acaricides. Release of chemicals into the air and water, to commodities containing pesticide residues. Therefore, the above-noted adverse effects have forced the scientific communities to focus on the development of mycoacaricide as an alternative eco-friendly measure.

Biopesticide use on a global scale is increasing by almost 10 per cent every year (Kumar *et al*., 2013). However, these pesticides are going to contribute noticeably to their global market consumption needs, which are to increase further in the future by substituting them for and thus reducing the over-reliance on chemical pesticides. Biopesticides offer powerful tools to create a new generation of sustainable agriculture products. These are the formulations based on the live organisms (e.g. fungi, bacteria, viruses) used to manage pests in agriculture.

There are many insect pests that attack various agriculture and horticulture crops among them mite pests become very serious pests in vegetables and fruit crops, especially red spider mite, *Tetranychus urticae* Koch. In modern agriculture, the management of mites by chemical acaricides is becoming ineffective and costly due to the development of resistance to most of the acaricides in a short time. Hence, there is a need to develop an effective and sustainable alternative control measure for mites. Of late, the role played by parasitic acaropathogenic fungi in the mite population has received worldwide attention.

More than 750 species of fungi, mostly Deuteromycetes and Entomophthorales from about 100 genera are pathogenic to insects. Among these, *H. thompsonii is* relatively less known but is gaining importance because of its target specificity and effectiveness in controlling many microscopic to sub-microscopic pests which are difficult to control by conventional management practices (Banik and Halder, 2013). The genus *Hirsutella* includes many species that are pathogens of insects, mites, and nematodes. There are about 50 entomopathogenic species under the genus *Hirsutella* (McCoy *et al*., 1988), whose potential to be developed as mite-controlling bioagents have been exploited. Of all the species, *H. thompsonii* is specific to the mite and is the most widely studied one (McCoy, 1981).

Mass production of *H. thompsonii* increases its availability with effectiveness in pest management programs. For mass production of *H. thompsonii* various media are used for its better growth and sporulation. It is necessary to test

the best medium for good growth and sporulation of *H. thompsonii*.

MATERIALS AND METHODS

For finding the best medium for the growth and sporulation of *H. thompsonii* fungus, five different synthetic media in the solid and liquid states of each were compared.

Solid media test

Five synthetic media in the solid state used for the study included: T_1: Potato Dextrose Agar medium, T_2: Sabaurad Dextrose Agar, T_3: Agar Agar (Bactro), T_4: Czapek's Dox Dextrose agar medium, and T_5: Sabaurad Dextrose with Yeast agar. These five media were tested in a Completely Randomized Design with four replications.

According to treatment and repetition, all the agar media (Table 1) were prepared in the required quantity and autoclaved at 121 ! temperature and 15 lbs pressure for 30 minutes. Then after the media was allowed for cooling; at a warm temperature of the media chloramphenicol antibiotic has been added in the trace for inhibited bacterial growth and shaken well for uniform mixing. For solidification, 20 ml of media was poured into 90 mm diameter disposable petri plates. The petri plates were inoculated aseptically in the laminar airflow by placing a five-millimeter diameter mycelial disc in the center from the 10-day-old culture of *H. thompsonii* with the help of a cork borer. The petri plates were incubated at 25±2 ! temperature for 10 days.

The radial growth (mm) of the fungus was recorded every two days after inoculation. The count of sporulation was recorded by taking 10 culture blocks of a five-millimeter diameter mycelial disc from the 10-day-old culture that was suspended in 20 ml sterilized distilled water, homogenize, and filtered through a muslin cloth. A drop from the such filtrate was kept on Neubauer's haemocytometer for counting the number of conidia present per milliliter. The data thus obtained were statistically analyzed.

Liquid media test

Five synthetic media in the liquid state used for the study included: T_1: Potato dextrose Broth, T_2: Sabaurad Dextrose broth, T_3: Potato dextrose Broth with Yeast extract, T_4: Czapek's Dox broth medium, and T_5: Sabaurad Dextrose with Yeast extract. These five liquid media were tested in a Completely Randomized Design with four replications.

According to treatment and repetition, all the liquid media (Table 2) were used as broth media with the same ingredients omitting agar-agar. A hundred milliliters of the liquid medium was filled in each 250 ml conical flask. These flasks were plugged with non-absorbent cotton and were sterilized at 121 ! with 15 lbs for 30 minutes in the autoclave.

The flasks were inoculated aseptically in laminar airflow by placing a five-millimeter diameter culture block cut with a cork borer from a two-week-old pure culture. The flasks were incubated at 25±2 !.

After 18 to 21 days of inoculation of mycelia, mats were harvested on previously weighed oven-dried Whatman's filter paper No. 42. The filter paper with mycelial mats was dried in a hot air oven for three days at 60 ! temperature up to the till constant weight was received and the dry weight of mycelium was recorded by deducting the weight of filter paper. The spore count was recorded at the end of the incubation period; the whole mycelia substrate was

Table 1: Composition of different solid media used for mass multiplication of *Hirsutella thompsonii*.

Agar media	Composition (g/l)
Potato dextrose agar	Potato = 200 + Dextrose = 20 + Agar = 30
Sabouraud dextrose agar	Peptone = 10 + Dextrose = 40 + Agar = 15
Agar-Agar	Potassium sulphate =1 + Borax = 0.20 + Alkyl benzoate = 0.10 + Sea weed extract = 14
Czapeks dox dextrose agar	Sucrose = 30 + Sodium nitrate = 2 + Dipotassium phosphate = 1 + Magnesium sulphate = 0.50 + Potassium chloride = 0.50 + Ferrous sulphate = 0.01 + Agar = 15
Sabouraud dextrose agar with yeast	Peptone = 10 + Dextrose = 40 + Agar = 15 + Yeast

Table 2: Composition of different liquid media used for mass multiplication of *Hirsutella thompsonii*.

Agar media	Composition (g/l)
Potato dextrose broth	Potato = 200 + Dextrose = 20
Sabouraud dextrose broth	Mixture of Peptone and Tryptone (1:1) = 10 + Dextrose = 20
Potato dextrose broth with yeast extract	Potato = 200 + Dextrose = 20 + Yeast
Czapeks dox dextrose broth	Sucrose = 30 + Sodium nitrate = 3 + Dipotassium phosphate = 1 + Magnesium sulphate = 0.50 + Potassium chloride = 0.50 + Ferrous sulphate = 0.01
Sabouraud dextrose broth with yeast extract	Mixture of Peptone and Tryptone (1:1) = 10 + Dextrose = 20 + Yeast

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homogenized on a mechanical mixer for 15 minutes after that 0.5 ml of Tween-80 solution was added to each flask. A clear spore suspension was obtained by filtering the shaken biomass through a double layer of muslin cloth. A drop from the such filtrate was kept on Neubauer's haemocytometer for counting the number of conidia present per milliliter. The data thus obtained were subjected to statistical analysis.

RESULTS AND DISCUSSION

Solid media test (Plate: 1)

Five different synthetic media in the solid state were tested for their suitability for the growth, spore formation, and biomass of *H. thompsonii*. The data of the media test are analyzed and summarized in Table 3.

Radial growth

The radial growth of *H. thompsonii* was measured after 10 days of inoculation in five different solid media (Table 3) The result indicated that maximum growth of *H. thompsonii* was observed in sabouraud dextrose agar (SDA) + yeast (84.25 mm) followed by potato dextrose agar (PDA) medium (80.75 mm) and sabouraud's dextrose agar (73.75 mm). The minimum growth of *H. thompsonii was* obtained in Czapek dox dextrose agar (CDA) with 53.75 mm of mycelial growth. No growth was observed in the agar-agar medium. The descending order of the radial growth of *H. thompsonii* on different synthetic solid media was $T_5 > T_1 > T_2 > T_4 > T_3$.

Sporulation

It is evident from the result presented in Table 3 that among the five tested solid media, the outstanding

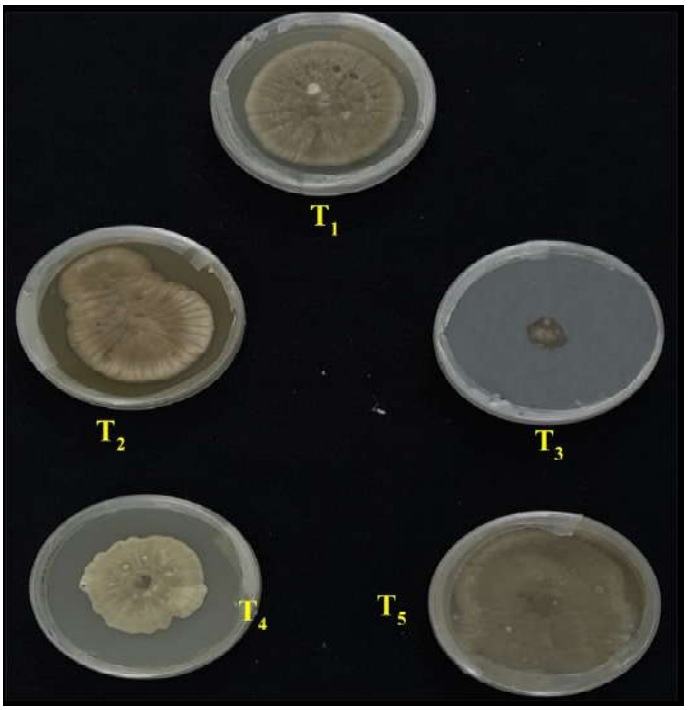

Plate 1: Growth of *Hirsutella thompsonii* on different solid media

sporulation of *H. thompsonii* (10.10×10^7 cfu/ml) was found in Sabouraud's dextrose agar (SDA) + yeast medium followed by potato dextrose agar (PDA) (8.60×10^7 cfu/ml) and (SDA) (6.40×10^7 cfu/ml). However, the significantly lowest sporulation (4.20×10^7 cfu/ml) was observed in Czapek's dox dextrose agar (CDA), whereas sporulation was not obtained in agar-agar medium. The descending order of

Table 3: Effect of different solid media on growth (radial diameter), sporulation and biomass of *Hirsutella thompsonii*.

Solid Media	Radial diameter of *H. thompsonii* (mm)	cfu/ml (×10⁷)	Wet weight of biomass (g)	Dry weight of biomass (g)
PDA	9.16[b]	3.01[b]	4.18[a]	1.44[b]
	(80.75)	(8.60)	(17.04)	(1.59)
SDA	8.61[c]	2.62[c]	3.95[b]	1.44[b]
	(73.75)	(6.40)	(15.14)	(1.58)
Agar Agar	0.71[e]	0.71[e]	0.71[d]	0.71[d]
	(0.00)	(0.00)	(0.00)	(0.00)
CDA	7.36[d]	2.16[d]	3.45[c]	1.26[c]
	(53.75)	(4.20)	(11.43)	(1.10)
SDA + Yeast	9.45[a]	3.25[a]	4.34[a]	1.56[a]
	(84.25)	(10.10)	(18.32)	(1.94)
S.Em.±	0.09	0.06	0.07	0.02
C.D. at 5%	0.27	0.19	0.20	0.07
C.V. (%)	2.55	5.43	3.96	3.37

PDA- Potato Dextrose Agar; SDA- Sabouraud Dextrose Agar; CDA- Czapek's dox Dextrose Agar; Figures outside parentheses are $\sqrt{(X+0.5)}$ transformed values; Figures inside the parentheses are original values; Treatment means with the same letter(s) are at par with each other.

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the spore production of *H. thompsonii* on different synthetic solid media was $T_5 > T_1 > T_2 > T_4 > T_3$.

Biomass

The results presented in Table 3 indicate that higher (18.32 g) wet biomass was obtained in SDA + yeast medium which was at par with PDA (17.04 g). The wet biomass in the SDA medium was 15.14 g followed by CDA (11.43 g). There was no growth of *H. thompsonii* in the agar-agar medium. The descending order of wet biomass *H. thompsonii* on different synthetic solid media was $T_5 \geq T_1 > T_2 > T_4 > T_3$. Similarly, a higher dry biomass of 1.94 g was recorded in SDA + yeast followed by PDA (1.59 g) which was found at par with SDA (1.58 g). The lowest dry biomass was obtained in CDA (1.10 g) and dry biomass was not obtained in agar-agar medium. The descending order of dry biomass *H. thompsonii* on different synthetic solid media was $T_5 > T_1 \geq T_2 > T_4 > T_3$.

There was no report of growth of *H. thompsonii* in different solid media. Therefore, the present findings are discussed well with another entomopathogen. The present findings on results of different solid media on growth and sporulation of *H. thompsonii* were closely correlated with work done by Deb *et al*. (2017) from Meghalaya who reported the maximum colony diameter of *B. bassiana* in SDYA (sabouraud dextrose agar + yeast). Similarly, Pandey and Kanaujia (2010) from Pantnagar, Uttarakhand, and Lal *et al*. (2010) from Bahraich, Uttar Pradesh also found sabouraud deõtrose medium with yeast extract superior by obtaining higher biomass and conidial count of *B. bassiana* and *M. anisopliae*. Banu and Rajalakshmi (2014) from Tamil Nadu, India reported the maximum growth and sporulation of *L. lecanii* and *Fusarium palllidoroseum* Cooke in SDAY (Sabouraud deõtrose agar with Yeast eõtract) and CZA (Czapek Doõ agar) medium. Dale and Shinde (2017) in Maharashtra, India reported that saboured dextrose agar and potato dextrose agar is the most suitable substrate for *B. bassiana* as it yielded the highest colony-forming unit (9.5×10^9 and 8×10^8 cfu/g, respectively). The present work is also in corroboration with Prabowo *et al*. (2020) who also found Potato Deõtrose Agar (PDA), Potato Deõtrose Agar Yeast (PDAY), Sabouraud Deõtrose Agar (SDA), Sabouraud Deõtrose Agar Yeast extract (SDAY) were superior for horizontal and vertical growth of *M. anisopliae* in Indonesia. From Brazil, Carolino *et al*. (2021) also reported the sabouraud dextrose agar media suitable for the growth of *M. anisopliae.* According to Soundarapandian and Chandra (2007) growth, sporulation, and biomass of *M. anisopliae* were maximum when cultured in both Czapeck doõ agar and potato deõtrose agar media at Theni, Tamil Nadu, which was more or less related to present findings.

Liquid media test (Plate: 2 & 3)

Five different liquid media were tested for their suitability for the growth, spore formation, and biomass of the *H. thompsonii*. The results of the analyzed data of the liquid media are presented in Table 4.

Sporulation

A significantly higher sporulation (10.80×10^8 cfu/ml) was observed in sabouraud dextrose broth. The next in order of merit was sabouraud dextrose broth + yeast (9.10×10^8 cfu/

Table 4: Effect of different liquid media on biomass and sporulation of *Hirsutella thompsonii.*

Treatments	cfu/ml ($\times10^8$)	Wet weight of biomass (g)	Dry weight of biomass (g)
Potato dextrose broth	2.98[b]	2.87[b]	2.09[b]
	(8.40)	(7.74)	(3.89)
Sabouraud dextrose broth	3.36[a]	3.30[a]	2.39[a]
	(10.80)	(10.37)	(5.20)
Potato dextrose broth + yeast	2.63[c]	2.56[c]	1.64[c]
	(6.40)	(6.10)	(2.18)
Czapek's dox dextrose broth	2.10[d]	2.22[d]	1.53[c]
	(3.90)	(4.43)	(1.85)
Sabouraud dextrose broth + yeast	3.10[b]	2.97[b]	2.12[b]
	(9.10)	(8.34)	(4.02)
S.Em.±	0.04	0.04	0.04
C.D. at 5%	0.13	0.13	0.12
C.V. (%)	3.11	3.13	4.15

Figures outside parentheses are $\sqrt{(X+0.5)}$ transformed values; Figures inside the parentheses are original values. Treatment means with the same letter(s) are at par with each other.

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ml) which was at par with potato dextrose broth (8.40×10^8 cfu/ml) and followed by potato dextrose broth + yeast (6.40×10^8 cfu/ml). A significantly lower spore (3.90×10^8 cfu/ml) was recorded in Czapek's dox dextrose broth. The descending order of the spore production of *H. thompsonii* on different synthetic liquid media was $T_2 > T_5 \geq T_1 > T_3 > T_4$.

Biomass

The higher wet weight (10.37 g) of the mycelia was obtained in sabouraud dextrose broth followed by sabouraud dextrose broth + yeast (8.34 g) and potato dextrose broth (7.74 g). The lowest wet weight (6.10 g) was gained in potato dextrose broth + yeast and Czapek's dox dextrose broth (4.43 g).

In the case of mycelial dry weight, a significantly higher dry weight (5.20 g) was recorded in sabouraud dextrose broth. The dry weight of sabouraud dextrose broth + yeast (4.02 g) was found at par with potato dextrose broth (3.89 g), while the lowest biomass was observed in potato dextrose broth + yeast (2.18 g), which was at par with Czapek's dox dextrose broth (1.85 g). The descending order of the wet weight *H. thompsonii* on different synthetic liquid media was $T_2 > T_5 \geq T_1 > T_3 > T_4$, as well as dry weight of biomass, was $T_2 > T_5 \geq T_1 > T_3 \geq T_4$.

In the present study, sabouraud dextrose broth and sabouraud dextrose broth + yeast was found the best media for sporulation and biomass of *H. thompsonii* which was in close agreement with the result of Bhadauria *et al*. (2012) at Uttarakhand who showed significantly higher spore production of *B. bassiana* in sabouraud dextrose broth. Similarly, Prasad and Pal (2014) from Meerut, Uttar Pradesh recorded the maximum spores (2.46×10^8 spores/ml) of *B. bassiana*, followed by *M. anisopliae* (1.57×10^8 spores/ml), and *V. lecanii* (1.80×10^8 spores/ml) in SDB. Patil (2015) from Nasik, Maharashtra showed Sabouraud's dextrose broth with yeast extract was superior, which gave significantly highest cfu ($8.33\text{x}10^8$ /ml) and biomass (6.10 g) of *N. rileyi* and Agale *et al*. (2018) at Chhattisgarh, India also noted significantly higher spore production of the *M. anisopliae* in SDB. The results of the present findings are more or less in agreement with Aghajanzadeh *et al*. (2006), who reported Sabouraud Deõtrose Broth as better than Potato Deõtrose Broth (PDB) liquid media in terms of mycelia production of *H. thompsonii* from Bengaluru, Karnataka.

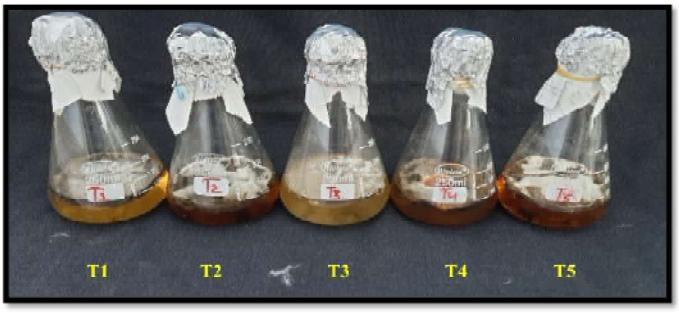

Plate 2: Growth of *Hirsutella thompsonii* on different liquid media

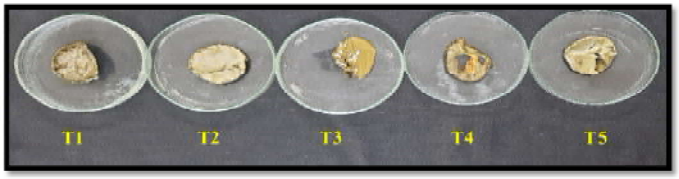

Plate 3: Dry mycelial mats of *Hirsutella thompsonii* from different liquid media

CONCLUSION

Sabouraud's dextrose agar + yeast solid medium supported higher radial growth and sporulation of *H. thompsonii* followed by PDA medium. The mycelial growth was not observed in agar agar medium. Whereas, the maximum wet biomass was obtained in SDA + yeast and found at par with the PDA medium. On the other hand, the maximum dry biomass was obtained in SDA + yeast followed by PDA and SDA medium. The order of the radial growth and spore production of *H. thompsonii* on different synthetic solid media was sabaurad dextrose agar with yeast > potato dextrose agar > sabaurad dextrose agar > czapek's dox dextrose agar > agar agar.

In the case of liquid media, the significantly highest sporulation, as well as wet and dry mycelial biomass, was observed in sabaurad dextrose broth, whereas the significantly lowest spore production and biomass (mycelial wet and dry weight) was recorded in Czapek's dox dextrose broth. The descending order of the spore production and wet weight was sabaurad dextrose broth > sabaurad dextrose broth with yeast ≥ potato dextrose broth > potato dextrose broth with yeast > Czapek's dox dextrose broth as well as mycelial dry weight was sabaurad dextrose broth > sabaurad dextrose broth with yeast ≥ potato dextrose broth > potato dextrose broth with yeast ≥ Czapek's dox dextrose broth.

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Research Article

Impact of botanicals, mycopathogens and insecticides for the management of thrips on roses under poly house condition

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ABSTRACT

Roses are unexcelled in their popularity as a garden flower and constitute an extremely important floriculture crop. The investigation was therefore undertaken for the management of rose thrips under poly house conditions. Seven days after spraying the treatments *viz*., Dichlorvos 76% EC @ 1 ml/l (82.41%), Spinosad 45% SC @ 0.5 ml/l (79.45%), neem oil @ 2% (74.99%), and neem seed kernel extract (NSKE) @ 5% (70.58) recorded high mortality on thrips population on leaflets. The chemical treatments of Dichlorvos 76% EC @ 1 ml/l and Spinosad 45% SC @ 0.5 ml/l were found superior which recorded 85.51 and 83.55 per cent thrips mortality on seven days after spraying followed by Neem oil @ 2% (73.07%) and NSKE @ 5% (70.86%), respectively in unopened rose buds. The population of thrips declined significantly in chemical treatments such as Dichlorvos 76% EC @ 1 ml/l (94.15%) and Spinosad 45% SC @ 0.5 ml/l (91.66 %) followed by botanicals like NSKE @ 5% (87.06%) and neem oil 2% (86.38%) on seven days after spraying in partially opened rose flowers. The impact of treatments *viz*., Spinosad 45% SC @ 0.5 ml/l, NSKE @ 5%, Dichlorvos 76% EC @ 1 ml/l and neem oil @ 2% was found effective in reducing thrips population upto the extent of 92.94, 90.40, 89.48 and 84.89 per cent on seven days after spraying in fully opened rose flowers.

Key words: Greenhouse, Management, Rose, *Rhipiporothrips cruentatus*, Thrips

Rose (*Rosa* spp.) is one of the most prevalent flowering shrubs in India just as in different countries. Esteemed for their wonderful and, often, fragrant blooms, roses have been cultivated in gardens for centuries as vines, shrubs, specimen plants, ground covers and container plants. Commercial rose cultivation under open-field and secured structures is acquiring significance with the region under its development expanding step by step. The thrips species, *Rhipiporothrips cruentatus* Hood, is a documented pest of many plants, including vegetables, fruits, roses, greenhouse-grown plants, and cotton (Hegde *et al.*, 2011). Thrips are one of the vital nuisance pests of roses, causing 28-95% damage (Gahukar, 2003). It is important to distinguish and oversee thrips on roses, even at low densities, on the grounds that even a couple of people on blossoms might bring about flower petal staining (Bertaux *et al.*, 2003). Thrips are tiny insects that reproduce rapidly, congregate in tight places that make pesticide coverage difficult, and feed with rasping-piercing-sucking mouth parts, resulting in the deformation of flowers and leaves (Duraimurugan and Jagadish, 2011). The capacity to bear thrips on floriculture crops is particularly low. Synthetic insecticides are utilized oftentimes to control thrips in greenhouses, and populations of certain thrips species have created resistance to different groups of insecticides. Fortunately, biopesticides have been acquiring interest among those concerned with developing environmentally friendly and safe integrated crop management systems, with compatible approaches and tactics for pest management (Copping and Menn, 2000). As of now, microbial insecticides are a principle component of the biopesticides industry (Shi, 2000). One significant constraint for utilizing microbial insecticides is that they are comparatively slow in action compared with other chemical insecticides. One mandate of researchers is to develop fast and environmentally safe pest management systems utilizing biopesticides.

MATERIALS AND METHODS

The experiment was conducted under polyhouse conditions at the Department of Horticulture, Adhiyamaan College of Agriculture and Research, Athimugam, Krishnagiri. The details of the material used and methods adopted during the period of investigation are explained below. For carrying out the present investigation materials like various insecticides, biopesticides, botanicals, rose plants, hand sprayers, separating curtains, and 10 X magnifying lens were used. The plant products were obtained from Adhiyamaan College of Agriculture, Krishanagiri. The chemicals were purchased from the local market. The biopesticides were obtained from RRS, Paiyur.

The field trial was laid out in Randomized Block Design with 13 treatments replicated thrice, having inter-replication

and inter-plot spacing of 60 cm. Dutch Rose variety of rose was used at a spacing of 30 x 45 cm with a net plot size of 1.5 x 0.9 m.

Preparation of botanical extracts

Neem seed kernel extract

Fresh neem seed kernels of 50 g were dried and ground to coarse powder then soaked in water overnight and filtered by using muslin cloth. The volume of the solution was adjusted to one litre to get 5% concentration.

Sweet flag rhizome extract

Sweet flag rhizome powder of 20 g was soaked in water overnight and filtered by using a fine muslin cloth. The volume of the filtrate was made to one litre of water to get 2% concentration.

***Vitex nedungo* leaf extract**

Matured 20 g of leaves of the *Vitex nedungo* plant was collected, ground and soaked in water overnight and filtered by using a muslin cloth. The volume of the filtrate was made to one litre of water to get 2% concentration.

Holy basil leaf

Matured 20 g of leaves of the holy basil leaf was collected, ground and soaked in water overnight and filtered by using a muslin cloth. The volume of the filtrate was made to one litre of water to get 2% concentration.

Eucalyptus leaves

Matured 20 g of leaves of the eucalyptus leaf was collected, ground and soaked in water overnight and filtered by using a muslin cloth. The volume of the filtrate was made to one litre of water to get 2 % concentration.

Karpuravalli leaf

Matured 20 g of leaves of the Karpuravalli leaf was collected, ground and soaked in water overnight and filtered by using a muslin cloth. The volume of the filtrate was made to one litre of water to get 2 % concentration.

Neem oil (NO)

To get a 2 per cent solution first mix 20 ml of neem oil with 3 ml of sticking agent teepol until the white emulsion is formed. Then add one litre of water and mix thoroughly for use as spray fluid.

Pongamia oil

To get a 2 per cent solution first mix 20 ml of neem oil with 3 ml of sticking agent teepol until the white emulsion is formed. Then add one litre of water and mix thoroughly for use as spray fluid.

Method of observation

From each plot, three plants were randomly selected and tagged. From each selected plant, three leaves from the upper, middle and bottom were selected at random and were tagged for recording observations. Thus, observations on the population of nymphs of thrips were recorded from three leaves of randomly selected three plants per plot. The number of nymphs of thrips present on the buds and flowers was also recorded. Pre-treatment observations were recorded 24 hrs before treatment. Post-treatment observations were recorded on 3, 5, and 7 days.

Time of application of treatment

In all, three applications (sprayings) were undertaken using a hand sprayer after February pruning for the management of thrips. The first application of various treatments was done 30 days after pruning on 13th Feb. 2020. The second application was done 21 days after the first spraying. The third application was done 21 days after the second spraying.

Calculations for the efficacy of treatments

Per cent reduction and the reduction in the nymphal population of thrips were worked out as per the following formula.

$$\text{Per cent reduction} = \frac{\text{Pre-treatment population- post-treatment population}}{\text{Pre-treatment population}} \times 100$$

RESULTS AND DISCUSSION

The nymphal population of thrips per plot in different treatments was recorded 24 hrs before each spraying to ascertain the original population on the rose plants for the calculation of the reduction level in the population of thrips after each spraying.

Effect of different treatments on percent reduction of thrips population in rose leaflets after spraying

The mean data of three sprays on rose leaflets with respect to the thrips population and its reduction are presented in Table 1.

All the treatments were significantly superior to the control (water spray). The highest mortality of the thrips was recorded three days after spraying Spinosad 45% SC @ 0.5 ml/l (45.80%) and Dichlorvos 76% EC @ 1 ml/l (48.13%).

The highest mortality of thrips was recorded on five days after spraying of Spinosad 45% SC @ 0.5 ml/l (66.35%), Dichlorvos 76% EC @ 1 ml/l (65.75%), NSKE 5% (59.79 %),

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and neem oil 2% @ 20 ml/l (59.61%) when compared all other treatments. Seven days after spraying, the treatment of Dichlorvos 76% EC @ 1 ml/l (82.45%), Spinosad 45% SC @ 0.5 ml/l (79.45%), neem oil 2% @ 20 ml/l (74.99%), and NSKE 5% (70.58) recorded high mortality of thrips. Water sprays failed to bring down of thrips population which recorded as high as 34.33 thrips per leaflet with only 1.03 per cent of mortality.

It concluded that the treatment like Neem oil @ 2%, NSKE @ 5%, Spinosad 45% SC @ 0.5 ml/l and Dichlorvos 76% EC @ 1 ml/l effectively controlled the thrips on rose leaflets, and other treatments reduced the thrips population marginally. The effectiveness of NSKE @ 5% and neem oil @ 2% products on thrips has been reported by several workers. Balasingam *et al* (2003) reported neem seed water extract and garlic sap extract to record the lowest thrips count across seasons and highest dry-pod yields in chilli which is in close agreement with the present study. Dhananjaya Kumar and Nandihalli (2009) reported that at 10 days after spray pongamia oil was superior followed by neem oil and NSKE which also supports the present study.

Effect of different treatments on per cent reduction of thrips population in rose buds after spraying

The effect of different treatments on thrips in rose buds under poly house conditions is furnished in Table 2. The thrips population did not vary significantly on the day before treatment imposition. However, the thrips population ranged from 23.33 to 27.00/bud.

Three days after spraying Dichlorvos 76% EC @ 1 ml/l and Spinosad 45% SC @ 0.5 ml/l recorded the lowest population which was highly significant over other treatments. Significantly the highest thrips were recorded in the water spray. A similar trend was also recorded five and seven days after the spray. The chemical treatments of Dichlorvos 76% EC @ 1 ml/l and Spinosad 45% SC @ 0.5 ml/l were found superior which recorded 44.72 and 38.13 per cent of mortality on three days after spraying followed by Neem oil @ 2% (34.61%) and NSKE @ 5% (31.63%), respectively.

Table 1. Effect of different treatments on rose thrips in leaflets under poly house conditions.

Tr. No	Treatment		Number of thrips/leaflets			% Reduction		
		1 DBS	3 DAS	5 DAS	7 DAS	3 DAS	5 DAS	7 DAS
T1	Holy basil leaf @ 2%	36.33	26.67	20.33	15.67	26.66	44.04	56.86
		(6.11)	(5.26)	(4.61)	(4.08)			
T2	Vitax leaf @ 2%	36.67	28.33	23.33	17.33	22.74	36.37	52.74
		(6.14)	(5.41)	(4.93)	(4.28)			
T3	Eucalyptus leaves @ 2%	34.33	28.00	23.33	18.00	18.43	32.04	47.56
		(5.94)	(5.38)	(4.93)	(4.35)			
T4	Karpuravalli leaf @ 2%	36.00	29.33	22.67	17.33	18.52	37.02	51.86
		(6.08)	(5.50)	(5.00)	(4.28)			
T5	Sweet flag @ 2%	33.67	27.67	24.00	17.67	17.82	28.71	47.52
		(5.89)	(5.35)	(3.87)	(4.32)			
T6	Neem oil @ 2%	34.67	20.67	14.00	8.67	40.38	59.61	74.99
		(5.97)	(4.65)	(5.13)	(3.10)			
T7	Pongamia oil @ 2%	36.67	30.33	25.33	17.33	17.28	30.92	52.74
		(6.14)	(5.59)	(5.13)	(4.28)			
T8	NSKE @ 5%	34.00	21.33	13.67	10.00	37.26	59.79	70.58
		(5.92)	(4.72)	(3.83)	(3.31)			
T9	Spinosad 45% SC @ 0.5 ml/l	35.67	19.33	12.00	7.33	45.80	66.35	79.45
		(6.06)	(4.50)	(3.60)	(2.88)			
T10	Dichlorvos 76% EC @ 1 ml/l	36.00	18.67	12.33	6.33	48.13	65.75	82.41
		(6.08)	(4.43)	(3.61)	(2.70)			
T11	*Verticillium lecanii* @ 2 ml/l	34.00	26.33	23.00	15.00	22.55	32.35	55.88
		(5.92)	(5.22)	(4.89)	(4.00)			
T12	*Metarrhizium anisopliae* @ 2 ml/l	34.00	26.00	23.67	14.33	23.52	30.38	57.85
		(5.92)	(5.19)	(4.96)	(3.91)			
T13	Water spray	34.33	34.33	32.33	32.00	-	-	1.03
		(5.94)	(5.94)	(5.77)	(5.74)			
	S.Em.±	-	1.02	1.10	0.98	-	-	-
	C.D. (0.05)	NS	2.09	2.28	2.02	-	-	-

Note: DBS: Day before spray, DAS: Days after spray; Figures in parentheses are $\sqrt{X+1}$ transformation values

Five days after spraying, the highest mortality of the thrips was recorded in Dichlorvos 76% EC @ 1 ml/l (67.11%) and Spinosad 45% SC @ 0.5 ml/l (65.81%) followed by neem oil @ 2% (56.42%) and NSKE @ 5% (55.67%) and remaining treatments were moderately effective against thrips. Seven days after spraying two botanicals (neem oil @ 2% and NSKE @ 5%) and chemicals (Dichlorvos 76% EC @ 1 ml/l and Spinosad 45% SC @ 0.5 ml/l) effectively reduced the thrips population. The neem oil @ 2% and NSKE @ 5% reduced the population of thrips up to 73.07 and 70.86 per cent, respectively and they were on par with each other. Dichlorvos 76% EC @ 1 ml/l and Spinosad 45% SC @ 0.5 ml/l also effectively reduced thrips population up to 83.55 and 85.51 per cent, respectively.

The mycopathogen treatments marginally reduced the population of thrips. The range of mycopathogens (*Metarrhizium anisopliae* @ 2 ml/l and *Verticillium lecanii* @ 2 ml/l) varied from 53.32 to 57.14 per cent of the decline in population of thrips.

Effect of different treatments on percent reduction of thrips population in rose partially opened flower after spraying

The data pertaining effect of different treatments on thrips in partially opened rose flowers under poly house conditions are furnished in Table 3. The population of thrips in pre-treatment ranged forms 57.67 to 57.00/partially opened rose flower in poly house conditions. The result showed that the population of thrips declined significantly in chemical treatments such as Dichlorvos 76% EC @ 1 ml/l (52.60%) and Spinosad 45% SC @ 0.5 ml/l (50.00%) followed by botanicals like neem oil @ 2% (44.96%) and NSKE @ 5% (40.58%) at three days after spray.

Five days after spraying, the population of thrips were reduced by both chemicals (Dichlorvos 76% EC @ 1 ml/l and Spinosad 45% SC @ 0.5 ml/l) with 77.37 and 79.76 per cent reduction, respectively. The next effective treatment included neem oil @ 2% and NSKE @ 5% with a decline in thrips population of 74.44 and 75.87 per cent, respectively.

Table 2. Effect of different treatments on rose thrips in buds under poly house conditions.

Tr. No.	Treatment	Number of thrips/Bud				% Reduction		
		1 DBS	3 DAS	5 DAS	7 DAS	3 DAS	5 DAS	7 DAS
T1	Holy basil leaf @ 2%	22.67 (4.86)	20.67 (4.65)	16.00 (4.12)	12.00 (3.60)	8.82	29.42	47.06
T2	Vitax leaf @ 2%	23.33 (4.93)	19.00 (4.47)	16.33 (4.16)	12.67 (3.69)	18.55	30.00	45.69
T3	Eucalyptus leaves @ 2%	24.67 (5.07)	22.67 (4.86)	18.33 (4.39)	13.33 (3.78)	8.10	26.05	45.96
T4	Karpuravalli leaf @ 2%	24.33 (5.03)	22.33 (4.83)	18.00 (4.35)	11.33 (3.51)	8.22	26.01	53.43
T5	Sweet flag @ 2%	25.67 (5.16)	24.33 (5.03)	16.33 (4.16)	12.00 (3.60)	5.22	36.38	53.25
T6	Neem oil @ 2%	26.00 (5.20)	17.00 (4.24)	11.33 (3.51)	7.00 (2.82)	34.61	56.42	73.07
T7	Pongamia oil @ 2%	25.00 (5.10)	19.33 (4.50)	16.67 (4.20)	12.33 (3.65)	22.68	33.32	50.68
T8	NSKE @ 5%	26.33 (5.23)	18.00 (4.35)	11.67 (3.55)	7.67 (2.94)	31.63	55.67	70.86
T9	Spinosad 45% SC @ 0.5 ml/l	26.33 (5.23)	15.00 (4.00)	9.00 (3.16)	4.33 (2.30)	44.72	65.81	83.55
T10	Dichlorvos 76% EC @ 1 ml/l	25.33 (5.13)	15.67 (4.08)	8.33 (3.05)	3.67 (2.16)	38.13	67.11	85.51
T11	*Verticillium lecanii* @ 2 ml/l	25.67 (5.16)	21.67 (4.76)	16.00 (4.12)	11.00 (3.46)	15.58	37.67	57.14
T12	*Metarrhizium anisopliae* @ 2 ml/l	25.00 (5.10)	22.33 (4.83)	17.33 (4.28)	11.67 (3.55)	10.68	30.68	53.32
T13	Water spray	27.00 (5.29)	26.33 (5.22)	26.33 (5.22)	26.67 (5.26)	2.81	2.81	1.22
	S.Em.±	-	1.26	0.97	1.06	-	-	-
	C.D. (0.05)	NS	2.57	2.01	2.18	-	-	-

Note: DBS: Day before spray, DAS: Days after spray; Figures in parentheses are $\sqrt{X+1}$ transformation values

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Table 3. Effect of different treatments on rose thrips in partially opened flowers under poly house conditions.

Tr. No.	Treatment	Number of thrips/Partially opened flower				% Reduction		
		1 DBS	3 DAS	5 DAS	7 DAS	3 DAS	5 DAS	7 DAS
T1	Holy basil leaf @ 2%	50.00 (7.14)	40.00 (6.40)	23.33 (4.93)	11.00 (3.46)	20.00	53.33	78.00
T2	Vitax leaf @ 2%	52.00 (7.28)	41.33 (6.50)	21.67 (4.76)	13.00 (3.74)	20.51	58.32	75.00
T3	Eucalyptus leaves @ 2%	51.67 (7.25)	38.67 (6.29)	19.33 (4.50)	14.33 (3.91)	25.15	62.58	72.26
T4	Karpuravalli leaf @ 2%	50.33 (7.16)	34.33 (5.94)	22.67 (4.86)	13.33 (3.78)	31.73	54.95	73.51
T5	Sweet flag @ 2%	54.67 (7.46)	37.00 (6.16)	20.67 (4.65)	12.00 (3.60)	32.32	62.19	78.05
T6	Neem oil @ 2%	56.33 (7.57)	31.00 (5.65)	14.67 (3.95)	7.67 (2.94)	44.96	74.44	86.38
T7	Pongamia oil @ 2%	53.00 (7.34)	39.00 (6.32)	19.33 (4.50)	13.33 (3.78)	26.41	64.09	74.79
T8	NSKE @ 5%	56.67 (7.59)	33.67 (5.88)	13.67 (3.83)	7.33 (2.88)	40.58	75.87	87.06
T9	Spinosad 45% SC @ 0.5 ml/l	56.00 (7.54)	28.00 (5.32)	12.67 (3.69)	4.67 (2.38)	50.00	77.37	91.66
T10	Dichlorvos 76% EC @ 1 ml/l	57.67 (7.65)	27.33 (5.38)	11.67 (3.55)	4.00 (2.23)	52.60	79.76	94.15
T11	*Verticillium lecanii* @ 2 ml/l	57.00 (7.61)	36.00 (6.08)	17.00 (4.24)	12.67 (3.69)	36.84	70.17	77.77
T12	*Metarrhizium anisopliae* @ 2 ml/l	55.67 (7.52)	37.67 (6.21)	17.33 (4.28)	11.67 (3.55)	32.33	68.87	79.03
T13	Water spray	55.23 (7.25)	54.12 (7.21)	54.45 (7.21)	54.60 (7.16)	2.00	1.41	1.14
	S.Em.±	-	1.08	1.11	1.02	-	-	-
	C.D. (0.05)	NS	2.23	2.29	2.11	-	-	-

Note: DBS: Day before spray, DAS: Days after spray; Figures in parentheses are $\sqrt{X+1}$ transformation values

Minimum control of the thrips population was recorded in control with 1.41 per cent.

The data of seven days after spraying showed that neem oil @ 2% (86.38%) and NSKE @ 5% (87.07%) were found effective in controlling on thrips population and likewise, chemical treatments Spinosad 45% SC @ 0.5 ml/l (91.66%) and Dichlorvos 76% EC @ 1 ml/l (94.15%) also effectively reduced thrips population.

The treatment of *Verticillium lecanii* (77.77%) and *Metarrhizium anisopliae* (79.03%) were found on par with each other. The other treatments *viz*., Holy basil leaf @ 2% (78.00%), Sweet flag @ 2% (78.05%), Vitax leaf @ 2% (75.00%), Karpuravalli leaf @ 2% (73.51%) and Eucalyptus leaves @ 2% (72.26%) showed a partial decline in the population of thrips.

Jagdish and Purnima (2011) recorded 35.58 and 74.37 per cent reductions in thrips population at three and five days after spraying of NSKE @ 2% under poly house conditions. Jayalaxmi *et al* (2011) reported that NSKE was found to reduce thrips density to the extent of 64-88%. These reports are similar to the present study.

Effect of different treatments on per cent reduction of thrips population in rose fully opened flowers after spraying

The data on the effect of different treatments against thrips on fully opened rose flowers under poly house conditions are given in Table 4. The population of thrips a day before treatment ranged from 50.67 to 45.67 per fully opened flower. The treatments *viz*., neem oil @ 2%, NSKE @ 5%, Spinosad 45% SC @ 0.5 ml/l and Dichlorvos 76% EC @ 1 ml/l were found effective in reducing thrips population upto the extent of 41.01, 43.14, 48.71 and 50.66 per cent at three days after spraying and also, they were found at on par with each other.

Subsequently, five days after spraying, the population of thrips was significantly reduced in neem oil @ 2% (67.62%) and NSKE @ 5 % (71.23%) followed by the treatment of Spinosad 45% SC @ 0.5 ml/l (84.61%), and Dichlorvos 76% EC @ 1 ml/l (79.61%). The treatments *Verticillium lecanii*

Table 4. Effect of different treatments on rose thrips in fully opened flowers under poly house conditions.

Tr. No	Treatment		Number of thrips/fully opened flower			% Reduction		
		1DBS	3 DAS	5 DAS	7 DAS	3 DAS	5 DAS	7 DAS
T1	Holy basil leaf @ 2%	46.67	36.67	24.33	16.33	21.42	47.80	65.00
		(6.90)	(6.13)	(5.03)	(4.16)			
T2	Vitax leaf @ 2%	47.00	36.33	22.33	15.67	22.70	47.29	66.65
		(6.93)	(6.11)	(4.83)	(4.08)			
T3	Eucalyptus leaves @ 2%	46.00	37.33	22.67	13.00	18.84	50.71	71.73
		(6.86)	(6.19)	(4.86)	(3.74)			
T4	Karpuravalli leaf @ 2%	47.67	38.00	22.00	12.00	20.28	53.84	74.82
		(6.98)	(6.24)	(4.79)	(3.60)			
T5	Sweet flag @ 2%	45.67	36.00	23.00	13.33	21.17	49.63	70.81
		(6.83)	(6.08)	(4.89)	(3.78)			
T6	Neem oil @ 2%	46.33	27.33	15.00	7.00	41.01	67.62	84.89
		(6.88)	(5.32)	(4.00)	(2.82)			
T7	Pongamia oil @ 2%	49.00	31.67	18.33	11.00	35.36	62.59	77.55
		(7.07)	(5.71)	(4.39)	(3.46)			
T8	NSKE @ 5%	48.67	27.67	14.00	4.67	43.14	71.23	90.40
		(7.05)	(5.35)	(3.78)	(2.38)			
T9	Spinosad 45% SC @ 0.5 ml/l	52.00	26.67	8.00	3.67	48.71	84.61	92.94
		(7.28)	(5.26)	(3.00)	(2.16)			
T10	Dichlorvos 76% EC @ 1 ml/l	50.67	25.00	10.33	5.33	50.66	79.61	89.48
		(7.19)	(5.09)	(3.36)	(2.51)			
T11	*Verticillium lecanii* @ 2 ml/l	50.33	28.67	17.00	12.00	43.03	66.22	76.15
		(7.16)	(5.44)	(4.24)	(3.60)			
T12	*Metarrhizium anisopliae* @ 2 ml/l	50.00	31.00	17.67	13.00	38.00	64.66	74.00
		(7.14)	(5.65)	(4.32)	(3.74)			
T13	Water spray	48.67	48.67	48.67	48.67	-	-	-
		(7.05)	(7.04)	(7.04	(7.04)			
	S.Em.±	-	1.20	1.13	1.08	-	-	-
	C.D. (0.05)	NS	2.47	2.34	2.23	-	-	-

Note: DBS: Day before spray, DAS: Days after spray; Figures in parentheses are $\sqrt{X+1}$ transformation values

(66.22%) and *Metarrhizium anisopliae* (64.66%) were found at par with each other.

Finally, data from seven days after spraying revealed that the highest mortality of thrips was significantly observed in NSKE @ 5% (90.40%) and Spinosad 45% SC @ 0.5 ml/l (92.94%) follow by neem oil @ 2% (84.89%) and Dichlovrvos (89.48%) when compared with other remaining treatments. The reaming treatments Eucalyptus leaves @ 2%, Karpuravalli leaf @ 2%, sweet flag @ 2%, *Verticillium lecanii* and *Metarrhizium anisopliae* were found on par with each other. Jagdish and Purnima (2011) recorded 40.34, 56.79 and 74.48 per cent reductions in thrips at one, three and five days after spraying of NSKE @ 2% under poly house conditions. Dhananjaya Kumar and Nandihalli (2009) reported that neem @ 2% effectively controlled the thrips population. These reports support the present study.

CONCLUSION

Currently rose is a one of the important flowers which can use for vast functions. But pests are considerably at yield loss in the garden. In this regard, suppressing the pest population in the garden by using chemical, mycopathogens and botanical pesticides is essential. To cut down the expenditure, botanical pesticides are feasible like neem oil @ 2% and NSKE @ 5%. In addition, Spinosad 45% SC @ 0.5 ml/l is also a good option for thrips management in roses as it is safe and eco-friendly.

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Research Article

Disease assessment and casing variables for the cultivation of white button mushroom [*Agaricus bisporus* (Lange)]

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ABSTRACT

An essential step in the cultivation of white button mushrooms for commercial use is the addition of substrate at the casing to boost quality and yield. The goal of this experiment was to investigate how different casing combinations affected the growth and yield of the white button mushroom [*Agaricus bisporus* (Lange)] strain U3 as well as conduct a wet bubble and green mould disease assessment. The various casing compositions differed significantly from one another. The casing mixture Cocopeat + Farmyard manure + Rice husk (Steam pasteurization) produced the highest overall yield (816.66 g), while Cocopeat produced the lowest (516.66 g). A gain of 1.48 was generated for every rupee spent on research thanks to the BC ratio. During the 2020-2021 survey years, the incidence of the disease of wet bubble and green mould varied from 2.98 to 33.33 and 14.83 to 64.92 per cent, respectively in Punjab's button mushroom farming farm districts. These studies will aid mushroom farmers in making the best casing material choices for the white button mushroom (*A. bisporus*), which has the best growth and yield potential.

***Keywords*:** Button mushroom, casing, wet bubble, green mould.

The most extensively farmed and consumed mushroom in the world is the button mushroom [*Agaricus bisporus* (Lange)], and India accounts for around 40% of global mushroom cultivation (Giri and Prasad, 2007; Kaur and Rampal, 2017). Today, the following species are farmed commercially in India: oyster, button, paddy straw, and milky mushrooms. Approximately 73% of the production is of white button mushrooms. Oyster mushroom, paddy straw mushroom, and milky mushroom share of other tropical mushrooms is 16, 7, and 3%, respectively (Verma 2013; Sharma *et al*., 2017; Jahan and Singh, 2019). Hundreds of seasonal producers produce button mushrooms during the winter, especially in the Northern States with a focus on major cities (Sing and Kamal, 2016). Mushrooms are extremely high in protein (12-35%), crude fibre (19%), vitamins, minerals and carbs (4-6%), but they are also low in calories and fat (less than 0.05%), making them a treat for those with diabetes, high blood pressure, and hypertension (Thakur, 2020). Mushrooms are an excellent food for humans to consume since they contain larger amounts of water (90%), ash (8-10%), and fat (2-8%) than other foods (Fekadu, 2015).

In order to produce fruiting bodies, *A. bisporus* needs two separate substrates: compost, which it consumes while it grows vegetatively, and casing soil, where the right physicochemical and biological conditions trigger the process that starts the construction of pin heads. The button mushroom will develop on a variety of composted soils (Delphina and Royse, 2008). The nutritionally deficient casing materials and the compost in which it develops vegetatively create the ideal physical, chemical, and biological conditions that promote the production of fruiting bodies. Compost used to grow white button mushrooms includes gypsum, straw-bedded horse manure, wheat straw, and chicken manure (Straatsma *et al*., 2000).

The selection of casing substance is crucial for the production of mushrooms since it has a big impact on the yield, quality, and price of the mushrooms. Mushroom mycelia development and growth are influenced by environmental factors, chemical composition, and microorganisms in addition to genetic considerations (Pardo *et al*., 2004). Although a casing layer can be made of a variety of materials, coco peat is thought to be the best option. Because of its special ability to store water (Kaur and Rampal, 2017).

The changing environmental conditions created by the casing layer, mostly because of the higher microbial activity in the casing material, favour the promotion of the reproductive development of mushrooms from the vegetative stage (Gulser and Peksen, 2003). Bacteria in casing dirt affect output, homogeneity, and product quality (Choudhary, 2011).

A severe pathogen that affects mushroom crop output is green mould. In India, it has reportedly resulted in significant crop loss (Shah *et al*., 2013). The fungus known as green mould is an active coloniser of dead mushroom tissue and organic matter. This fungus' spores are dispersed

by water, air, and reckless handling (Munshi *et al*., 2010). The management of this illness involves the use of carbendazim, bitertanol, and captan. Effective opponents of virulent *Trichoderma* strains include some bacteria, particularly *Bacillus* species which are naturally present in the casing (Gupta *et al*., 2018).

Mycogone perniciosa is the pathogen that causes Wet Bubble illness. The formation of white mycelial growth on button mushroom fruiting bodies is the disease's primary identifying feature. It spreads and completely envelops the cap. The occurrence of amber watery droplets just on the surface of warped mushrooms is another defining sign of the illness (Munshi *et al*., 2010). The use of contaminated spawn or casing soil can also be blamed for the disease's early development (Fletcher *et al*., 1989). Wet bubble disease can be prevented by spraying 0.8% formalin on the surface of the casing (Gupta *et al*., 2018).

MATERIALS AND METHODS

Survey on disease incidence

The surveys on diseases green mould and wet Bubble were carried out in different parts of Punjab during the normal button mushroom growing season (Nov. to Feb.). The areas where the survey was carried out are Amloh, Maneli, Landran, Ranwan, Nabha, Khamano, Agoul, Behlolpur, Chunni, Sarana, Ludhiana, Dakala, Khanna, Malerkotla, Patiala, Sherpur, Sirah, Haibowal Kalan, Sanaur, and Ropar.

$$\text{Disease Incidence} = \frac{\text{No. of infected bags in the sampling unit}}{\text{Total no. of bags in the sampling unit}} \times 100$$

Casing treatment

Three different case materials of 45 mm thickness were used. The compost was kept at a temperature of 22 to 25 °C, which is ideal for the mycelial colonisation of the casing layer. Eight casing mixtures were prepared, T0 (Control) Cocopeat, T1 Cocopeat + FYM + Rice husk (2% formalin), T2 Cocopeat + FYM (2% Formalin), T3 Cocopeat + Rice husk (2% Formalin), T4 Rice husk + FYM (2% Formalin), T5 Cocopeat + FYM + Rice husk (Steam pasteurisation), T6 Cocopeat + FYM (Steam pasteurisation), T7 Cocopeat + Rice husk (Steam pasteurisation), T8 Rice husk + FYM (Steam pasteurisation). All the treatments were used in 1:1 ratio. These eight treatments were replicated thrice.

Yield data

Following the development of the pin head and the days of the spawn run, yield data considering the total number and weight of fruiting bodies were set down up to three flushes. Estimation of the cost-benefit ratio, cost of cultivation, gross monetary return, and net monetary return were also worked out.

Statistical analysis

Various data were statistically analysed as per the procedure set by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Prevalence of green mould and wet bubble of button mushroom

Surveys were conducted throughout the typical button mushroom growing season, from November to January in 2020-2021, to determine the extent of green mould and moist bubble of button mushrooms at various places (Fig. 1), including the F1 and F20 districts of Punjab. The information on disease incidence and prevalence is shown in Table 1.

It is clear from a review of the data in Table 1 that when compared to other studied farms in Punjab districts, F5 and F18 had a greater prevalence of wet bubble illness and green mould. The average incidence of green mould and wet bubble of button mushrooms in two years was maximum at F 5 (12.55 and 13.28%), whereas it was minimum at F 20 (0.90%) and F 11 (1.04%) districts of Punjab. During the 2019-2020 survey years, the incidence of the disease of wet bubble and green mould varied from 2.98 to 33.33 and 14.83 to 64.92 per cent, respectively, in Punjab's button mushroom farming farm districts (Table 1).

$$\text{Prevalance} = \frac{\text{No. of bags with disease infection in the unit}}{\text{Total no. of unit surveyed}} \times 100$$

The impact of various casing materials on white button mushroom production

In CP+FYM+RH steam pasteurisation (1:1:1) (17 days), early pin head emergence was seen. This was followed with CP+FYM+RH 2% formalin (1:1:1) (18 days). With a total of 21 days following casing, delayed pin head production was

a. b.

Fig. 1. Symptoms of (a) green mould and (b) wet bubble on bags.

Table 1: Prevalence of green mould and wet bubble at different button mushroom growing farms of Punjab during 2020-2021 crop seasons.

Sl. No.	Farmers	Disease incidence (%)					
		Trichoderma			Wet Bubble		
		2019	2020	Mean	2019	2020	Mean
1	F1 (Amloh)	2.09	4.08	3.08	2.72	3.07	2.90
2	F2 (Maneli)	2.23	2.71	2.47	2.11	3.30	2.71
3	F3 (Landran, Mohali)	2.12	3.40	2.76	2.21	3.72	2.97
4	F4 (Ranwan)	2.21	2.61	2.41	2.72	3.10	2.91
5	F5 (Nabha)	8.10	17.00	12.55	18.80	22.33	20.57
6	F6 (Khamano)	0.75	1.21	0.98	1.40	2.40	1.90
7	F7 (Agoul, Patiala)	5.17	5.83	5.50	4.15	4.40	4.27
8	F8 (Behlolpur)	1.00	1.17	1.08	1.73	1.73	1.73
9	F9 (Chunni)	4.12	4.70	4.41	5.34	12.63	8.98
10	F10 (Sarana)	2.17	2.10	2.13	2.82	3.43	3.13
11	F11 (Ludhiana)	0.84	0.71	0.78	1.20	0.88	1.04
12	F12 (Dakala, Patiala)	0.54	0.64	0.59	1.43	2.85	2.14
13	F13 (Khanna)	3.72	5.50	4.61	4.50	7.30	5.90
14	F14 (Malerkotla)	1.52	2.57	2.04	2.68	2.41	2.55
15	F15 (Patiala)	2.71	3.20	2.95	3.85	3.76	3.81
16	F16 (Sherpur, Sangrur)	8.97	14.83	11.90	11.43	11.57	11.50
17	F17 (Sirah, Ludhiana)	2.50	3.13	2.82	4.48	3.76	4.12
18	F18 (Haibowal Kalan, Ludhiana)	11.00	15.57	13.28	7.23	8.51	7.87
19	F19 (Sanaur, Patiala)	1.57	2.33	1.95	1.81	2.71	2.62
20	F20 (Ropar)	0.60	1.21	0.90	8.40	8.52	8.46
	Mean	3.19	4.72		4.55	5.62	

seen in the pasteurisation processes of steam pasteurisation and CP+RH 2% formalin. Similar findings were reported by Rehman *et al*. (2016) and Noble *et al*. (2003). The whole length of the spawn run is 13 to 19 days. Our results support the conclusion of Ram and Kumar (2010), who reported that within 20 days mushroom bags were completely colonized by mushroom mycelium. The treatment T5 (CP+FYM+RH steam pasteurisation), which was followed by treatment T1 (CP+FYM+RH 2% formalin), produced the highest yield (Table 2). Similar findings were reported by Chandra *et al*. (2014), Rehman *et al*. (2016), Dhar *et al*. (2006), Colak (2004), and Kaur and Rampal (2017). The largest pileus diameter was observed in T5 CP+FYM+RH steam pasteurisation (Table 3). The longest stipe ever measured was in treatment T5 (Table 4).

Estimation of cost of cultivation, gross income, net profit and benefit-cost ratio

A high cost-benefit ratio of 1.48 was shown by T5 and T6 (Table 5). Similar results were found by Barmon *et al*. (2012) who found mushrooms to be a profitable agricultural enterprise with a benefit-cost ratio of 1.55. Netam *et al*. (2018) found that mushroom cultivation under controlled conditions revealed a total benefit-cost ratio of 1.98. The use of family labour for different operations may further reduce the expenditure making the farmer self-confident in raising the crop with great remuneration.

Table 2: Impact of various casing materials on overall weight (g).

Treatments	Combinations	1st Flush	2nd Flush	3rd Flush	Total weight
T0	COCOPEAT	166.66	170.00	180.00	516.66
T1	CP+FYM+RH (2% Formaldehyde)	243.33	246.66	256.66	746.65
T2	CP+FYM (2% Formaldehyde)	220.00	223.33	233.33	676.66
T3	CP+RH (2% Formaldehyde)	196.66	200.00	210.00	579.66
T4	RH+FYM (2% Formaldehyde)	183.33	186.66	196.66	566.65
T5	CP+FYM+RH (Steam pasteurization)	266.66	270.00	280.00	816.66
T6	CP+FYM (Steam pasteurization)	230.00	233.33	243.33	739.33
T7	CP+RH (Steam pasteurization)	203.33	206.66	216.66	626.65
T8	RH+FYM (Steam pasteurization)	190.00	193.33	203.33	586.66
S.Em.±		6.99	8.14	10.00	-
C.D. (5%)		20.98	24.34	30.02	-

CP= Cocopeat, FYM= Farmyard manure, RH= Rice husk.

Table 3: Impact of different casing materials on the diameter of pileus (mm).

Treatments	Combinations	1st Flush	2nd Flush	3rd Flush
T0	COCOPEAT	25.00	20.00	21.23
T1	CP+FYM+RH (2% Formaldehyde)	39.43	34.00	36.00
T2	CP+FYM (2% Formaldehyde)	36.33	30.33	32.00
T3	CP+RH (2% Formaldehyde)	31.86	26.00	29.30
T4	RH+FYM (2% Formaldehyde)	29.33	22.00	24.93
T5	CP+FYM+RH (Steam pasteurization)	44.00	36.00	38.00
T6	CP+FYM (Steam pasteurization)	38.10	32.33	34.00
T7	CP+RH (Steam pasteurization)	33.53	28.33	30.00
T8	RH+FYM (Steam pasteurization)	30.33	24.00	27.23
S.Em.±		1.44	0.56	0.41
C.D. (5%)		4.31	1.69	1.23

CP= Cocopeat, FYM= Farmyard manure, RH= Rice husk.

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Table 4 : Impact of different casing materials on the length of the stipe (mm).

Treatments	Combinations	1st Flush	2nd Flush	3rd Flush
T0	COCOPEAT	11.33	8.00	6.66
T1	CP+FYM+RH (2% Formaldehyde)	24.83	20.66	17.16
T2	CP+FYM (2% Formaldehyde)	20.00	16.53	13.06
T3	CP+RH (2% Formaldehyde)	17.00	12.66	10.00
T4	RH+FYM (2% Formaldehyde)	14.00	9.16	7.60
T5	CP+FYM+RH (Steam pasteurization)	27.33	23.36	21.23
T6	CP+FYM (Steam pasteurization)	22.10	18.20	15.06
T7	CP+RH (Steam pasteurization)	18.66	14.33	11.90
T8	RH+FYM (Steam pasteurization)	15.66	10.86	8.66
S.Em.±		0.86	0.85	0.78
C.D. (5%)		2.62	2.54	2.34

CP = Cocopeat, FYM = Farmyard manure, RH = Rice husk.

Table 5 : Economics of different combinations.

Treatments	Combinations	COC	GMR	NMR	B:C
T0	COCOPEAT	97.5	103	5.5	1.05
T1	CP+FYM+RH (2% Formaldehyde)	110	149	39	1.35
T2	CP+FYM (2% Formaldehyde)	100	135	35	1.35
T3	CP+RH (2% Formaldehyde)	100	116	16	1.16
T4	RH+FYM (2% Formaldehyde)	95	113	13	1.18
T5	CP+FYM+RH (Steam pasteurization)	110	163	53	1.48
T6	CP+FYM (Steam pasteurization)	100	148	48	1.48
T7	CP+RH (Steam pasteurization)	100	125	25	1.25
T8	RH+FYM (Steam pasteurization)	95	117	22	1.23
S.Em.±		-	5.06	5.06	0.01
C.D. (5%)		-	15	15	0.03

CP = Cocopeat, FYM = Farmyard manure, RH = Rice husk, COC= Cost of cultivation; GMR = Gross monetary return; NMR = Net monetary return; B:C = Benefit-cost ratio.

CONCLUSION

The outcomes from various casing blends conclude that reintroducing fresh casing mixtures in mushroom production is feasible. Given that Punjab is a major rice-growing region, farmyard waste and rice husks are both readily available there. Economically, treatments 5 and 6 exhibited the highest BC ratio and benefited farmers the most. In order to grow white button mushrooms, the mixtures Cocopeat + Farmyard manure + Rice Husk and Cocopeat + Farmyard manure can be employed.

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Research Article

Incidence of *Botryosphaeria dothidea* on mango fruits

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ABSTRACT

Mango (*Mangifera indica* L.) is the world's fifth most important fruit crop with production of about 54.83 million metric tonnes. India contributes over 45 per cent to Global production, where Uttar Pradesh is the number one mango producer state with productivity of 17.14 tonnes/ha and production of around 4.55 million tonnes. Occurrence of black tip disorder of mango fruits is common in India but similar symptoms on the shoulder region of mango *cv.* Langra fruits followed by the dropping of affected fruits was first observed at Saharanpur in 2012. Incidence of disease was regularly monitored in several districts of Uttar Pradesh and pathogenic involvement of *Botryosphaeria dothidea* was confirmed. The incidence of *B. dothidea* was found widespread on mango *cvs.* Langra, Chousa, Dashehari, and it was also found highly pathogenic to mango *cv.* Amrapali in pathogenicity test. *In-vitro* testing indicated that the pathogen can be managed by the application of commonly used pre-harvest spray fungicides (propineb, propiconazole, and difenoconazole).

***Keywords*:** Mango, black tip, *Botryosphaeria dothidea,* fruit rot, propineb, propiconazole, difenoconazole.

Mango (*Mangifera indica* L.) is the world's fifth most important fruit crop. Global production of mango fruits was estimated at about 54.83 million metric tonnes (Statista, 2020). In India, the production of mango was estimated at 20.386 million tonnes from a cropped area of 2.317 million hectares during 2020-21 (Anonymous, 2022). Uttar Pradesh has the highest productivity (17.14 tonnes /ha) and production (4.55 million tonnes) of mango in India (Anonymous, 2018). The black tip of mango fruits has been considered as a disorder caused by pollutants emitted from brick kilns (Ram 1989; Zhang *et al*., 1995). The symptoms of this disorder appear as characteristic necrosis and blackening of mango fruits at the distal end and no pathogenic involvement was reported in the past. However, we observed similar symptoms on the shoulder region of mango *cv.* Langra fruits at Saharanpur during July 2012 (Fig. 1) followed by the dropping of affected fruits. We regularly monitored the problem, isolation efforts were made and the study could be concluded during 2020 after confirming the pathogenic association of *Botryosphaeria dothidea* in the black tip portion during the later stages of fruit development.

Fig. 1. Black tip type symptoms recorded during 2012

The members of the Botryosphaeriaceae family, *viz. Botryodiplodia theobromae* (Prakash and Srivastava, 1987), *Botryosphaeria ribis* (Prakash and Eckert, 1992), *Lasiodiplodia theobromae* (Shabaz *et al*., 2009) and *Neofusicocum mangiferae* (Phillips *et al*., 2013) have been reported to cause dieback, twig drying and fruit rot of mango in India. *Botryosphaeria dothidea* has been reported to cause severe fruit rot of mango in Australia (Slippers *et al*., 2005), Brazil (Marques *et al*., 2013) and Taiwan (Ni *et al*., 2010); leaf spot in India (Rabari *et al*., 2015); and gummosis followed by decline of mango trees in China (Mo *et al*., 2013). Pre- and post-harvest diseases of mango caused by different fungi including *B. dothidea* have been a great constraint, particularly in areas of rain during fruit maturity (Diedhiou *et al*., 2007; Prakash, 2004; Prakash *et al*., 2011; Shukla *et al*., 2018). Keeping in view the different symptoms observed on fruits of mango followed by the dropping of affected fruits, we have conducted systematic studies to understand the disease and establish the pathogenic potential of *B. dothidea* on mango fruits.

MATERIALS AND METHODS

Surveys and collection of infected fruits

Mango orchards located in districts of Uttar Pradesh were surveyed from 2012 to 2020 for the incidence of diseases with mango crop. Symptoms and extent of the incidence of fruit diseases were recorded in orchards during April to

August every year and samples were brought to the laboratory for isolation of associated pathogens. The incidence (%) of diseases was calculated by the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of fruits having disease symptoms}}{\text{Total number of fruits observed}} \times 100$$

Isolation of pathogen

Fruit samples collected from orchards were kept in tagged paper bags before isolation. For isolation, a technique described by Armentrout and Baudoin (1990) was adopted. Infected fruits were divided into three categories based on the stages of symptoms development and used for the isolations. The first category included samples with grayish small lesions and hard tissue in the shoulder portion of immature fruits. The fully developed mature fruits were kept in the second category which had enlarged black lesions and comparatively softer diseased tissue. The third category of fruit samples were having black enlarged lesions with softer tissues and cracked epicarp after initiation of the ripening process (Fig. 2a & b). Isolations were also made from other types of symptoms. Fruit samples were thoroughly washed in a gentle stream of tap water. Small bits of pulp was taken after removing the peel of the lesion on the fruit and were transferred to potato dextrose agar (PDA) amended with streptomycin sulfate (300 ppm). Isolation was also done from the black tip at the distal end of the fruits and from dark brown to black disease spots on black tip symptom-free fruits in a similar manner. The plates were then incubated at 25 ± 2°C in the BOD incubator for initiation of fungal growth. After 1-3 days, as the growth of the fungus was observed around the incubated tissue, mycelial tips were transferred to fresh PDA to develop pure cultures.

Fig. 2a Black tip affected fruits of mango *cv.* Dashehari used for isolation during 2019.

Fig. 2b Shoulder black affected fruits of mango *cv.* Langra used for isolation during 2019.

Pathogenicity test

Koch's postulates of two *B. dothidea* isolates were established through wetting of surface sterilized mature mango fruits *cv.* Amrapali with a 15-day-old culture suspension on the tree itself (Koch 1884). No physical injury was made on the fruit epicarp. Ten replicate fruits for each isolate along with the control were enveloped with perforated polythene bags separately for 48 hours after inoculation and observations were made every day.

Molecular characterization

The DNA isolation of fungal isolates which have successfully produced symptoms upon artificial inoculation was performed using the dried mycelium mat obtained by inoculating a small mycelial bit of fungal isolates in potato dextrose broth (HiMedia Laboratories Pvt Ltd, Mumbai) followed by incubation at 25°C±1 for 7-10 days. The collected fungal mat was crushed into fine powder in a sterilized pestle mortar using liquid nitrogen. The fungal DNA was extracted using HiPurA™ Fungal DNA Purification Kit (Himedia) following the manufacturer's instructions. The amplification of the ITS region including the 5.8S gene of the ribosomal RNA of the pathogen was done using the primers ITS1 (52 - TCCGTAGGTGAACCTGCGG-32) and ITS4 (52 - TCCTCCGCTTATTGATATGC-32) (White *et al*., 1990). PCR was performed using 25 µl 2X EmeraldAmp MAX PCR Master Mix (Takara), 1 µl of each primer (10 mM), 2 µl of DNA template. The final volume was adjusted to 50 µl using nuclease-free sterile distilled water. The PCR amplification procedure followed was 94 °C denaturation for 3 min, 94 °C denaturation for 15 s, 52 °C annealing for 40 s, 72 °C extension of 1 min, 35 amplification cycles, and 72 °C extension for 5 min. The PCR product was further sequenced from Eurofins Genomics India Pvt Ltd. The sequences thus obtained were subjected to NCBI nBLAST analysis and submitted to the NCBI database. The accession numbers were thus obtained.

***In vitro* evaluation of fungicides**

Commonly used fungicides, effective against fruit rot diseases of mango, *viz*. difenoconazole @ 0.1 percent (Score 25 EC, Syngenta India Limited), propineb @ 0.2 percent (Antracol 70 WP, Bayer Crop Science) and propiconazole @ 0.1 percent (Tilt 25 EC, Crystal Crop Protection Ltd) were evaluated under laboratory conditions at lower concentrations (0.05 and 0.025%) using food poison technique (Falck 1907; Grower and Moore, 1962). Five mm mycelium discs were transferred on PDA amended with fungicides at desired concentrations in each Petri plate 24

hours after pouring. Five replicates were taken for each treatment along with control (without fungicide). The plates were then incubated at 25±1 °C in the BOD Incubator and data were recorded at 10 days after inoculation. Mycelial growth inhibition was calculated using the formula:

$$\text{Mycelial growth inhibition (\%)} = \frac{\text{Mycelial diameter in control} - \text{Mycelial diameter in treated}}{\text{Mycelial diameter in control}} \times 100$$

Statistical analysis

We used a completely randomized block design with four replicates. The data were analyzed by ANOVA using OPSTAT software (Sheoran *et al.*, 1998), and the differences among the treatments were tested by the least significant difference test at P≤0.05.

RESULTS AND DISCUSSION

Black tip disease of mango fruits has been known as a non-pathogenic disorder (Ram, 1989; Zhang *et al.*, 1995). This disorder is characterized as necrosis and blackening of mango fruits at the distal end, however, we have recorded the incidence of the same symptoms in the shoulder region of mango fruits. Over 50 percent of the affected fruits were dropped down during developmental stages, however, remaining sustained on the tree till maturity. Since such symptoms were never before documented; we regularly monitored and ultimately confirmed the pathogenic involvement of *B. dothidea*.

Black tip-like symptoms on the shoulder region near the stem end of the developing fruits of mango *cv*. Langra were first time recorded during the survey in Saharanpur district. The lesions were grayish-black in colour, 4-14 mm in diameter, irregular in shape, depressed, and hard. Dropping of such fruits was observed at over 50 per cent, however, the remaining fruits sustained till maturity. The underlying tissues of some of the affected mature fruits became soft and eventually, the fruits dropped. The incidence of such symptoms was recorded at 0.3% in Amroha, 1% in Bijnor, 0.1% in Moradabad, 0.5% in Muzaffarnagar, 0.4% in Meerut and 1.0-1.5% in Saharanpur district however, it was rare in Ayodhya, Barabanki, Lucknow, Sitapur, Unnao districts. The severity of these symptoms was found more on *cv*. Langra followed by *cv*. Chausa (<0.1%) but very rarely on *cv*. Dashehari.

No pathogen could be isolated from the fruit samples belonging to category 1 (described in the materials and method section), hence it is considered a black tip similar symptom. However, we have isolated *B. dothidea* from category 2 and 3 symptoms. The fungus was constantly obtained from such fruit samples; as well as from the lesions without black tip-like symptoms on fruits collected from Lucknow and neighboring districts. The growth of the fungus in PDA was initially observed as white up to 6-9 days and later it gradually became grayish to black in the next 6-7 days (Fig. 3). Colony characters of *B. dothidea* isolated from Bijnor and Saharanpur were found slightly different and both were maintained. However, morphological and molecular studies indicated no difference.

Fig. 3. Culture of *B. dothidia.*

Results of the pathogenicity test indicated that both the isolates were highly virulent pathogens. A period of 72 hours was enough for the fungus to colonize the epicarp and reach the pulp (Fig. 4). First fruit drop was also recorded 72 hours after inoculation. All the replicates were picked 5 days after inoculation for observation and re-isolation of the pathogen. All the replicates of both the fungal isolates were found infected but no infection was observed in control fruits. Fungal colonies with similar morphology were obtained upon re-isolation. The results presented here have confirmed the association of *B. dothidea* with the symptoms described above.

Fig. 4. Infection of *B. dothidia* in artificially inoculated fruits of mango *cv.* Amrapali

An amplification product of ~600 bp was obtained in the case of both isolates. The sequences have been submitted in the NCBI database vide accession numbers MW259067 (Saharanpur isolate) and MW259068 (Bijnor isolate). Upon

BLASTn analysis both the isolates were identified as *Botryosphaeria dothidea*. The Saharanpur isolate (MW259067) showed maximum similarity (99.65%) with an isolate from Sri Lanka (JX139033.1), however, the Bijnor isolate (MW259068) showed 100 percent similarity with the isolates from Tamil Nadu, India (KY702575.1); Malaysia (KM998726.1), Sri Lanka (JX139033.1, KM357558.1) and Iran (KR816212.1). A phylogenetic tree was constructed using the MEGA X software (Fig. 5). The tree has divided all the isolates into two major clusters separating the *Lasiodiplodia theobromae* and *B. obtusa* from *B. dothidea* and other species of *Botryosphaeria viz*., *B. scharifii* and *B. berengeriana*. The present isolates were clustered along with all the other *B. dothidea* isolates collected from different parts of the world.

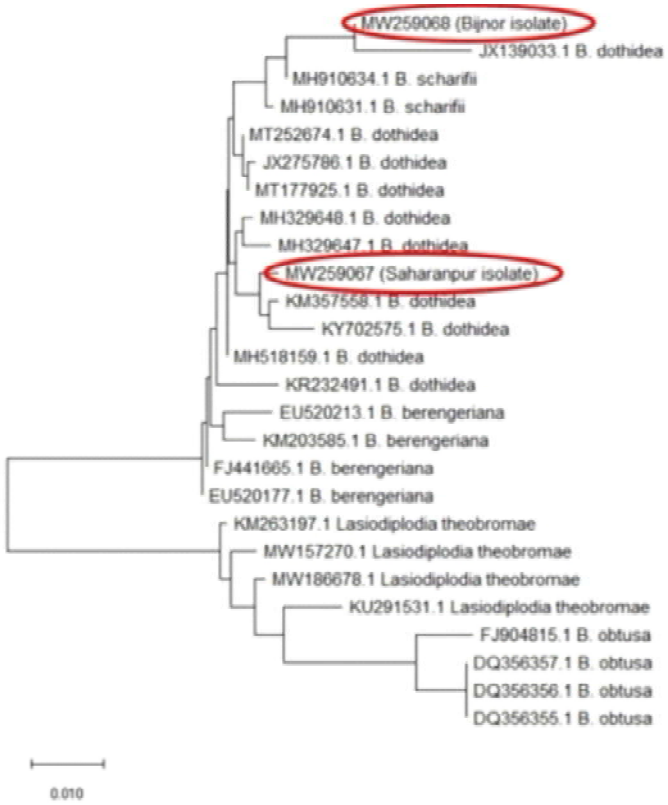

Fig. 5. Phylogentic tree generated from ITS region of wilt causing pathogens including CISH isolate.

We understand that the initial symptoms were non-pathogenic disorders which later proved to be a soft target for the fungus to cause infection. It was hypothesized that as *B. dothidea* is an opportunistic pathogen, therefore the black tip type symptoms which could be a disorder as we could not isolate any microbe in early stages, have triggered the secondary infection by *B. dothidea* due to the co-occurrence of rains and maturity of fruits. It was also noticed that the fruits which were not showing the black tip type symptoms on the shoulder region of fruits at the unripe stage majority of those remained free from the infection of *B. dothidea* after ripening. This observation has supported our hypothesis. *B. dothidea* has been recently recognized primarily as an endophyte of the healthy tissue of woody plants and remains dormant until the onset of suitable conditions (Marsberg *et al*., 2016). The same may be true for necrotic tissues on fruits, where due to high levels of acidity, *B. dothidea* might have not been able to cause infection in immature fruits during the early stages of symptom development. It is well known that as the black tip symptoms get older, the chemical composition of the underlying tissues of the mesocarp changes, and it becomes sweeter as it also happens during the ripening of fruits. The citric acid and malic acid are reduced; and the content of glucose, fructose, and sucrose is increased with the maturity and ripening stage of fruits (Medlicott and Thompson, 1985). Thus, the isolation of fungus in later stages might be possible due to the active growth of *B. dothidea* with the increase in the sugar content of fruits. The hypothesis is also supported by our observation of symptoms developed on the ripening of fruits and the presence of *B. dothidea* in such symptoms. Among various pathogens of mango, the members of the Botryosphaeriaceae family, *viz. Botryodiplodia theobromae* (Prakash and Srivastava, 1987), *Botryosphaeria ribis* (Prakash and Eckert, 1992), *Lasiodiplodia theobromae* (Shabaz *et al*., 2009) and *Neofusicocum mangiferae* (Phillips *et al*., 2013) have been reported to cause dieback, twig drying and fruit rot of mango in India. *Botryosphaeria dothidea* have been reported to cause severe fruit rot of mango in Australia (Slippers *et al*., 2005), Brazil (Marques *et al*., 2013) and Taiwan (Ni *et al*., 2010) but, it was the first report of *B. dothidea* causing fruit rot in India. The single previous report on *B. dothidea* from India was on mango leaf spots (Rabari *et al*., 2015). The isolation of the fungus achieved in later stages of fruit maturity after the onset of monsoon is also supported by previous reports (Diedhiou *et al*., 2007; Prakash, 2004; Prakash *et al*., 2011; Shukla *et al*., 2018).

Since, the pathogen, *B. dothidea* was identified for the first time in Uttar Pradesh, it was found highly virulent in the pathogenicity test and it was also found widely distributed in mango growing areas, efficacy of commonly used pre-harvest spray fungicides for the management of mango fruit rot was evaluated. The fungicides like difenoconazole, propiconazole, metalaxyl + mancozeb, myclobutanil, carbendazim, thiophanate methyl, *etc*. have been used for the pre-harvest spray to reduce postharvest fruit rot in India (Prakash, 2004; Shukla *et al*., 2018). We

applied the three most popular fungicides for testing against *B. dothidea* and found them effective. In the bioassay test, the growth of fungus, *B. dothidea* in untreated Petri plates reached the maximum (82-90 mm) on the 10th day after inoculation and varied degree of suppression was recorded in treated plates (Table 1, Fig. 6). Propiconazole @ 0.025 per cent was highly suppressive to both the isolates. Difenoconazole @ 0.025 per cent was highly effective against Bijnor isolate but moderately against Saharanpur isolate. Propineb was also found effective @ 0.05 per cent but less effective than the first two fungicides (Fig. 6, Table 1).

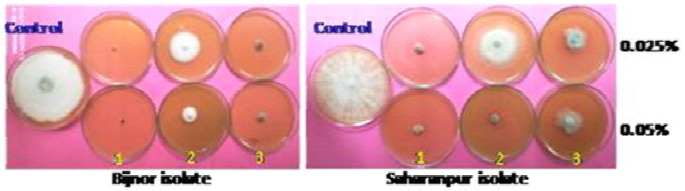

Fig. 6. Effect of fungicides (propiconazole (1), propineb (2), difenoconazole (3) on radial growth of *B. dothidia* isolates in Bijnor and Saharanpur isolates.

Table 1: Radial growth (mm) of *B. dothidia* on PDA treated with fungicides (10 days after inoculation).

Treatments	Bijnor isolate		Saharanpur isolate	
	0.025%	0.050%	0.025%	0.050%
Control	86.0[a]	86.0[a]	89.0[a]	89.0[a]
Difenoconazole	3.0[d]	0.0[d]	12.0[d]	10.0[c]
	(96.5)*	(100.0)	(86.5)	(88.8)
Propiconazole	43.0[b]	21.0[b]	65.0[b]	12.0[c]
	(50.0)	(75.6)	(27.0)	(86.5)
Propineb	11.0[c]	10.0[c]	33.0[c]	35.0[b]
	(87.2)	(88.4)	(62.9)	(60.7)
C.D. at 5%	2.01	1.81	2.52	2.38

Superscripts over number indicate statistically significant differences among different alphabets,
Transformed values are given in parentheses.

CONCLUSION

The black tip-type symptoms were recorded near the stem end on the shoulder region of fruits and the pathogenic association of *B. dothidea* was confirmed at the maturity stage of fruits. The black tip-affected fruits were considered more susceptible to *B. dothidea* due to reduced acidity in comparison to black tip-free fruits. The fungicides, difenoconazole, propiconazole, and propineb found effective against *B. dothidea* may help in reducing inoculum build-up and disease incidence by the pre-harvest spray a month prior to harvesting.

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Research Article

Molecular characterization of *Macrophomina phaseolina* from black gram seeds

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ABSTRACT

Diseases are one of the most significant restrictions in commercial crop production, as they cause significant productivity losses. Black gram, like other crops, is susceptible to biotic (fungi, bacteria, and viruses) as well as abiotic stresses. Fungi *Macrophomina phaseolina,* have been linked to black gram seeds. Genetic identification was done to identify the fungal pathogen at the species level. The dominant mycoflora developed on black gram seed samples during the seed mycoflora study was separately cultured on Potato Dextrose Agar and was used. Molecular identification through Internal Transcribed Spacer (ITS) rDNA region of dominant seed mycoflora isolated from black gram cultivars/genotypes revealed 95.46 per cent similarities with *Macrophomina phaseolina* isolate.

Keywords: Black gram, cultivar, mycoflora, internal transcribed spacer, *Macrophomina phaseolina.*

Black gram belongs to the Leguminosae or Fabaceae family. Black gram or urdbean is reported to have originated in India (De Candolle, 1986; Piper and Morse, 1914). Black gram is an important legume crop in Asia that has many nutritional benefits and provides easily digestible high-quality protein (Ghafoor and Ahmad, 2005). It is also a green manuring crop. Black gram seeds are highly nutritious with protein (25-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids, and vitamins. Black gram has been distributed mainly in tropical and sub-tropical countries, where it is grown mainly in the summer season. It is grown in India, Pakistan, Sri Lanka, Burma, and some countries in South East Asia. In India, black gram is very popularly grown in Andhra Pradesh, Bihar, Madhya Pradesh, Maharashtra, Uttar Pradesh, West Bengal, Punjab, Haryana, Tamil Nadu and Karnataka, and it produces about 23.4 lakh tonnes of black gram annually from 46.7 lakh hectares of area, with average productivity of 501 kg per hectare in 2020-21 (agricoop.nic.in). Blackgram area accounts for about 15.7 per cent of India's total pulse acreage and contributes 9.09 per cent of total pulse production. In Gujarat, black gram is grown in an area of 1.17 lakh ha with a production of 0.88 lakh tonnes during 2020-21 with a productivity of 746 kg\ha^{-1} (Anonymous, 2022).

Diseases are one of the most significant restrictions in commercial crop production, as they cause significant productivity losses. Black gram, like other crops, is susceptible to biotic (fungi, bacteria, and viruses) as well as abiotic stresses. Fungi such as *Aspergillus flavus, A. niger, Alternaria alternata, Macrophomina phaseolina, Colletotrichum lindemuthianum, Curvularia* spp., *Penicillium* spp., *Cladosporium cladosporioides, Fusarium* spp., *Drechslera* spp., *Rhizopus* spp. have been linked to black gram seeds (Rahman *et al.,* 1999; Shailbala and Tripathi, 2004; Rathod *et al.,* 2012).

MATERIALS AND METHODS

The procedure was undertaken at the Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand during 2021-22. For this purpose, genetic identification was done to identify the fungal pathogen, and Internal Transcribed Spacer (ITS) rDNA was employed to identify the fungal pathogen at the species level.

For DNA extraction, the pathogen was grown on Potato Dextrose Agar medium at 25 ± 2 °C for 8 days. Mycelia were harvested by filtering through Whatman filter Paper No. 1, washed repeatedly with distilled sterilized water to remove excess salts adhering to it. DNA extraction was based on the cetyle trimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980). Mycelium (1 g) was crushed in liquid nitrogen and transferred into 7.5 ml pre-warmed (65 °C) DNA extraction buffer [1 M TrisHCl (pH 8.0), 5 M NaCl, 0.5 M EDTA (pH 8.0) and 2% CTAB], mixed well and incubated in a water bath at 65 °C with gentle shaking for 45 min. An equal volume of chloroform: isoamyl alcohol (24:1 %v/v) was added and mixed gently to denature proteins and centrifuged at 12,000 rpm for 15 min. at 4 °C. DNA was

precipitated with 0.6 volume of chilled ethanol and 0.1 volume of 3 M sodium acetate (pH 5.2) and centrifuged at 18,514 g for 15 min. The pellets were washed twice with chilled 70% ethanol, dried at room temperature, and re-suspended in 100 ìl sterile TE (10 mM Tris-HCl buffer and 1 mM EDTA-pH 8). Two microliter DNase-free RNase was added to dissolved DNA stock and incubated in a water bath at 37 °C for 45 min. followed by 65 °C for 10 minutes for enzyme inactivation. The DNA was quantified and qualified using NanoDrop Spectrophotometer and agarose gel electrophoresis. The sample was stored in a deep freezer at -20 °C temperature for long-term usage.

The dominant seed mycoflora was identified tentatively on a morphological level and was characterized at the molecular level using rDNA-ITS region sequencing. The ITS 1 and ITS 4 primer pair were used for PCR amplification of the pathogen. The PCR reaction contained 50 ng genomic DNA, 1X PCR buffer (Thermo Scientific), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 ìM of each primer, and 1.0 unit Dream Taq DNA Polymerase (Thermo Scientific) in a 50-ìL reaction volume. The PCR program was: one cycle at 94 °C for 2 min., 35 cycles at 94 °C for 30 min., 55 °C for 1 min. and 72 °C for 1 min. and one cycle of 72 °C for 10 min. and then held at 4 °C. The sequencing of the amplified product was carried out in an automated sequencer ABI 3730 genetic analyzer at Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka, India.

The sequence identity matrix of dominant mycoflora was generated using Bioedit Sequence Alignment Editor (version 5.0.9) (Hall, 1999). After multiple alignments, phylogenetic analysis was done in MEGA 4.0 (Tamura *et al*., 2007) software using the default parameters of one character-based algorithm. The bootstrapped consensus phylogenetic tree was generated for each of these algorithms with 500 replication.

RESULTS AND DISCUSSION

The ITS region of fungal DNA was identified at the molecular level for the dominant fungus *Macrophomina phaseolina* (rDNA).

DNA extraction and quantification

The CTAB (Cetyl Trimethyl Ammonium Bromide) DNA extraction methodology was used to isolate DNA, as described in the materials and methods section. The amount of DNA in the sample (1734.98 ng/µl) was measured using NanoDrop Spectrophotometer at A260 nm/A280 nm and A260 nm/A230 nm. For further examination, samples with a high concentration of DNA and no RNA/protein contamination were chosen.

Polymerase chain reaction (PCR)

The ITS rDNA region was amplified using universal primer pairs ITS1 and ITS4 for PCR amplification. The primer combination of ITS1 and ITS4 produced an amplicon of 565bp for *M. phaseolina*. The amplified product was analyzed on 1.8 per cent agarose gel.

Sequencing result of sample

The results were aligned and examined in the NCBI nucleotide BLAST to determine the similarity of newly sequenced samples to the GenBank databases, and they were matched at various global similarity levels. The ITS rDNA sequence of the sample was confirmed as *M. phaseolina* region of specific (Accession No. ON778591) covered the internal transcribed spacer-1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer-2, complete sequences, and 28S ribosomal RNA gene, partial sequence. A BLAST search for similarities of *M. phaseolina* identification showed 95.46 per cent and the percentage of similarity of the isolates ranged from 95.22 to 95.84 per cent. Therefore, the species name assigned was according to the closest BLAST search. The pathogen of interest showed 95.46 per cent similarity with the already reported *M. phaseolina* (MK113066.1) sequence.

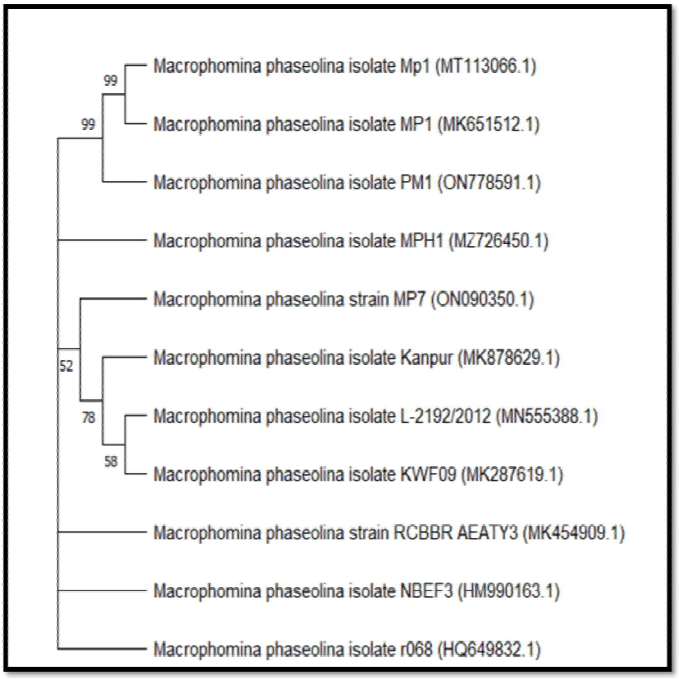

Fig. 1. Phylogenetic tree based on the sequence of rDNA ITS region of *M. phaseolina*

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Bhupathi and Theradimani (2018) reported that among the seven isolates screened, K2m7 collected from Kallipatti in Namakkal district was identified as a virulent culture. Variability among the seven *M. phaseolina* isolates analyzed by RAPD-PCR technique grouped them into two clusters. Cluster A had six isolates and Cluster B had one isolate with a genetic similarity of 15-64 per cent.

Phylogenetic analysis

The phylogenetic tree was constructed with nucleotide sequence from the sequenced ITS region of *M. phaseolina* (Fig. 1) and compared with other similar worldwide fungal isolates available in the NCBI database.

CONCLUSION

The dominant mycoflora developed on black gram seed samples during the seed mycoflora study was separately cultured on PDA and was used. Molecular identification through ITS rDNA region of dominant seed mycoflora isolated from black gram cultivars/genotypes revealed 95.46 per cent similarities with *Macrophomina phaseolina* isolate.

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Research Article

Role of new fungicide pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC)) in the management of powdery mildew and anthracnose diseases in grapes

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ABSTRACT

Grape is a fruit crop of commercial significance in the arena of Indian horticulture. Powdery mildew (c.o. - *Erysiphe necator*) and anthracnose (c.o. - *Elsinoe ampelina/ Colletotrichum gloeosporioides*) are the most important biotic constraints in grapes. The combination fungicide pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC) was evaluated for two seasons (2016-17 & 2017-18) in the vineyards of ICAR-National Research Centre for Grapes, Pune, Maharashtra against both the diseases. Different concentrations of pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC) *viz*., 400, 500 and 600 ml\ha^{-1} were compared with its component fungicides, pydiflumetofen 7.5% and difenoconazole 12.5% w/v (200 SC) separately. Experimental results showed that pydiflumetofen (7.5% + difenoconazole 12.5% w/v (200 SC) applied @ 600ml ha^{-1} manifested the lowest pooled PDI of 10.00 in the case of leaves and 10.31 in case of bunches, respectively with a corresponding yield of 10.99 t ha^{-1}. The test fungicide was not phytotoxic to the grapevines up to the dose of 1200 ml ha^{-1}. Against anthracnose, the pooled data showed that pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) @ 600 ml ha^{-1} as foliar spray showed the lowest PDI of 10.81. Hence, the treatment pydiflumetofen (7.5% + difenoconazole 12.5% w/v (200 SC) @ 500-600 ml ha^{-1} was effective against powdery mildew as well as anthracnose.

***Keywords*:** Powdery mildew, Pydiflumetofen, Difenoconazole, grapes, anthracnose.

Grape is an important fruit crop in India with production of 3229 thousand MT in the year 2021-22 from an average 152 thousand ha cultivated area (Anonymous, 2021). Powdery mildew (c.o- *Erysiphe necator* previously known as *Uncinula necator*) is one of the most important diseases in grapes limiting the commercial production of the crop in most of the places in India. Powdery mildew appears on leaves as chlorotic spots on the upper leaf surface. As spores are produced, the infected areas appear white, powdery or dusty patches. On fruit and rachis, the pathogen appears as white, powdery masses that may colonize the entire berry surface. Anthracnose caused by *Elsinoe ampelina/ Colletotrichum gloeosporioides* is characterized by lesions on shoots, leaves and berries. Lesions first appear on young shoots, showing up as small black circular spots with a yellow border that will later become larger and create grey lesions which appear sunken. Triazoles like Penconazole 10 EC @ 1 ml l^{-1} and Hexaconazole 5 EC @ 1 ml l^{-1} were most effective to control the powdery mildew disease (Thind *et al*., 2010, Narayana *et al*., 2005). Sulphur (Pearson and Goheen, 1988, Keller and Hrazdina, 1988), Sterol biosynthesis inhibitors (Yang *et al*., 2011), strobilurins (Bartlett *et al*., 2002), metrafenone (Capriotti *et al*., 2006), Polyoxin D Zinc Salt 5% (Deore *et al*., 2021) and succinate dehydrogenase inhibitors (Sierotzki and Scalliet, 2013, Stammler *et al*., 2015) have been used successfully in controlling the disease but repeated applications of chemical fungicides result in the development of pathogen resistance (Ghule *et al*., 2018) and festered environmental conditions (Thomas, 1986). Pydiflumetofen is the newest member of the succinate dehydrogenase inhibition (SDHI) family of fungicides that belongs to FRAC Group 7. Pydiflumetofen disrupts energy production by inhibiting the functioning of succinate dehydrogenase, an enzyme involved in both the citric acid cycle and the mitochondrial electron transport chain. It is a medium to high-risk fungicide exhibiting cross-resistance and is claimed to be effective against the powdery mildew of grapes. Difenoconazole (FRAC-3) belongs to the chemical group triazoles. It functions by inhibiting the sterol biosynthesis in fungal cell membranes. It is a De-Methylation inhibitor with a medium risk for resistance development. The "premix" types of products can provide better disease control due to their horizontal spectrum. It also provides disease control security if there is field resistance to one of the two active ingredients, as well as aids to prevent resistance if there is not (Reddy *et al*., 2017). In the present investigation, a ready mix formulation of Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) was tested against powdery mildew

and anthracnose of grapes to evaluate its efficacy in field conditions.

MATERIALS AND METHODS

Anthracnose

A field experiment was conducted for evaluating the bio-efficacy of Pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC) against anthracnose of grapes during 2016-17 and 2017-18 in a vineyard of Thompson Seedless variety at ICAR-National Research Centre for Grapes, Pune (latitude: 18.52°N, longitude: 73.86°E, elevation: 560 m MSL) India. The field experiment was laid out in Randomised Block Design with four replications. Eight treatments *viz.*, pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC) @ 400, 500 and 600 ml ha^{-1}, pydiflumetofen (20% SC) and difenoconazole (25% EC @ 225 and 300 ml ha^{-1}), respectively along with check fungicides Carbendazim (12%) + mancozeb (63% WP @ 1500 g ha^{-1}) and (Thiophanate methyl (70% WP) @ 715 g ha^{-1} were compared with the untreated control. Applications were made at 7-day intervals using an inter knapsack sprayer. The water volume used for sprays was 1000 l/ha and the sprays were directed at both the adaxial and abaxial leaf surfaces. Border vines were considered as guard plants and observations were recorded on the central vine. Untreated control plants were included which did not receive any fungicide application. Disease severity was recorded before the first spray application and subsequently on the 10^{th} day after each fungicide application using a 0–4 index scale, where 0 = no incidence, 1 = trace to 25, 2 = 26 to 50, 3 = 51 to 75, and 4 = more than 75 per cent leaf area infected (Horsfall and Heuberger, 1942). Five canes each were selected from the eastside and the westside arms and the disease ratings were recorded on 10 leaves on each cane.

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Number of leaves observed} \times \text{Maximum rating}} \times 100$$

The mean of percent disease index (PDI) in both seasons was calculated and percent disease control was tabulated using the formula of Vincent (1947).

$$I=C–T/C\times100$$

Where,

I=Percent disease control, C= PDI in untreated control, T=PDI in fungicide treatment.

Powdery mildew

The field experiment of bio-efficacy and phytotoxicity of pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC)) against powdery mildew of grapes was carried out during 2016-17 and 2017-18 in a vineyard of Tas-A-Ganesh variety grown on bower system of training at ICAR-National Research Centre for Grapes, Pune (latitude: 18.52°N, longitude: 73.86°E, elevation: 560 m MSL) India. The field experiment was laid out in Randomised Block Design with four replications. The test fungicide pydiflumetofen (7.5%) + difenoconazole 12.5% w/v (200 SC) @ 400, 500 and 600 ml ha^{-1}, pydiflumetofen (20% SC) @ 225 ml ha^{-1} difenoconazole 25% EC @ 300 ml ha^{-1} as component fungicide and hexaconazole 1000 ml/ha as standard check comprised the six treatments. A water-sprayed untreated control was also maintained. Fungicide application was done with the visibility of initial symptoms (65 and 68 days after fruit pruning in both 2016-17 and 2017-18, respectively). A total of four sprays including one preventive spray were given at an interval of 10 days with a knapsack sprayer. Water volume used for spray 1000 l/ha at the full canopy. The test fungicide pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC)) was supplied by Syngenta India Pvt. Ltd.

Powdery mildew incidence on leaves was recorded by adopting 0-4 scale, where 0 means no disease present and 4 means more than 75 per cent leaf area is infected (Ghule *et al.*, 2018). Percent Disease Index (PDI) was calculated by the formula of Wheeler (1969), which is mentioned before. The ratings on a minimum of 10 leaves and a bunch were recorded on randomly selected canes. Ten such canes per plant were observed. The observations on eight plants spread over four replications were recorded. Thus, the data presented on PDI is average of randomly selected 80 canes. After maturity, bunches were harvested and fruit yield was calculated in tonnes per hectare. The mean of PDI of both seasons was calculated and percent disease control was tabulated using the formula of Vincent (1947) as given above.

All data obtained were subsequently analyzed statistically by ANNOVA using SAS 9.3 software.

Phytotoxicity

Phytotoxicity observations were recorded from the powdery mildew trial. Grape vines were sprayed twice with pydiflumetofen (7.5% + difenoconazole 12.5% w/v (200 SC)) @ 600 and 1200 ml/ha. Sprayed vines were critically observed for the presence of phytotoxic effects such as chlorosis, tip burning, necrosis on leaves and berries, epinasty and russeting on berries up to 10 days after each spray. Observations were recorded at 0, 1, 3, 5, 7 and 10 days after each application in the form of visual ratings on 0-10 scale where, 0=No Phytotoxicity, 1=0-10%, 2=11- 20%, 3=21-30%, 4=31- 40%, 5=41- 50%, 6=51- 60%, 7=61- 70%, 8=71-80%, 9=81- 90%, 10=91- 100%.

RESULTS AND DISCUSSION

Anthracnose

The results indicated that all treatments were significantly superior to the untreated control. Pydiflumetofen (7.5%) + difenoconazole 12.5% w/v (200 SC) @ 500- 600 ml ha^{-1}, showed the lowest PDI values in both 2016-17 and 2017–18 i.e. 13.88 and 8.93 with mean PDC of 57.77 and 13.38 and 8.93 respectively with a mean percent disease control (PDC) of 59.97 (Table 1., Fig 1). It was followed by the dose of 500 ml/ha where the PDI values were 13.88 and 8.93 with a mean PDC of 57.77. Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) @ 600 ml/ha and @ 500 ml ha^{-1} were at par with each other. However, the lowest dose of Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) @ 400 ml ha^{-1} had a PDC of 31.24. Component fungicides *i.e.*, pydiflumetofen (20% SC) and difenoconazole (12.5% w/v (200 SC) showed a PDC of 35.31 and 46.2, respectively. Standard check fungicides, carbendazim (12%) + mancozeb (63% WP) and thiophanate methyl (70% WP) which exhibited a PDC of 41.07 and 44.66, respectively were at par with each other. The untreated control had the maximum PDI of 25.5 and 28.5 in the two consecutive seasons under study.

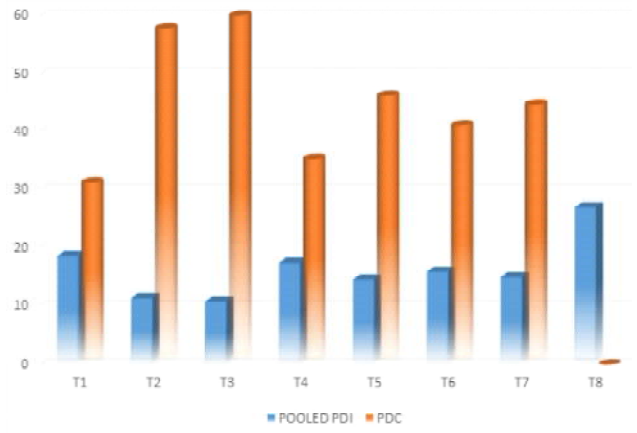

Fig. 1. Bio-efficacy of pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) against Anthracnose

Powdery mildew

In leaves, the results indicated that all treatments were significantly superior over untreated control on leaves and gave a better marketable yield/vine. The test fungicide Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) @ 500- 600 ml/ha, manifested the lowest PDI values in both 2016-17 and 2017–18 i.e. 12.38 and 8.00 with PDC 79.32 and 12.25, 7.75 respectively with a mean percent disease control (PDC) of 79.70 (Table 2., Fig 2). Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) @600 ml/ha and @500 ml/ha were at par with each other. However, it was followed by the lowest dose of Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) @400 ml/ha had a PDI of 15.25 and 22.68 during the aforementioned period with a PDC of 61.50. Component fungicides viz; Pydiflumetofen 20% SC and difenoconazole 12.5% w/v (200 SC) exhibited a PDC of 66.01 and 71.85 respectively. Standard check fungicide, Hexaconazole 5 EC which had a PDC of 72.45 was at par with the dose @300 ml/ha of difenoconazole. The untreated control had the maximum PDI of 25.5 and 28.5 in the two consecutive seasons under study. In bunches, same trend

Table 1: Bio-efficacy of Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) in the control of anthracnose of grapes after foundation pruning.

Tr. No.	Treatment details	Dose (ml ha^{-1} or g ha^{-1})	PDI of Anthracnose on leaves (%) 2016-17	2017-18	Pooled PDI	Pooled PDC
T1	Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC)	400	18.06 (25.14)^{cd}	19.05 (26.08)^{e}	18.56 (25.52)^{e}	31.24 (33.93)^{e}
T2	Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC)	500	13.88 (21.78)^{a}	8.93 (17.38)^{b}	11.41 (19.73)^{a}	57.77 (49.47)^{a}
T3	Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200SC)	600	13.38 (21.44)^{a}	8.25 (16.47)^{b}	10.81 (19.18)^{a}	59.97 (50.75)^{a}
T4	Pydiflumetofen 20% SC	225	20.06 (26.57)^{e}	14.87 (22.65)^{d}	17.47 (24.70)^{d}	35.31 (36.45)^{d}
T5	Difenoconazole 25% EC	300	15.88 (23.39)^{b}	13.18 (21.15)^{c}	14.53 (22.40)^{b}	46.20 (42.82)^{b}
T6	Carbendazim 12% + Mancozeb 63% WP	1500	19.44 (26.15)^{de}	12.37 (20.59)^{c}	15.91 (23.49)^{c}	41.07 (39.83)^{c}
T7	Thiophanate methyl 70% WP	715	17.19 (24.47)^{bc}	12.68 (20.85)^{c}	14.94 (22.73)^{e}	44.66 (41.93)^{b}
T8	Untreated control	-	25.50 (30.17)^{f}	28.5 (32.24)^{f}	27.00 (31.30)^{f}	0.00 (0.00)^{f}
	C.D. (P = 0.05)		1.12	0.75	0.66	1.83

Figures with the same letter in a column are not significantly different from each other, Figures in parentheses indicate arcsine transformed averages.

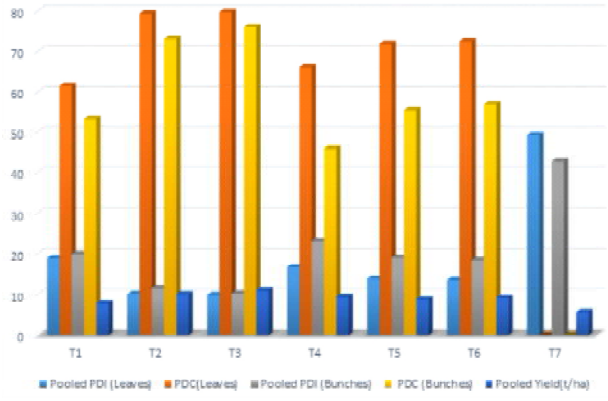

Fig. 2. Bio-efficacy of pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) against powdery mildew on leaves and bunches

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was observed. The test fungicide Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) @ 500- 600 ml/ha, showed the lowest PDI of 15.00 and 8.12 with a pooled PDC of 73.00 and 13.13 and 7.50 respectively with pooled PDC of 75.96 (Table 1). The untreated control had the highest pooled PDI of 42.81.

The test treatment pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC) @ 600 ml ha^{-1} gave the highest yield *i.e.*, 19.57 t ha-1 which was followed by @ 500 ml ha^{-1} with 17.13 t ha^{-1}. (Table 2). There was no occurrence of any phytotoxicity symptoms *i.e.*, chlorosis, wilting, vein clearing, epinasty, hyponasty, necrosis and scorching on leaves berries and after spray.

The results indicated that all treatments were significantly superior over untreated control for PDI on leaves and enhanced marketable yield per vine. The test fungicide pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC) @ 500-600 ml ha^{-1}, manifested the lowest PDI values in both powdery mildew and anthracnose in 2016-17 and 2017-18, respectively. Pydiflumetofen is a novel succinate dehydrogenase inhibitor discovered by Syngenta (Buxton *et al*., 2016). The succinate dehydrogenase (SDH) enzyme, also known as succinate ubiquinone oxidoreductase is an essential part of the tricarboxylic cycle and the mitochondrial electron transfer chain of fungal metabolism and is the primary target of SDHIs (Piqueras *et al*., 2014, Rehfus *et al*., 2016). Raut *et al*. (2020) reported that pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC) @ 0.6 ml l^{-1} was most effective against anthracnose and powdery mildew diseases in mango. Pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC) was also reported to be successful in the management of powdery mildew of tomato (Ekbote *et al*., 2019). These reports are in congruence with present findings wherein grape powdery mildew and anthracnose were successfully controlled by pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC). Pydiflumetofen is a broad-spectrum fungicide for the control of powdery mildew and Alternaria disease of vegetables, fruit crops and trees as well as wheat leaf blight and wheat (Stierli *et al*., 2019). Difenoconazole is a broad-spectrum systemic fungicide and acts as a sterol demethylation inhibitor that prevents the development of the fungus by inhibiting membrane ergosterol biosynthesis. Rawat *et al*. (2020) reported that Pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC) @ 600 ml/ha was most effective against the apple fruit scab, premature leaf fall and powdery mildew and at the same time improved yield of the apple crop. Banyal *et al*. (2020) also observed maximum average disease control as well as tuber yield in the early blight of potato after the use of Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC). Fungicide resistance not only causes a decrease in the control efficacy of fungicides on plant diseases but also increases the dosage of fungicides in the field, leading to environmental pollution and residue issues. Therefore, searching for new chemical alternatives with different modes of action and high fungicidal activity is necessary for resistance management.

Table 2: Bio-efficacy of Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC) in the control of powdery mildew on leaves and bunches of grapes after fruit pruning.

Tr. No.	Treatment details	Dose (ml/ ha^{-1})	Leaves				Bunches				Fruit Yield (t ha^{-1})		
			2016-17	2017-18	Pooled PDI	Pooled PDC	2016-17	2017-18	Pooled PDI	Pooled PDC	2016-17	2017-18	Pooled Yield
T1	Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC)	400	15.25 (22.97)^{b}	22.68 (28.44)^{e}	18.97 (25.81)^{d}	61.50 (51.65)^{d}	16.25 (23.56)^{b}	23.75 (29.07)^{d}	20.00 (26.56)^{b}	53.26 (46.87)^{b}	15.31^{cd}	12.90^{c}	14.10^{d}
T2	Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC)	500	12.38 (20.59)^{a}	8.00 (16.42)^{a}	10.19 (18.60)^{a}	79.32 (62.96)^{a}	15.00 (22.75)^{ba}	8.12 (16.41)^{a}	11.56 (19.87)^{a}	73.00 (58.70)^{a}	19.52^{ab}	17.13^{b}	18.33^{ab}
T3	Pydiflumetofen 7.5% + difenoconazole 12.5% w/v (200 SC)	600	12.25 (20.48)^{a}	7.75 (16.15)^{a}	10.00 (18.43)^{a}	79.70 (63.22)^{a}	13.13 (21.22)^{a}	7.50 (15.78)^{a}	10.31 (18.69)^{a}	75.96 (60.68)^{a}	20.17^{a}	19.57^{a}	19.87^{a}
T4	Pydiflumetofen 20% SC	225	14.44 (22.33)^{b}	19.06 (25.88)^{d}	16.75 (24.15)^{c}	66.01 (54.34)^{c}	27.50 (31.61)^{d}	18.75 (25.42)^{c}	23.13 (28.73)^{c}	45.94 (42.66)^{c}	17.26^{bc}	17.02^{b}	17.14^{bc}
T5	Difenoconazole 25% EC	300	14.31 (22.21)^{b}	13.43 (21.49)^{c}	13.88 (21.85)^{b}	71.85 (57.97)^{b}	23.12 (28.71)^{d}	15.00 (22.45)^{d}	19.06 (25.85)^{b}	55.43 (48.13)^{b}	14.86^{d}	17.21^{b}	16.04^{c}
T6	Hexaconazole 5 EC	1000	14.81 (22.62)^{b}	12.31 (20.53)^{b}	13.56 (21.59)^{b}	72.45 (58.38)^{b}	22.87 (28.39)^{c}	14.06 (21.84)^{d}	18.44 (25.40)^{b}	56.93 (48.99)^{b}	16.19^{cd}	17.32^{ab}	16.75^{bc}
T7	Untreated control	-	56.50 (48.73)^{c}	42.06 (40.43)^{f}	49.28 (44.58)^{e}	0.00 (0.00)^{e}	44.37 (41.76)^{e}	41.25 (39.96)^{a}	42.81 (40.86)^{d}	0.00 (0.00)^{d}	11.71^{e}	8.86^{d}	10.28^{e}
	C.D. (*P = 0.05*)		0.80	0.77	0.77	1.90	3.54	4.92	1.98	3.64	2.38	2.34	1.84

Figures with the same letter in a column are not significantly different from each other, Figures in parentheses indicate arcsine transformed averages.

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CONCLUSION

It was concluded that the mixture of pydiflumetofen (7.5%) + difenoconazole (12.5% w/v (200 SC)) gave a good control in anthracnose and powdery mildew of grapes and could be an effective tool for resistance management.

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Research Article

Frequency distribution of mycotoxigenic fungal contamination in maize-based dairy and poultry feeds

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ABSTRACT

Contamination of dairy and poultry feeds with mycotoxigenic fungi is a major threat to animal and human health. In Karnataka, there is rather little information about the natural occurrence of mycotoxigenic fungi and mycotoxins in feedstuffs. This study was conducted to determine the incidence of mycotoxigenic fungi especially *Aspergillus* spp. and *Fusarium* spp. in maize-based dairy and poultry feeds. A total of 48 feed samples consisting of 24 each of dairy feeds and poultry feeds were collected in major dairy and poultry farming districts of Karnataka. The feed samples were collected in a moisture-free bag with proper labelling and the collected feed samples were inoculated to the Petri dishes containing potato dextrose agar to determine the mycoflora present in the feed. Majorly five fungi genera were isolated and identified as, *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus,* and *Trichoderma*, with their isolation frequencies of 20.83, 14.58, 10.41, 12.5, and 10.41 per cent, respectively. *Aspergillus* spp. and *Fusarium* spp. were most frequently associated with feeds among the leading mycotoxigenic fungi. Hence, the present study reveals the significance of mycotoxins production in veterinary and public health as well as attending to its economic losses.

***Key words*:** *Aspergillus*, dairy, feed, *Fusarium*, poultry, mycotoxigenic fungi.

Maize is one of the most important cereal crops in the world and contributes to food security in most developing countries. In India, maize is emerging as the third most important crop after rice and wheat. Its importance lies in the fact that it is not only used as human food and animal feed but at the same time it is also widely used for corn starch in industry, corn oil production and baby corn *etc*. The increasing use of maize as feed and the increasing interest of the consumer in nutritionally enriched products which have raised the demand for maize as the core driving forces behind the emerging importance of maize crop in India (Anonymous, 2014).

Among all cereals, the most prominent source of energy is maize. Between 70 to 80 per cent of its production is used as a feed ingredient worldwide and among the raw materials involved in animal feed, maize is the main component, constituting between 50 to 70 per cent of the diets of cattle and poultry. But this cereal is often contaminated with mycotoxigenic fungi or mycotoxins in both field and storage conditions and the consumption of these mycotoxin-contaminated feedstuffs by animals and poultry birds leads to adverse effects on their health (Gremmels, 2008).

Forage and cereal crops are often contaminated by fungi in the field or during processing, transportation, and storage when conditions such as temperature and relative humidity are favourable. Temperature and relative humidity of above 30 °C and 80-100 per cent, respectively are favourable for fungal growth. Other conditions include nutrient availability (Njobeh, 2003) and oxygen supply (Filtenborg *et al*., 2000). Most of the fungi invade only a minor fraction of feed particles with appropriate conditions for their growth.

The presence of visible mould spores may not likely serve as a reliable guide to mycotoxin content (Smith and Seddon, 1998) but must not be disregarded when assessing the occurrence of mycotoxins. This is because it provides a foundation for the evaluation of the risk of mycotoxin contamination. Observations of fungi in food and animal feed are likely indications of the presence of mycotoxins and it is only through the cultivation of fungi under laboratory conditions during the presence or absence of fungi can be appreciated.

Pathogenic microorganisms and their secondary metabolites (mycotoxins) in the general chain of nutrition represent the most important potential risk to animal and human health. Because of the harmful effect of mycotoxins in the nutrition chain of humans and animals, in this paper, the presence of potentially mycotoxigenic fungi in samples of poultry and dairy feeds was determined.

MATERIALS AND METHODS

Collection of samples

The survey was conducted in the major poultry and dairy farming districts of Karnataka to collect the maize-based poultry and dairy feed samples (Table 1). A total of 48 feed samples were collected from 12 districts of Karnataka, India. Out of these 48 samples, 24 samples comprised cattle feed, and 24 samples were of poultry feed origin. Approximately 300 g each of poultry and cattle feed samples were collected in a dry, moisture-free bag. These samples were collected randomly from different sources like KMF units, milk collection units, feeds shops as well as dairy farmers. The feed samples collected were brought to the plant pathology laboratory for further processing and analysis. These samples were transported to the laboratory in moisture-free conditions for fungi isolation and identification.

Table 1: Collection of maize-based feed samples from major dairy and poultry farming districts of Karnataka

Districts	No. of samples collected
Ballari	4
Belagavi	4
Bengaluru	4
Chamarajanagara	4
Chikkaballapur	4
Davanagere	4
Dharwad	4
Kalaburagi	4
Kolar	4
Mandya	4
Mysuru	4
Raichur	4

Isolation and identification of fungi

Fungi were isolated and cultured according to the method described by Pitt and Hocking (1997). About one gram of the feed sample was diluted in 9 ml of sterile distilled water which was followed by five other serial dilutions. One milliliter of the extract was placed at random in each of the Petri dishes containing potato dextrose agar (PDA) and chloramphenicol (500 mg liter^{-1}). The pure culture was used for identification between five and seven days of incubation. Determination of each species of fungi was done by referring to the observations of Thom and Raper (1945) for *Aspergillus* spp., Rodrigues *et al*. (2007) for *Fusarium* spp., and Pitt and Hocking (1997) for *Penicillium* and other genera. This was done by observing both the microscopic characteristics of the colonies on the PDA media used as well as the microscopic morphology under a microscope. The pure culture of different isolates (identified fungi) was aseptically sub-cultured in potatoes dextrose agar slant and incubated.

RESULTS AND DISCUSSION

Feed samples were collected and the isolation of mycotoxigenic fungi from the feeds was done to know the frequency of their occurrence and the results are presented here under.

Forty-eight feed samples collected were subjected to isolation of mycotoxigenic fungi, of which 33 samples were found positive for the presence of mycoflora (Table 2). *Aspergillus* spp. was isolated from 10 feed samples followed by *Fuasrium* spp. in seven samples, *Rhizopus* from six samples, whereas *Penicillium* spp. and *Trichoderma* spp. were isolated from five samples each. Among 33 feed samples, a maximum number of samples that were detected with mycoflora contamination were collected from dairy farmers (12 feed samples), followed by feed samples collected from feeds shop (10 samples) and milk collection unit (8 samples), three feed samples were detected with mycoflora contamination which were collected from KMF (Karnataka Milk Federation) (Fig. 1).

This data will help to understand the level of mycotoxigenic fungal contamination in feeds at different stages or levels they cross till it reaches the final consumer. This observation revealed that feeds stored by dairy farmers were more likely to get contaminated with toxigenic mycoflora and this stage was considered as the most vulnerable stage for the contamination with toxigenic mycoflora. This might be due to improper storage methods followed by the farming community and also might be due to improper handling of feed samples at dairy farmers' houses apart aware among the farming community regarding mycotoxin contamination of feeds is very less, and as a result, the chances for feed contamination at dairy farmer level is more.

In this study, five fungal genera (Table 3) were isolated in both poultry and dairy feeds, which were collected from 12 major dairy and poultry farming districts of Karnataka. The frequency of isolated fungal genera includes *Aspergillus* (20.83 %), *Fusarium* (14.53 %), *Rhizopus* (12.5 %), *Penicillium* (10.41 %), and *Trichoderma* (10.41 %). This result is similar to the results obtained by Makun *et al*. (2010) and Kpodo *et al*. (2000) who reported that some of the fungal genera such as *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus,* and *Mucor* were contaminated the cereal crops (maize, sorghum, paddy) which are the ingredients of feed production.

The frequency of isolated fungal genera in feed samples presented in Table 3, reveals that the maximum number of 10 samples were contaminated with *Aspergillus* with a frequency of 28.83 per cent followed by 7 feed samples

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Table 2: List of dairy and poultry feed samples detected with the presence of mycotoxigenic fungi.

Name of the district	Sample 1	Sample 2	Sample 3	Sample 4
Ballari	**Dairy farmer**	**Feeds shop**	**Milk collection unit**	**Dairy farmer**
Mycoflora detected	*Aspergillus* spp.	*Aspergillus* spp.	*Penicillium* spp. *Trichoderma* spp.	*Rhizopus* spp.
Belagavi	**Dairy farmer**	**Feeds shop**	**Milk collection unit**	**Feeds shop**
Mycoflora detected	*Aspergillus* spp.	*Penicillium* spp. *Trichoderma* spp.	Not detected	Not detected
Bengaluru	**Feeds shop**	**Feeds shop**	**Milk collection unit**	**KMF**
Mycoflora detected	*Penicillium* spp.	Not detected	*Trichoderma* spp.	Not detected
Chamarajanagara	**Dairy farmer**	**Feeds shop**	**Milk collection unit**	**Dairy farmer**
Mycoflora detected	*Trichoderma* spp.	*Aspergillus* spp.	*Fusarium* spp.	*Rhizopus* spp.
Chikkaballapur	**Dairy farmer**	**Feeds shop**	**Milk collection unit**	**Dairy farmer**
Mycoflora detected	*Rhizopus* spp.	*Trichoderma* spp.	Not detected	Not detected
Davanagere	**Dairy farmer**	**Feeds shop**	**Milk collection unit**	**Feeds shop**
Mycoflora detected	Not detected	*Penicillium* spp.	*Aspergillus* spp. *Fusarium* spp.	*Aspergillus* spp.
Dharwad	**Dairy farmer**	**Feeds shop**	**Milk collection unit**	**Dairy farmer**
Mycoflora detected	*Fusarium* spp.	*Rhizopus* spp.	*Aspergillus* spp.	*Penicillium* spp.
Kalaburagi	**KMF**	**Feeds shop**	**Milk collection unit**	**KMF**
Mycoflora detected	*Aspergillus* spp.	Not detected	Not detected	*Fusarium* spp.
Kolar	**Dairy farmer**	**Feeds shop**	**Milk collection unit**	**Dairy farmer**
Mycoflora detected	*Fusarium* spp.	Not detected	*Penicillium* spp.	*Rhizopus* spp.
Mandya	**Dairy farmer**	**Feeds shop**	**Dairy farmer**	**KMF**
Mycoflora detected	*Aspergillus* spp.	*Fusarium* spp.	Not Detected	*Penicillium*spp.
Mysuru	**Dairy farmer**	**Feeds shop**	**Milk collection unit**	**Dairy farmer**
Mycoflora detected	*Rhizopus* spp.	*Aspergillus* spp.	*Fusarium* spp.	Not detected
Raichur	**Dairy farmer**	**Feeds shop**	**Milk collection unit**	**Dairy farmer**
Mycoflora detected	Not detected	Not detected	*Rhizopus* spp.	Not detected

contaminated with *Fusarium* to an extent of 14.53 per cent frequency. This result is similar to the results obtained by Rosa *et al*. (2009), who conducted the survey to find out the presence of mycobiota in feedstuffs and finished swine feed and to determine the ability of *Aspergillus* and *Penicillium* isolates to produce ochratoxin A (OTA). Corn, brewers' grains, and finished swine feed samples were collected from different factories. Fungal counts were higher than 2.8×10^4 CFU/g. *Fusarium*, *Aspergillus,* and *Penicillium* genera were isolated at high levels.

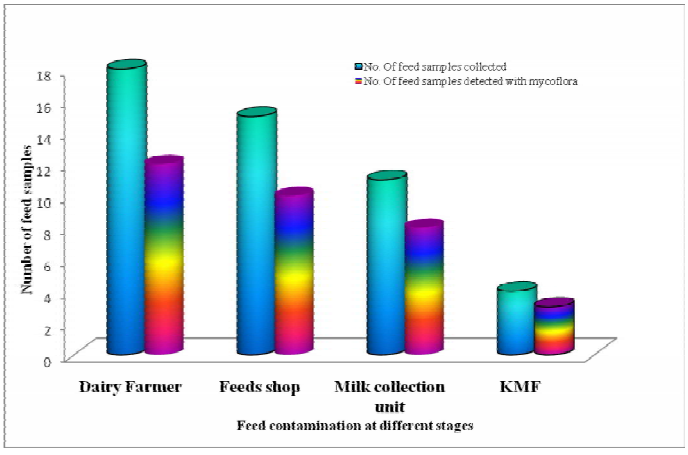

Fig. 1. Mycotoxigenic fungal contamination of dairy and poultry feeds at different stages

Data from the mycological analysis revealed that *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp., were the prevalent fungal species isolated along the corn-based poultry and dairy feed supply chain (Surenrdanatha Reddy *et al*., 2011). *Aspergillus* spp. and *Fusarium* spp. were known fungal genera, commonly isolated from corn throughout the world. Although most *Fusarium* spp., are known as field fungi and invade plant tissues in the field, under favorable conditions, some are able to grow on harvested and stored grains and *Aspergillus* has been shown to be predominant in

Table 3: Occurrence and frequency of fungal genera isolated from maize-based poultry and dairy feeds.

Contaminated mycoflora	No. of feed samples contaminated	Frequency (%)
Aspergillus spp.	10	20.83
Fusarium spp.	7	14.53
Rhizopus spp.	6	12.5
Penicillium spp.	5	10.41
Trichoderma spp.	5	10.41

cereals and other ingredients used in producing poultry, and dairy feeds in tropics (Pitt and Hocking, 1997). This may explain their prevalence along the corn-based poultry and dairy feed supply chain. The occurrence of these mycotoxigenic fungi in feeds has significant implications for livestock health as these species are potential producers of many important mycotoxins such as aflatoxin and fumonisin.

CONCLUSION

It is important to note that the impact of mycotoxigenic fungi on animals extends beyond the symptoms. The mycotoxin produced by the fungi poses a serious economic impact worldwide. The economic impact result from lowered productivity, and reduced feed conversion efficiency which causes reduced weight gain, less meat and egg production, and increased disease incidence because of immunosuppression. This study helps to identify toxigenic fungi and their occurrence in maize feeds collected from major dairy and poultry farming districts of Karnataka and it will help to understand the fungal ecology which is critical in the development of efficient and innovative control strategies.

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Research Article

Perpetuation of *Phoma naikii* in the crop debris of pigeon pea

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ABSTRACT

Infected debris such as leaves, stem, petiole, and twig could be an important source of survival for the pathogens and a source of inoculum for the subsequent spreads from one season to the next and from one area to another area. This study was conducted to determine the length of survival of *Phoma naikii* in the infected pigeon pea debris under different storage conditions *viz*., room, refrigerator, pot, and field conditions. Under pot conditions, the samples were buried under 5 and 7.5 cm, and one set was placed on the surface of the soil. These samples were subjected to isolation at 15 days intervals and incubated at 25 ±2 °C. The observations were recorded for the growth and development of pathogens in the tissues after a week. The results revealed that the frequency of recovery of the pathogen decreased over time and the fungus was capable of overwintering for 23 weeks (% reduction was 86.66-6.66) in infected debris buried in sterilized soil regardless of burial depth. It has survived for 27 weeks (% reduction was 93.33-13.33) in field conditions, 30 weeks (% reduction was 100.0-20.0) on the surface of the soil, 34 weeks (% reduction was 100.0-20.0) in room conditions and 36 weeks (% reduction was 100.00-26.66) in the refrigerated conditions. Hence, the present study indicates the variation in the viability of the pathogen in the infected debris stored at six different conditions.

***Keywords*:** *Phoma naikii*, pigeon pea, storage, survival.

Pigeonpea [*Cajanus cajan* (L.) Millsp.], a diploid legume crop species ($2n = 2x = 22$) and member of the tribe Phaseoleae and family Fabaceae. Based on the range of genetic diversity of the crop in India, Vavilov (1951) concluded that pigeon peas originated from India. Eastern Africa has been considered as a secondary center of origin (Zeven and Hukovsky, 1975). The global pigeon pea production accounts for an area of 6.99 m ha with a production of 5.96 m t and productivity of 852 kg ha^{-1} (Anonymous, 2018). In India, the total area, production, and productivity account for 4.80 m ha, 4.28 m t, and 892 kg ha^{-1}, respectively. There are numerous constraints in the production of pigeon peas. Where, the crop is known to be affected by more than 200 pathogens of which 83 are fungi (Nene *et al*., 1989) and more recently the blight of pigeon pea causal agent has been identified as *Phoma naikii* by Savitha *et al*. (2022) which is becoming a serious menace in parts of North-Eastern Karnataka due to mono-cropping. The flowering to pod formation stages is normally prone to be attacked by the disease and about 200 true *Phoma* taxa have been defined and recognized which are divided into two large groups, the first one is saprophytic or weakly parasitic (opportunistic) and the second one is a specific pathogen of cultivated plants (Vander and Van Kesteren, 1971).

The pathogen produces various manifested symptoms *viz*., leaf spot, stem canker, twig blight, blossom blight, and die back on pigeon pea, and survival of the pathogen in these infected parts plays an important role in disease epidemics. Many plant pathogenic fungi and bacteria survive during the off-season as saprophytes on diseased plant debris or organic material present in or on the soil. Pathogens of annual crops must be capable of survival during the off-season when host plants are absent. If the pathogens do not possess mechanisms that enable them to survive during the off-season or during periods when the environment is unfavourable the disease cycle will be broken and the pathogen will not survive. If survival or spread is prevented the disease will not occur (West *et al*., 1999). Thus, knowledge of survival often reveals a weak link in the disease cycle that can be utilized in managing the disease. Therefore, to manage *Phoma* blight epidemics effectively, the survival of the pathogen on debris needs to be better understood. Hence, this study was conducted to compare the length of time the pathogen *P. naikii* would survive in infested debris that was buried or left on the soil surface and other conditions. The goal of this research was to more precisely define the length of time a field must be rotated out of pigeon pea and other host crops of the pathogen to reduce or eliminate infested debris from previous crops as a source of inoculum of the pathogen.

MATERIALS AND METHODS

Survivability of pathogen in infected debris under different conditions

To study the ability of the fungus to survive in soil with host tissues, the sterilized soil was taken in the earthen pots and one set of *P. naikii* infected pigeon pea leaves were placed on the surface of the soil, buried to a depth of 5 and 7.5 cm, respectively and kept in glass house condition. Another set of dried infected samples was kept in the refrigerator (-4 °C) and room condition in blotter paper cover. The third set of infected samples was placed in field condition. Infected leaves from all sets of experiments were taken and subjected to isolation at 15 days intervals. Infected leaf debris was surface sterilized with $HgCl_2$ (0.1 %) and washed twice with sterile water. Five pieces of infected tissues were kept in a Petri plate containing malt extract agar medium and incubated the plates at 25 ± 2 °C. The observations were recorded for the growth and development of pathogens in the tissue after a week. The percentage of survivability was calculated by using the formula.

$$\text{Survivability (\%)} = \frac{\text{No. of tissues retrieved}}{\text{Total no. of tissue plated on media}} \times 100$$

RESULTS AND DISCUSSION

Survivability of pathogen in infected debris under different conditions

This study was conducted to determine the length of survival of *Phoma* pathogen in the infected pigeon pea debris under different storage conditions *viz*., room, refrigerator, pot, and field conditions. The results revealed that the frequency of recovery of pathogens decreased over a period of time. However, the decline in recovery followed a different pattern for each treatment. At the beginning of the experiment, *Phoma* pathogen was recovered from 100.0, 100.0, 93.33, 100.0, 86.66, and 86.66 per cent of the debris kept in the refrigerator, room, field surface, buried under 5 and 7.5 cm, respectively. The percentage of recovery of the pathogen was greater from debris kept in refrigerator condition when compared to all other treatments throughout the experiment. Percent recovery from refrigerated condition did not change during the first 17 weeks of the experiment where the pathogen has been recovered from 100 per cent of the debris but then declined rapidly in the next 20 weeks of the experiment with retrieval percent reducing from 100 to 26.66 (Table 1, Fig. 1).

Table 1: Survivability of *Phoma naikii* in leaf debris.

Recovery intervals (days)	Recovery intervals (weeks)	Percent retrieval under different storage conditions					
		Refrigerator	Room	Field	Pot condition		
					Surface	5 cm	7.5 cm
15	2	100.00	100.00	93.33	100.00	86.66	86.66
30	4	100.00	100.00	93.33	100.00	86.66	86.66
45	6	100.00	100.00	93.33	100.00	86.66	80.00
60	8	100.00	93.33	86.66	100.00	73.33	73.33
75	10	100.00	93.33	86.66	86.66	73.33	73.33
90	12	100.00	93.33	86.66	86.66	73.33	66.66
105	15	100.00	93.33	80.00	80.00	66.66	66.66
120	17	100.00	93.33	73.33	73.33	66.66	66.66
135	19	93.33	93.33	60.00	60.00	46.66	46.66
150	21	80.00	80.00	53.33	53.33	26.66	20.00
165	23	80.00	73.33	40.00	46.66	6.66	6.66
180	25	73.33	60.00	26.66	40.00	0.00	0.00
195	27	60.00	53.33	13.33	26.66	0.00	0.00
210	30	46.66	40.00	0.00	20.00	0.00	0.00
225	32	40.00	26.00	0.00	0.00	0.00	0.00
240	34	33.33	20.00	0.00	0.00	0.00	0.00
255	36	26.66	0.00	0.00	0.00	0.00	0.00
270	38	0.00	0.00	0.00	0.00	0.00	0.00

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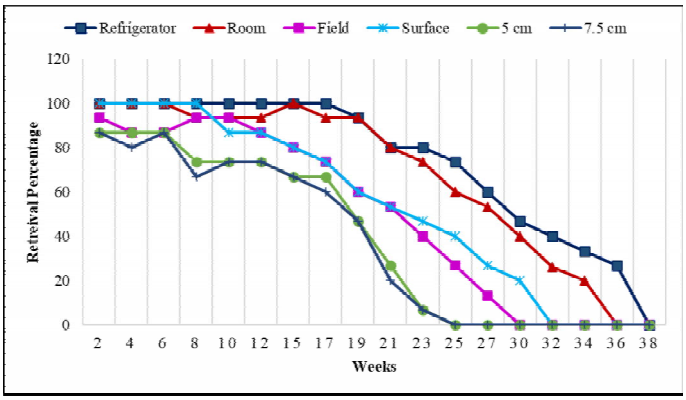

Fig. 1. Survivability of *Phoma naikii* under different conditions.

The percent recovery of the debris kept in room condition varied between zero and 35 weeks. In the period between zero to 19th week, there was a constant decline in the survival of the pathogen coupled with a rapid decline in the percent recovery between 20th to 35th week, by the end of 35th week 20 per cent of debris yielded colonies of the pathogen. Thereafter, there was no recovery of the fungus. For debris from field condition, the percent recovery declined steadily during the initial 12 weeks and then declined rapidly with the retrieval percentage reducing from 93.33 in the first week to 13.33 per cent in the 28th week. The pathogen survived for 30 weeks from debris on the soil surface. An initial recovery of 100 per cent was dropped down to only 20 per cent after 30th week, and thereafter there was no recovery. Percent recovery was lower from the buried debris as compared to other treatments where retrieval percentage declined rapidly from 86.66 per cent in the first week to 6.66 per cent in the 24th week irrespective of the burial depth.

Hence, the present study indicated the variation in the viability of the pathogen in the infected debris at six different storage conditions. It is evident that there might be potential primary inoculum in the field conditions for infection of pigeon pea crop up to 28 weeks. Similar findings were observed by Dilbag (1985) who revealed that *P. eupyrena* was viable on diseased leaves for six months in field and laboratory conditions. However, the viability of the pathogen gradually decreased every month. Cerkauskas (1987) observed that *Phoma* was capable of overwintering for five months in parsnip petioles buried in muck or mineral soil at Vineland Research Station, regardless of burial depth or soil type. Khani *et al*. (2016) found that *P. koolunga* was recovered on amended PDA from 93 per cent of stubble sections retrieved from the soil surface, 36 per cent of buried stubble sections, and 100 per cent of pseudo sclerotia buried in field soil after one month. The frequency of recovery of *P. koolunga* decreased over time and the fungus was neither recovered from the stubble on the soil surface at 15 months nor from stubble buried in the soil at 11 months indicating the role of stubble in the survival of the pathogen.

Environmental conditions such as rainfall and temperature might have affected the survival and growth of fungi (Baird *et al*., 2003). A steady, gradual, or sharp decline in the recovery of the fungus from infected debris has resulted possibly due to the combined effect of temperature, desiccation, and humidity. Non-saprophytic growth of the fungus also contributed to its elimination. The saprophytic survival ability is greatly reduced in soil, which might be due to the poor competitive saprophytic ability of the fungus, probably because of low oxygen tension and greater chances of encountering antagonists. In comparison, the pathogen survived better in stubble left on the soil surface than in stubble buried in soil, which appeared to be due to lesser decomposition of the stubble on the soil surface. Burial of stubble may hasten decomposition and promote colonization of stubble by antagonistic microorganisms. Carbon released from decomposed residue promotes soil microbial activity and so increases pathogen degradation (Raaijmakers *et al*., 2009).

The decline of pathogens in the buried debris is a potential example of natural biological control, in which the antagonistic activity of saprophytic soil microorganisms might eliminate pathogens resident in infected host crop debris (Baker and Cook, 1974). On the basis of this study, a simple way to reduce the survival of pathogens is to incorporate the infected crop debris into the soil with thorough tillage after harvest is completed. Depth of incorporation is less important than completely covering debris with soil (Keinath, 2002). Ntahimpera *et al.* (1997) found that the disease was consistently higher when fields were chisel ploughed, which allowed more debris to remain on the soil surface, rather than roto tilled or mouldboard ploughed and infected debris should be disced into the soil as soon as possible after harvest to promote decay of infested debris and to reduce survival of the pathogen (Steekelenburg, 1983).

CONCLUSION

The pathogen has survived for 36 weeks (8.5 months) in the refrigerator condition, 34 weeks (8 months) in the room condition, 27 weeks (6.5 months) in the field condition, 30 weeks (7 months) when kept on the surface of the soil and 24 weeks (5.5 months) when buried in the soil, irrespective of burial depth. Therefore, infected debris such as leaves, stem, twig, and blossom could be an important source of

overwintering inoculum. Removal and destruction of infected debris at harvest could reduce a source of perennation of the fungus.

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Research Article

Effect of biocontrol agents and botanical extracts on antioxidant activity in chilli fruits

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ABSTRACT

The experiment was carried out to evaluate the effect of biocontrol agents and botanical extracts on antioxidant activity in chilli fruits. Chilli seedlings were treated individually and in combination with effective biocontrol agents and botanical extracts in field conditions *i.e.,* applied as seedling treatment and foliar spray. Results revealed that the chilli plants applied with seedling treatment and foliar spray with *T. viride* + *P. fluorescens* + Neem + Garlic showed higher β-carotene and capsaicin content in chilli fruits in comparison to the control. Maximum β-carotene (3.56 mg ml^{-1}) and capsaicin (2831.67µg g^{-1}) contents were recorded in chilli fruits, respectively. These antioxidant sources are responses to disease resistance against pathogens and benefit the digestive tracts, promote a healthy heart, relieve joint pains, promote weight loss, mitigate migraine, reduce cancer risk, prevent allergies *etc.*

***Key words*:** Bio control agents, plant extracts, chilli fruit, carotene and capsaicin

Chilli (*Capsicum annum* L.) is known as the universal spice of India and has diverse utilities as a spice, condiment, nutritional supplement, medicinal and economic benefits of its production and ornamental plant. Chilli fruits are rich in vitamins, especially vitamins A and C. They are also enriched with potassium, magnesium and iron. Indian foods are incomplete without chillies in their spices. Several type of carotenoids life β-carbotane, violaxanthin, lutin accumulate in the fruit during its ripe stages. These are integral and the most important ingredient in many different cuisines around the world as it adds pungency, taste, flavour and colour to the dishes. *Capsicum* species are used fresh (green) or dried (red), whole or ground, and alone or in combination with other flavouring agents. Pungency in chilli is due to the alkaloid "capsaicin" contained in the pericarp and placenta of fruits, it produces mild to intense spice when eaten. It is also used in the food industry as chilli paste, curry powder and other resources of food and bakers. Capsicum is also carotenogenic fruit, during the ripening transformation of the chloroplast into chromoplast occurs, chlorophylls disappear and more and new carotenoids are formed.

Indian foods are incomplete without chillies in their spices. There are many nutritional, medicinal and economic benefits of its production and it is also used for culinary applications. They benefit the digestive tract, promote a healthy heart, relieve joint pains, promote weight loss, mitigate migraine, reduce cancer risk, prevent allergies *etc.* Especially, it is used in pharmaceutical industries, preparation of oleoresin, cosmetics, and other industrial resources. Chillies, high in vitamin C and a good source of vitamin E, potassium and folic acid, are cholesterol-free and low in sodium. Fresh green chilli peppers have more vitamin C than citrus fruits, and fresh red chilli has more vitamin A than carrots (Marin *et al*., 2004). Chilli is extensively grown for dry chilli powder and also harvested green. The main determinants of pepper fruit colour are chlorophyll, carotenoids, and anthocyanins (Aza-Gonzalez *et al*., 2011), whereas the main determinants of the typical red colour of mature fruit are the carotenoids capsanthin and capsorubin (Kumar *et al*., 2011). Many other types of carotenoids accumulate in the fruit during the ripening process, for example, β-carotene, violaxanthin, lutein, and zeaxanthin (Ha *et al*., 2007).

Chilli fruits are good antioxidant sources and hold promise as natural ingredients in functional foods. Phenolics (20.54 to 20.75 mg/100 g sample), carotenoids (1.00 to 1.26 mg 100 g^{-1} sample) and ascorbic acid contents (187.24 to 281.73 mg 100 g^{-1} sample) varied between genotypes. Trolox equivalent antioxidant capacity (TEAC) ranged from 1.55 to 3.23 mM/mg sample. During the 120 min decolourization trial, antioxidant capacity decreased over time in the studied genotypes. Values ranged from 36 to 57 per cent β-carotene bleaching during the first 30 minutes (Campos *et al*., 2013). They were analysed to quantify their dry fruit yield, ascorbic acid contents, capsaicin content, α-Carotene and β-carotene.

The result indicated that dry fruit yield (0.01-0.04 kg plant^{-1}), ascorbic acid contents (92.07-301.11 mg 100 g^{-1}), capsaicin content (0.75-4.65 %), α-Carotene (1.02-5.26 mg l^{-1}) and β-carotene (0.97-4.45 mg l^{-1}) varied between the sixteen genotypes (Mena *et al*., 2018). Capsaicinoids content with a view to assess their relative potency and/or hotness in order to ensure the functional as well as the nutritional quality of capsicum in which capsaicinoids content of *Bhut Jolokia* (2.45%) was still higher than that of *Dhan Jolokia* (2.14%) (Sarwa *et al*., 2013).

MATERIALS AND METHODS

Chilli seedlings were treated individually and in combination with effective biocontrol agents and botanical extracts in field conditions, applied as seedling treatment and foliar spray. The biocontrol agents and botanical extracts were sprayed 21 days after transplanting and one day after the pathogen was inoculated. The treatments also included, plants inoculated only with pathogens were maintained for control. From each replication of the treatment, randomly fresh chilli fruits were harvested carefully without causing any damage. Collected chilli fruits were dried and ground in power for determination of antioxidant activity capsaicin and carotene content in chilli fruits. Systemic resistance in chilli plants is elicited due to some antioxidants created by biocontrol agents and botanical extracts.

About 0.5 g of dried, powdered (30-40 mesh) capsicum fruits were extracted with 25 ml of acetone and the mixture was shaken for 10 min, kept for 2 hr and then filtered through a glass-wool plug in a short-stemmed funnel. Volume was made up to 25 ml. 2 g of material was used for low capsaicin content (less than 0.1%). About 2 ml of the extract was passed through a basic alumina column, and 1.5 g in a 10.0-0.9 cm column. The column was washed three times with 5 ml portions of acetone after loading. Pure capsaicin was eluted with the methanol-acetone-water mixture (75:25:1), collecting 50 ml. About 10 ml (or other suitable volumes) was evaporated to dryness (temperature ≤ 65 °C) and colour was developed for the calibration curve (Bajaj and Kaur, 1979).

A 2 g of fresh sample was extracted with a solution (3% acetone in petroleum ether and a pinch of Na_2SO_4). The extracted sample was filtered 2-3 times till it turned colourless and then its volume was made to 50 ml. The extract was poured into a separating funnel along with water and kept overnight. When the impurities were separated the pure aliquot was collected in a beaker and the volume was made to 50 ml with petroleum ether. The OD was taken at 443, 475 and 492 nm. The change in absorbance was recorded at 443, 475 and 492 nm and the results were expressed as mg/l (Kaur *et al*., 2011).

RESULTS AND DISCUSSION

Carotene content

The quantity of β carotene was estimated from chilli fruit harvested among all treatments presented in Fig. 1. The β-carotene content of chilli fruit varied significantly between the treatments. The observation recorded maximum β-Carotene content was found in fruit of treated chilli plant with *T. viride* + *P. fluorescens* + neem + garlic (3.56 mg l^{-1}) treatment followed by *Trichoderma viride* + neem + garlic (3.45 mg l^{-1}), *Trichoderma viride* + *Pseudomonas fluorescens* (Tv+Pf) (3.44 mg l^{-1}) and *Pseudomonas fluorescens* + *Trichoderma viride* (Pf+Tv) (3.42 mg l^{-1}) whereas minimum amount was recorded in control (2.90 mg l^{-1}), respectively.

Table 1: Combination of treatments used for seedling treatment and foliar application.

Treatment	Treatment details
Neem (N)	Neem (ST + FS)
Garlic (G)	Garlic (ST + FS)
Trichoderma viride (Tv)	*Trichoderma viride* (ST + FS)
Pseudomonas fluorescens (Pf)	*Pseudomonas fluorescens* (ST + FS)
Trichoderma viride + neem (Tv+N)	*Trichoderma viride* (ST) + neem (FS)
Trichoderma viride + garlic (Tv+G)	*Trichoderma viride* (ST) + garlic (FS)
Pseudomonas fluorescens + neem (Pf+N)	*Pseudomonas fluorescens* (ST) + neem (FS)
Pseudomonas fluorescens + garlic (Pf+G)	*Pseudomonas fluorescens* (ST) + garlic (FS)
Trichoderma viride + *Pseudomonas fluorescens* (Tv+Pf)	*Trichoderma viride* (ST) + *Pseudomonas fluorescens* (FS)
Pseudomonas fluorescens + *Trichoderma viride* (Pf+Tv)	*Pseudomonas fluorescens* (ST) + *Trichoderma viride* (FS)
Trichoderma viride + *Pseudomonas fluorescens* + neem + garlic (Tv+Pf+N+G)	*Trichoderma viride* +*Pseudomonas fluorescens* (ST) + neem + garlic (FS)
Trichoderma viride + neem + garlic (Tv+N+G)	*Trichoderma viride* (ST) + neem + garlic (FS)
Control (C)	Pathogen Control

ST= Seedling treatment, FS= Foliar spray

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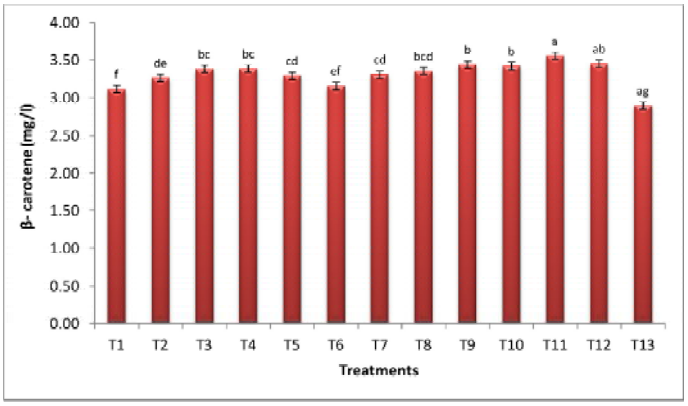

Fig. 1. β- carotene activity in chilli plants treated with biocontrol agents and botanical extracts against *Colletotrichum capsici.* (Results are expressed as means of three replicates and vertical bars indicate the standard deviation of the means. Different letters indicate significant differences among treatment results taken at the same time interval according to Duncan's multiple range test (P<0.05))

[**Treatment details:** T1= Neem (ST + FS), T2= Garlic (ST + FS), T3= *T. viride* (ST + FS), T4= *P. fluorescens* (ST + FS), T5= *T. viride* (ST) + Neem (FS), T6= *T. viride* (ST) + Garlic (FS), T7= *P. fluorescens* (ST) + Neem (FS), T8= *P. fluorescens* (ST) + Garlic (FS), T9= *T. viride* (ST) + *P. fluorescens* (FS), T10= *P. fluorescens* (ST) + *T. viride* (FS), T11= *T. viride* + *P. fluorescens* (ST) + Neem + Garlic (FS), T12= *T. viride* (ST) + Neem + Garlic (FS), T13= Control (C) whereas, ST- Seedling treatment, FS- Foliar spray, T- *Trichoderma* and P- *Pseudomonas*]

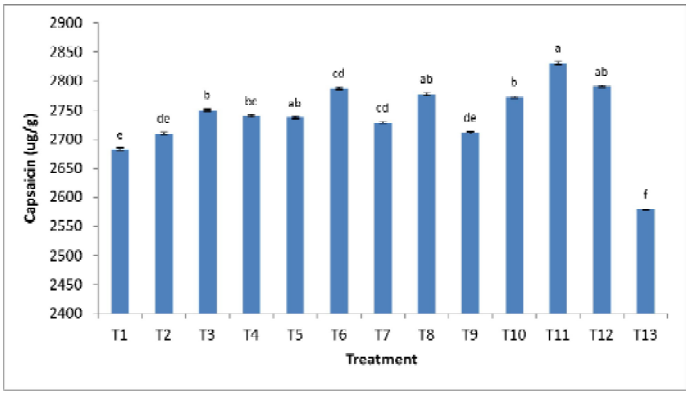

Fig. 2. Capsaicin (ug/g) activity in chilli plants treated with biocontrol agents and botanical extracts against *Colletotrichum capsici.* [Results are expressed as means of three replicates and vertical bars indicate the standard deviation of the means. Different letters indicate significant differences among treatment results taken at the same time interval according to Duncan's multiple range test (P<0.05)].

(**Treatment details:** T1= Neem (ST + FS), T2= Garlic (ST + FS), T3= *T. viride* (ST + FS), T4= *P. fluorescens* (ST + FS), T5= *T. viride* (ST) + Neem (FS), T6= *T. viride* (ST) + Garlic (FS), T7= *P. fluorescens* (ST) + Neem (FS), T8= *P. fluorescens* (ST) + Garlic (FS), T9= *T. viride* (ST) + *P. fluorescens* (FS), T10= *P. fluorescens* (ST) + *T. viride* (FS), T11= *T. viride* + *P. fluorescens* (ST) + Neem + Garlic (FS), T12= *T. viride* (ST) + Neem + Garlic (FS), T13= Control (C) whereas, ST- Seedling treatment, FS- Foliar spray, T- *Trichoderma* and P- *Pseudomonas*)

Capsaicin content

The quantity of capsaicin from chilli fruits harvested from all treatments is presented in Fig. 2. Capsaicin amounts varied significantly between the treatments. The results revealed that maximum capsaicin content was found in fruits of treated chilli plant with *T. viride* + *P. fluorescens* + neem + garlic (2831.67 µg g^{-1}) followed by *Trichoderma viride* + neem + garlic (2790.83 µg g^{-1}), *Trichoderma viride* + Garlic (2787.50 µg g^{-1}) and *Pseudomonas fluorescens* + garlic (2778.42 µg g^{-1}) whereas, minimum amount was recorded in the fruit of untreated chilli plant (2578.53 µg g^{-1}), respectively.

The observation recorded maximum β-Carotene content *i.e.,* 3.56 mg l^{-1} was found in chilli fruit treated with *Trichoderma viride* + *Pseudomonas fluorescens* (seedling treatment @ 5% each) + neem + garlic (foliar spray @ 15%). Carotenoids are known to play an important role in preventing oxidative damage. The pungency of chilli which is measured in terms of capsaicin, observation recorded maximum capsaicin content *i.e.,* 2831.67 µg g^{-1} was found in harvested fruits of chilli plant with *T. viride* + *P. fluorescens* + neem + garlic (seedling treatment + foliar spray). Several studies also showed similar results (Sanatombi and Sharma 2008).

CONCLUSION

Efficacy of botanical extract and biocontrol agents on antioxidant activity of fruits of chilli plant that were applied seedling treatment and foliar spray with *T. viride* + *P. fluorescens* + Neem + Garlic showed higher â-carotene and capsaicin content in chilli fruits in comparison to control. Maximum β-carotene (3.56 mg ml^{-1}) and capsaicin (2831.67µg g^{-1}) contents were recorded in chilli fruits, respectively. These antioxidant sources are responses to disease resistance against pathogens and benefit the digestive tract, promote a healthy heart, relieve joint pains, promote weight loss, mitigate migraine, reduce cancer risk, prevent allergies *etc.*

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Research Article

Response of pH on indigenous biocontrol activity of fungal and bacterial biological agents against *Rhizoctonia solani*

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ABSTRACT

Root rot incited by *Rhizoctonia solani* is one of the major diseases of peas. As there are no long-term management strategies for this soil-borne pathogen, therefore, the study was carried out to determine the effect of pH on the antagonistic activity of fungal and bacterial isolates (Th3 and Th5 (*Trichoderma harzianum*), An1 (*Aspergillus niger*), Po3 (*Penicillium oxalicum*) and PS1, PS2, and PS5 (Flourescent *Pseudomonas*) against *Rhizoctonia solani* at four different pH levels (5.0, 6.0, 7.0 and 8.0). All seven indigenous fungal and bacterial isolates evaluated for their antagonistic potential against root-rot pathogen significantly retarded the radial growth of *R. solani* in comparison to control at four different pH levels (5.0, 6.0, 7.0, and 8.0). The observations of the study revealed that all bio agents significantly inhibited the growth of *R. solani*. Similarly, *Trichoderma* species had significant effects on the number and size of sclerotia. The pH range of 6.0-8.0 was found optimum for *Trichoderma* spp. while Fluorescent *Pseudomonas* showed the best antagonistic activity at pH 7.0. As far as the effect of pH levels is concerned, all the promising fungal and bacterial isolates caused maximum growth inhibition of test pathogens at pH 6.0 to 7.0.

***Keywords*:** pH, *Rhizoctonia solani, Trichoderma harzianum,* Fluorescent, Pseudomonas, *Aspergillus niger*, *Penicillium oxalicum,*

Root rot is one of the most important destructive diseases of peas. The crop losses in peas are probably greater from root disease than from any other type of disease. Root rot may start when the pea plant is in the pre-or post-emergent seedling stage. Root decay generally begins on the fine feeder roots and progresses gradually to the main taproot. Infected plants produce peas which are irregular in size and variable in maturity with a lowered sugar content (Oyarzun, 1993). The fungal pathogen *Rhizoctonia solani* causes seed, seedling, and root rots in peas. On seedlings, reddish-brown, sunken lesions are formed on roots, lower stems, and root tips. The pathogen survives in soil and the disease is favoured by cool, wet conditions that slow germination and emergence (Markell *et al*., 2016). Considering the limitations and after-effects of fungicides and also the soil-borne nature of the disease has rendered disease management through fungicides a difficult task. Therefore, the thrust is laid on developing an integrated management approach. Since biological control is one of the essential components of Integrated Disease Management so, there has been a growing awareness towards eco-friendly strategies for plant disease management *i.e.* biological control (Chattopadhyay *et al*., 2002; Hamid, 2012)

Biological control of plant pathogens brings about a reasonably good degree of reduction to crop damage by plant pathogens, ensuring the sustainability of production, cost-effectiveness, and a healthy ecosystem. One of the major mechanisms known for the biological control of root diseases of crops is the production of antimicrobial compounds (Cook, 1993) and a variety of secondary metabolites which may be involved in the promotion of root growth, suppression of pathogen and induction of plant defense system against the pathogen's invasion.

Though biocontrol agents include all classes or groups of organisms existing in an ecosystem, yet, the maximum emphasis for developing biocontrol programmes has invariably gone to fungal and bacterial bioagents. The role that naturally occurring rhizosphere microbial communities play in the improvement of root health is poorly understood. Moreover, the discovery of naturally occurring microbial biocontrol agents for plant diseases starts with the principle that effective antagonistic can be found in local soils or more likely associated with local crop plants (plant-associated micro-organisms). Thus, the present study was undertaken to characterize and identify the predominant pathogen associated with root rot in peas. The aim of the study was to assess the potential of eco-friendly control methods, the indigenous biocontrol fungus, and bacteria for controlling root rot disease of peas caused by *Rhizoctonia solani*.

MATERIALS AND METHODS

The present investigation was undertaken during *rabi* season under field and laboratory conditions in the Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, U. P. (India). The study was undertaken to study the antagonistic potential of naturally occurring rhizospheric fungi and fluorescent *Pseudomonas* against wilt and root-rot pathogens of peas.

Isolation of pathogens was done in culture media from diseased plant parts collected from the field. Pure cultures were obtained by subculturing on Potato Dextrose Agar medium.

The rhizospheric soil of peas collected from different localities was brought to the laboratory in polythene bags for the isolation of naturally occurring rhizospheric fungi and bacteria. One gram of each soil sample was suspended in 9 ml of sterilized water and shaken thoroughly to get the soil particles uniformly dispersed in the suspension. One ml of suspension from the first dilution ($1:10^{-1}$) was aseptically transferred to another tube (10^{-2}) and this procedure was further repeated till the dilution of 10^{-6} was obtained. For the isolation of rhizospheric fungi, 0.1 ml of soil suspension from the dilution of 10^{-4} to 10^{-6} was transferred in sterilized Petri plates containing potato dextrose agar medium. The isolated fungal species were identified and confirmed on the basis of their morphological, cultural, and biochemical characteristics as documented in the literature.

The rhizobacterium, *Pseudomonas* was isolated by the method suggested by Collins and Lyne (1987). One gram of each soil sample was suspended in 9 ml of sterile normal saline solution and shaken vigorously for 10 minutes, thereafter, 0.1 ml aliquots was placed in the Petri plates containing selective media of appropriate dilution. These Petri plates were incubated at 35±2 ^{o}C for 24 h. After incubation, the suspected bacterial colonies were picked up and subcultured for their identification and further use in other studies. Identification of rhizobacterium was made on the basis of their cultural, morphological, and biochemical characteristics.

The effect of different pH was studied *in-vitro* on the antagonistic activity of fungal and bacterial isolates against root-rot pathogen (*Rhizoctonia solani*). The isolates of *Trichoderma harzianum* (Th3 and Th5), *Aspergillus niger* (An1), *Penicillium oxalicum* (Po3), and Fluorescent *Pseudomonas* (PS1, PS2, and PS5) were used for their efficacy in inhibiting the radial growth of *Rhizoctonia solani* at four different pH levels. The efficacy of indigenous fungal and bacterial biocontrol agents on radial growth inhibition of test pathogens *i.e.*, *R. solani* was studied *in-vitro*, through dual culture technique using 2 isolates of *T. harzianum* and 3 isolates of Fluorescent *Pseudomonas*, one isolates of *P. oxalicum* and *Aspergillus niger*. With the help of a cork borer, fungal mycelial discs having a diameter of 5 mm were cut from the young cultures of fungal bioagents and test fungus. These discs were placed in the Petri plates containing P.D.A., maintaining a distance of 4 cm between the discs of the test fungus. Petri plates were incubated for five days at 28±^{o}C. Each treatment was replicated thrice. Observations of radial growth inhibition of test pathogens were measured at intervals of 24 h for 5 days. The bio-efficacy of *Pseudomonas* against pathogens was determined by the dual culture in different techniques. In the Petri plate, four discs of the fungal pathogen were placed in the periphery of the Petri plate at equal distances thereafter, the blotting paper discs having a diameter of 10 mm were dipped in bacterial suspension and placed in the center of the Petri plates. In control, no blotting paper was placed. These Petri plates were incubated at 28±1^{o}C for 5 days. Each treatment had three replications. Observations of radial growth of the pathogens were recorded after every 24 h interval.

The observations were recorded on radial growth and percent inhibition over control was also calculated. The percent inhibition over check noted after 5 days of incubation was calculated by the following formulae (Vincent, 1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition

C = Colony diameter in check

T = Colony diameter in treated petri plate

RESULTS AND DISCUSSION

Isolation and identification of causal pathogens and rhizospheric fungal and bacterial bioagents

Causal pathogens

Infected seedlings as well as adult plants of pea showing the symptoms of root-rot, frequently yielded the growth of *R. solani* on potato dextrose agar medium. Repeated isolations from infected plants also exhibited the frequency of these pathogens. Identification of these causal pathogens was confirmed on the basis of their cultural and morphological characteristics recorded in pure cultures as well as in temporary slides, respectively, and as testimonised with the literature (Kraft 1994; Singh and Gurha 1996).

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Rhizospheric fungal and bacterial bioagents

The rhizospheric soil of peas collected from 5 different localities, more frequently yielded fungal and bacterial colonies on the medium. Among them, fungal populations included 2 isolates of *T. harzianum,* one isolate of *A. niger,* and *P. oxalicum* (Table 1). These fungal species were identified and confirmed on the basis of their cultural and morphological characteristics as documented in the literature (Bineeta, 2001; Mondal, 1998; Mondal *et al*., 2000).

The rhizospheric soil collected from different fields of pea yielded 15 different bacterial colonies. Among these, 3 isolates were identified as Pseudomonads. These isolates of Pseudomonas were characterized on the basis of their cultural, morphological, and biochemical characteristics. Pseudomonas isolates grown on medium produced pale yellow to greenish yellow and mucoid colonies. The isolates of Pseudomonas were found to be gram-negative,

Table 1: Microscopic features of fungal isolates.

Isolates No.	Tentative Identification	Source/ Vegetation	Colour on PDA	Colony reverse color on PDA
Th3	*Trichoderma harzianum*	Rhizosphere of Pea	Dark Green Colour	Yellowish green color
Th5	*Trichoderma harzianum*	Rhizosphere of Pea	Dark Green Colour	Yellowish green color
An1	*Aspergillus niger*	Rhizosphere of Pea	Black in colour	Black in colour
Po3	*Penicillium oxalicum*	Rhizosphere of Pea	Light Brown in colour	Light Brown in colour

chaemohetrotrophic motile rods with polar flagella. Fluorescent *Pseudomonas* spp. make up a diverse group of bacteria that can be generally distinguished from other Pseudomonads by their ability to produce water-soluble yellow-green pigment (Table 2) (Kloepper *et al.,* 1980a; 1980b). It is evident from Table 2 that all the isolates of *Pseudomonas* designated as Ps1, Ps2, and Ps5 were found to be positive for catalase, oxidase tests, gelatin hydrolysis, Methyl red production, citrate utilization, maltose utilization, acid production, Nitrate reductase activity. All *Pseudomonas* isolates (Ps1, Ps2, Ps5) were also tested for their PGPR activity such as the production of HCN, Siderophore, ammonia, and indole acetic acid production. Among isolates of *Pseudomonas*, three isolates (Ps1, Ps2, Ps5) produced hydrogen cyanide and turned piric acid paper to brown orange colour (Table 2). Hydrogen cyanide is produced by many rhizobacteria and has been found to play a very significant role in the biological control of soil-borne pathogens (Weller, 1988; Voisard *et al.,* 1989).

Table 2: Biochemical characterization of pseudomonas isolates.

Test	Ps1	Ps2	Ps5
Gram Staining	Gram-negative	Gram-negative	Gram-negative
Shape	Rod	Rod	Rod
Motility	+	+	+
Fluorescent Pigment	+	+	+
Starch Hydrolysis	+	+	+
Gelatin Hydrolysis	+	+	+
Indole Production	+	+	+
Methyl red production	+	+	+
Citrate utilization	+	+	+
Acid production test	+	+	+
Catalase Activity	+	+	+
Oxidase production	+	+	+
Nitrate Reductase Activity	+	+	+
Ammonia Production	+	+	+
HCN production	+	+	+
Siderophore Production	+	+	+

+ Present, – Absent

It is evident from Table 3 that all promising isolates exhibited significant effects on the growth inhibition of *R. solani* at varying levels of pH. However, the maximum reduction (1.5 to 2.2) in the growth of pathogens due to fungal isolates was recorded only at pH 6.0. Here too, both isolates of *T. harzianum i.e.,* Th3 and Th5 were found to be statistically at par in their efficacy at all pH levels, except at pH 7.0 (Table 3), other isolates *viz*., An1 and Po3, however, registered maximum reduction of 1.9 and 2.2 cm in radial growth at pH 6.0 exhibiting 68.8 to 73.0 per cent inhibition, respectively (Table 3).

Among, *Pseudomonas* isolates, Ps5 appeared to be most effective followed by resulting insignificantly maximum reduction of 1.4 and 1.5 cm in radial growth and 75.5 and 74.3 per cent inhibition, respectively at pH 7.0. The differences in the antagonistic potential of these isolates (Ps2 and Ps5), here too, were found to be non-significant at each level of pH (Table 3).

The results, so obtained in this study clearly indicate that growth inhibition of root-rot pathogens due to promising fungal and bacterial isolates is significantly influenced by different pH levels. Among the promising fungal isolates, Th3 proved to be most effective against the pathogens followed by Th5, An1, and Po3. Though, isolate Ps3 of Pseudomonas resulted in their maximum antagonistic potential followed by Ps2 and Ps1 against *R. solani.*

Besides this, there are several instances where the efficacy of these rhizospheric fungal and bacterial bioagents has been studied for their antagonistic potential against

Table 3: Effect of pH on radial growth inhibition of *Rhizoctonia solani* due to Fungal and Bacterial bioagents.

Fungal and Bacterial bioagents	pH levels							
	pH 5		pH 6		pH 7		pH 8	
	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)	*RG (cm)	Inhibition (%)
Th3	1.9	70.4	1.5	79.0	1.9	68.3	2.3	60.1
Th5	2.0	67.9	1.6	73.0	2.0	66.1	2.4	58.4
An1	2.1	66.8	1.9	73.0	2.2	63.3	2.7	52.0
Po3	2.3	64.2	2.2	68.8	2.2	59.0	3.3	42.7
Ps1	3.3	48.1	3.2	54.8	1.7	71.5	1.9	67.0
Ps2	3.1	50.7	3.0	57.6	1.5	74.3	1.7	69.3
Ps5	3.0	52.8	3.0	58.1	1.4	75.9	1.6	71.0
Control	6.4	0.0	7.1	0.0	6.1	0.0	5.7	0.0
C.D. at 5%	0.30	-	0.25	-	0.24	-	0.18	-

*RG= Radial Growth, Mean of Three replicates.

these soil-borne pathogens (Kredics *et al*., 2000; Elad *et al*., 1983b). The experimental finding of Elad *et al*. (1983a, 1983b), Harman *et al*. (1980, 1989), and Sharma *et al*. (2005) concluded that the fast-growing *Trichoderma* sp. caused more inhibition of the pathogens probably due to mycoparasitism and competition for nutrients and had suggested that mycoparasitism was principal mechanism involved in controlling *Pythium* in pea seeds. *In vitro* hyphal parasitism by *Trichoderma* sp. was also observed by many scientists (Dennis and Webster, 1971a; 1971b).

Elad *et al*. (1983a) and Kumar and Dubey (2001) studied a positive correlation between the lytic activity of several strains of *T. harzianum* on the cell wall of *R. solani*. Similarly, Wang *et al*. (1995) also found that *Trichoderma* spp. had strong antagonistic activity towards *F. solani* f.sp *pisi* under in vitro conditions and reported that *T. harzianum* allowed mini-mum pathogen growth in dual culture followed by *T. viride* and *P. fluorescence*

In another study, Harman *et al*. (1981) treated the seeds with *T. hamatum* and found satisfactory control of seed rot of pea and radish in soil infested with *Pythium* spp. and *R. solani*. However, Khara and Hadwan (1989) observed maximum antagonism against *R. solani* by T. *koningii* and *T. pseudokoningii*. They further stated that the maximum inhibition of the growth of *R. solani* was due to the culture filtrate of both species of *Trichoderma* which produced gliotoxin and glucose peptone. According to them, maximum inhibition has resulted at 30°C temperature but optimum pH for inhibition ranged from 4.5 to 5.0 for *T. koningii* and *T. pseudokoningii*, respectively.

CONCLUSION

The present assessment indicated that all the native or indigenous fungal and bacterial bioagents examined showed robust antagonistic activity against *R. solani*. The study also indicated that all fungal and bacterial bioagents may require a specific pH for their activity that allows us to better understand the biocontrol agent's behaviour in the control of *R. solani*. Consequently, they could be used as alternative methods of chemical application for the management of the root rot disease of peas caused by *R. solani*. Integration of all cultural, protective, and prophylactic measures can be adopted in integration with biological control for successful management of the disease.

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Research Article

Biological control of stem bleeding disease caused by *Thielaviopsis paradoxa* in coconut

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ABSTRACT

Stem bleeding disease caused by *Thielaviopsis paradoxa* is also one of the important diseases in coconut in Andhra Pradesh. Bio efficacy of native fungal and bacterial bioagents on stem bleeding pathogen *Thielaviopsis paradoxa* under *in vitro* conditions revealed that *Trichoderma asperellum* [Ambajipeta] was significantly superior with 79.17% growth inhibition followed by *T. reesei* [Ambajipeta] with 78.33%. Field evaluation of bio-control agents against Stem bleeding disease in coconut indicated that the cake application of *T. harzianum* and *T. reesei* formulation completely brought down the disease index by 100 per cent within 50 days of cake application. Paste application of bioagents *viz*., *T. harzianum*, *T. reesei,* and chemical fungicide copper oxychloride application also substantially reduced the disease.

Keywords: Stem bleeding disease, coconut, *Thielaviopsis paradoxa*, Trichoderma coir pith cake, biocontrol.

Coconut palm (*Cocos nucifera* L.) is an important plantation crop in India and is often described as 'Kalpavriksha' because of the multifarious uses of every part of it in the commercial sector. Coconut palms are successfully grown in the tropical countries of the world and are hence referred to as "King of tropical palms". The South Pacific and South Africa are often cited as possible centers of origin. Coconut provides food, drink, shelter, and industrial raw materials. Coconut is grown in almost 94 countries in the world of which 90% of the production comes from Asian and Pacific countries.

Coconut plays a vital role in the rural horticulture economy with a total production of 13411 T tonnes in India and 1112 T tonnes as a share of 8.27% and ranks fourth in Andhra Pradesh with an area and production (1.15 lakh ha; 1378 million nuts) after Kerala, Karnataka, and Tamil Nadu with productivity of 11957 nuts/ha in 2021-22. In Andhra Pradesh, East Godavari (50,490 ha), West Godavari (21,818 ha), Srikakulam (14,753 ha), and Visakhapatnam (7300 ha) districts occupy the major area in the forefront in coconut cultivation [Anonymous, 2022].

Stem bleeding of coconut is a debilitating disease and is prevalent in all coconut-growing regions in the tropics. The disease was first reported in Srilanka (Petch, 1906) and later reported in India (Sundraraman, 1922) and other countries. The disease is caused by a fungal pathogen, *Thielaviopsis paradoxa* (de Seynes) von Hohnel. The disease has been found to occur in all soil types, but more in laterite soils and sandy soils on the seashore or backwater areas (Nambiar, 1994). Stem bleeding disease in coconut was recorded by up to 15% in Andhra Pradesh (Srinivasulu *et al*., 2005). The pathogen is a soil-borne pathogen and enters the plant through growth cracks present on the stem and causes cortical decay. The disease is characterized by the development of dark brown patches appearing at the basal portion of the trunk. A dark reddish-brown liquid exudes from the longitudinal growth cracks present on the stem bark and forms irregular streaks of exudation. These streaks may coalesce and form larger lesions. No oozing is seen from old lesions. The exudates eventually dry up to form black encrustations with brownish-orange margins. The tissues beneath the discoloured patch show decay. As the decay progresses, the tissues become black and fibrous. As a result of this, cavities are formed from which liquid comes out when the bark is pressed. Severe infection may lead to reduced yield and death of young palms. Symptoms also occur in the crown region. The outer whorl of leaves becomes yellow rather prematurely, droop, and finally dry up. The trunk gradually tapers towards the apex and the crown size is reduced. The bleeding patches spread spirally about halfway up the stem and sometimes reach the crown and cause the death of palms. In severe cases, the bleeding patches reach the crown and kill the palm [Plates 1, 2 & 3].

MATERIALS AND METHODS

Isolation and identification of antagonistic fungi from the rhizospheric region of coconut

Soil samples were collected from the rhizospheric region of coconut in mandals of Dr. B.R. Ambedkar Konaseema district, Andhra Pradesh. Serial dilution and plate count method was used for the isolation of antagonistic fungi. The collected soil samples were subjected to serial dilutions using sterile distilled water and 0.5 ml of each sample at 10^{-3} and 10^{-4} dilutions were spread on Petri dishes containing Trichoderma-specific medium (TSM) (Elad *et al*., 1983). Two plates were maintained for each dilution. The plates were then incubated at 28 °C and were examined after four days. The hyphal tip method was adopted for the pure culture of organisms. The isolated antagonistic fungi were identified up to the level of genus or species based on growth, colour, and philides characters on the PDA medium.

Isolation and identification of antagonistic bacteria

Samples were serially diluted and 0.1 ml of the sample was spread on plates containing King's B medium. The isolate was purified by streaking and was maintained further. Identification of bacterial bioagents was made as per the description and physiological status suggested by Schaad *et al*. (2001).

In vitro antagonism on fungal pathogens of coconut

Dual cultures of the fungal and bacterial antagonists and the test pathogen were prepared by inoculating PDA discs from the growing margins of fresh fungal cultures onto Petri dishes containing PDA (Gams *et al*., 1987) and incubating them. The dual cultures were observed for antibiosis and agar blocks from the regions where the colonies merged were observed for typical interactions under the light microscope. In the case of bacterial antagonists, 8 mm mycelial discs of the pathogens were placed individually at the center of the plates and bacterial strain was streaked at three positions 2 cm away from the edge of the Petri plates with PDA medium and incubated. The mycelial growths of the test pathogens were measured at 48 hrs and subsequently one week after incubation (Nandakumar *et al*., 2000). Mycoparasitism of test pathogen isolates by fungal antagonists was studied using the dual culture technique developed by Dennis and Webster (1971) and described by Sanchez *et al*. (2007). The pathogen growth was measured after 4±days of incubation in both cases at 29.1°C and percent inhibition was calculated by using the formula as given by Vincent (1947).

$$\% \text{ inhibition} = \frac{\{\text{Mean growth in control} - \text{Mean growth in treatment}\}}{\text{Mean growth in control}} \times 100$$

Trichoderma coir pith cake [TCPC]

Trichoderma reesei [Ambajipeta] and *Trichoderma harzianum* [Ambajipeta] were used for the study. The Trichoderma coir pith cakes were prepared following Mohanan *et al.* (2013) with slight modifications. Trichoderma biomass was prepared using potato dextrose broth. The broth was distributed into 500 ml conical flasks with a quantity of 250 ml and plugged tightly with cotton. This was autoclaved at 15 p.s.i. for 20 minutes, cooled to room temperature, and inoculated with 5 mm culture disc of three days old Trichoderma inoculated flasks were incubated for 7 days at room temperature in a slanting position so as to get the highest surface area of the medium for fungal growth. Seven days after inoculation, the spent medium with fungal biomass was blended in an electric mixer for 1-2 minutes to homogenize the contents. This fungal biomass slurry was used for the preparation of coir pith cake formulation. Fine Coir pith after removing fibre from husk was collected from a coir industry. It was mixed with neem cake @ 25% and moistened with water to a moisture level of 70-75 per cent. The substrate was taken in a polypropylene bag sealed with an electric sealing machine and was autoclaved at 20 p.s.i. for 30 minutes and allowed to cool at room temperature. Maida flour was used as a nutrient-cum-binding material for Trichoderma coir pith cake preparation. For this, maida flour was boiled in water to get a sticky thick paste. Maida flour of 250 g was used to mix with 250 ml of sterile water to get a thick paste-like consistency and it was autoclaved at 20 p.s.i. for 30 minutes and allowed to cool at room temperature. The sterilized coir pith neem mixture @ 1000 g, Trichoderma biomass slurry @ 250 ml, and sterilized maida paste @ 250 ml were thoroughly mixed in a plastic tray. This mixture was used for making the product.

To prepare the formulation as a solid cake, 50 g mixture was compressed manually using a chakodi maker which was usually used in the Indian kitchen for snack preparation. Such an apparatus with provision for making more cakes at a time is being made locally with 5 cm dia. To prepare a single solid cake, 50 g mixture was used, and then it was compressed to get a solid cake. The cakes thus prepared were dried in an oven at 38-40 °C for 4 days (provided ventilation for the first two days for the moisture to escape). The colony-forming units of Trichoderma in the cake were determined after 4 days of drying by dilution plate technique (Pramer and Schmidt, 1956). The dried Trichoderma coir pith cakes (TCPC) were packed in polythene bags and stored at room temperature (26-30 °C). The treatments were arranged

in a completely randomized block design with 3-5 replications in the field. The data were analyzed statistically.

Talc formulation of *Trichoderma* spp.

Talc formulation of native *Trichoderma* spp was prepared. Potato dextrose broth was prepared and sterilized by autoclaving at 15 PSI (121.6 °C) for 15 minutes. Eight mm diameter mycelial discs of the antagonist were inoculated and incubated at 28 °C for 7 days. The homogenate (1×10^8 spores/ml) was mixed with talc powder at 1:2 ratio along with 0.5% carboxy methyl cellulose and dried in shade, following the method described by Jayarajan *et al*. (1994) with slight modification. The product was used for soil application studies.

Field evaluation of Trichoderma formulations under natural conditions

A field experiment was conducted at Dr. YSRHU-HRS, Ambajipeta, Dr. B.R. Ambedkar Konaseema district of Andhra Pradesh by imposing treatments along with untreated control in coconut. The experiment on the evaluation of cake formulations of bioagent, Trichoderma was tested against stem bleeding disease of coconut. The effect of Trichoderma cake formulation and paste application along with positive control (paste application of copper oxychloride) was tested against the stem bleeding disease of coconut. In the case of cake application, the treatment was given only once during the study period. In the case of the paste application, the paste application was carried out every month. Cake formulations were used to place on the slightly scraped portion of the bleeding patches. The bleeding patches are covered with fresh leaves of any available broad leaf and tightened with thread or trash [Plates 7 & 8]. The sprinkling of water was done to moisten the cake so as to proliferate the Trichoderma coir pith cake. Talc formulations of Trichoderma and Copper oxychloride were smeared on the bleeding patches as another treatment for comparison. Bleeding patches of nearly equal size were selected for the palms and the perimeter of the bleeding patches was taken into account for judging the degree of disease incidence one conspicuous bleeding patch was selected for each palm imposing treatments on the stem keeping in view the chances of appearance of more than one bleeding patch on the same stem. The initial perimeter of the bleeding patches was recorded prior to imposing the treatments and subsequent observations were made at monthly intervals up to three months. The efficacy of the treatments was determined by comparing the reduction in the perimeter of the bleeding patch after recording the final observations. Every month the treated palms were observed for the disease symptom and the percent recovery of the treated palms were observed. The palms were well managed with a regular package of practices. Treatments were imposed along with untreated control in Randomized Block Design with six replications.

RESULTS AND DISCUSSION

Isolation of coconut pathogens

The disease symptom of stem bleeding caused by *T. paradoxa* is depicted in Plates 1, 2 & 3. The infected stem portion of the palm where bleeding symptoms were conspicuous was chiselled out and surface sterilized with 0.1% sodium hypochlorite followed by 3 washes in sterilized distilled water (SDW) and then the stem bits were placed on Potato Dextrose Agar (PDA) media plates for *T. paradoxa*. The plates were then incubated for three days at 29±1°C and the test pathogen was isolated by purification.

***In vitro* antagonism of microbial bioagents on coconut stem bleeding pathogens**

The results presented in Table 1 on *in vitro* antagonism of biocontrol agents on coconut stem bleeding disease pathogen *T. paradoxa* (Plates 4, 5 & 6), it was observed that inhibition of *T. paradoxa* ranged from 47.22 to 79.17% reduction over control. It was observed that significantly

Table 1: *In vitro* evaluation of bioagents against *Thielaviopsis paradoxa*

Tr. No.	Code	*Thielaviopsis paradoxa*	% Reduction over control
T1	*Trichoderma reesei –[Ambajipet]*	19.50 (26.18)*	78.33
T2	*T. reesei –[Tirupathi]*	47.50 (43.54)	47.22
T3	*T. harzianum– [Ambajipeta]*	26.25 (30.77)	70.83
T4	*T.asperellum – [Ambajipeta]*	18.75 (25.63)	79.17
T5	*Pseudomonas fluorescens – [Ambajipeta]*	28.75 (32.40)	68.06
T6	*Pseudomonas striata- [Ambajipeta]*	28.50 (32.24)	68.33
T7	*Pseudomonas aeruginosa– [Tirupathi]*	52.00 (46.13)	42.22
T8	*Bacillus subtillis- 1[Tirupati]*	45.50 (42.39)	49.44
T9	*Bacillus subtillis- 2[Tirupati]*	90.00 (71.53)	0.00
T10	Control	90.00 (71.53)	0.00
	S.Em.±	0.87	
	C.D. at 1%	2.52	

*Values in the parentheses are angular transformed values

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Plate 1: Stem bleeding infection at the bottom portion of the stem

Plate 2: Stem bleeding infection at the top portion of the stem

Plate 3: Initiation of stem bleeding at bottom potion of stem

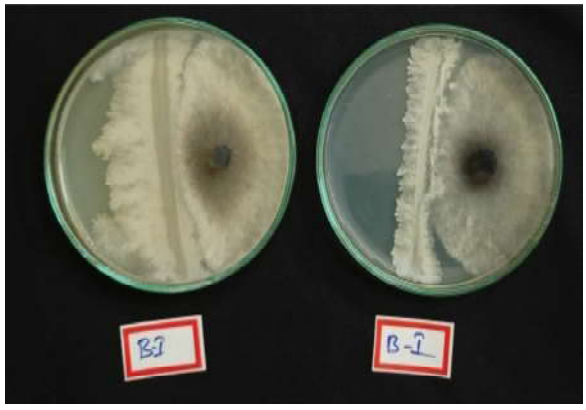

Plate 4: Dual culture test *T.Paradoxa* Vs *Bacillus subtillis*-I [Tirupathi]
T.Paradoxa Vs *Bacillus subtillis*-II [Tirupathi]

Plate 5: Dual culture test*T. paradoxa* Vs *Pseudomonas fluorescens [Ambajipeta]*

Plate 6: Dual culture test*T.Paradoxa* Vs *T. asperellumT.Paradoxa* Vs *T.reesei*

Plate 7: Chiselling/scraping of stem bleeding patch

Plate 8: Wrapping with fresh leaf on Trichoderma coir pith cake

Plate 9: Paste application of copper oxychloride on stem bleeding infected patch

Plate 10: Paste application of Trichoderma paste on stem bleeding infected patch

maximum growth inhibition of *T. paradoxa* were observed with *Trichoderma asperellum* [Ambajipeta] to a percent inhibition of 79.17 followed by *T. reesei* [Ambajipeta], *Pseudomonas fluorescens* [Ambajipeta], *Pseudomonas striata* [Ambajipeta], *Pseudomonas aeruginosa* [Tirupathi], *Bacillus subtillis*-1 [Tirupathi], *T. reesei* [Tirupathi] to 78.33, 68.06, 68.33, 52.00, 49.44 and 47.22%, respectively. However, no growth inhibition of *T. paradoxa* was observed with *Bacillus subtillis*-2 [Tirupathi]. The results corroborate with earlier workers who reported the potential of biocontrol agents against coconut pathogens (Tapwa1 *et al*., 2011). Sudarshan *et al*. (2019) reported that *Trichoderma viridae* was found to be most effective on *T. paradoxa* with 61.62% inhibition followed by *T. harzianum* and *T. virens* with 60.80 and 59.49 per cent inhibition, respectively.

Field evaluation of bio-control agents against Stem bleeding disease in coconut

As per the data in Table 2, the results of the experiment conducted during 2018-19 and 2019-20, the application of *T. harzianum* cake formulation completely brought down the disease index of 6.20 and 7.96 to zero per cent and reduced the disease by 100 % in respective years within 50 days of cake application. The disease index was reduced to 32.17

and 20.52% in the case of paste application of copper oxychloride, in respective years, and the disease index was reduced to 18.81 and 100 % in case of paste application of *T. reesei* against stem bleeding disease of coconut during 2018-19 and 20119-2,0 respectively (Plates 7, 8, 9 & 10).

The experiment was conducted during the year 2020-21 and 2021-22 with *T. reesei* cake formulation and *T. reesei* paste application against stem bleeding disease of coconut. The experiment results revealed that application of *T. reesei* cake formulation disease index of 15.07 and 11.28% was brought down to 0.00 per cent within 50 days of cake application. Whereas paste application of *T. reesei* as 19.50 and 10.14 was reduced to 1.66 (91.48% reduction over control) and 1.64 (83.82% reduction over control), respectively. In the case of copper oxychloride application disease was reduced by 66.93 and 72.53% over control. However, the treatments differed significantly at 50 DAT (Table 3).

Field evaluation of bio-control agents against stem bleeding disease in coconut

Trichoderma asperellum produces several groups of antibiotics and toxins, and then the growth of the pathogen is inhibited (Eziashi *et al*., 2010). Also, it can inhibit or reduce the growth of the pathogen through competition for space, nutrients, or oxygen. George *et al*. (2012) reported *Pseudomonas fluorescens*, a potential inhibitory biocontrol agent against *Gnanoderma* under *in vitro* conditions. The inhibition of mycelial growth of the pathogen by *P. fluorescens* may be due to the production of antibiotics. Production of antibiotics HCN, pyrrolnitrin, phenazine, and 2, 4-diacetyl

Table 2: Evaluation of *Trichoderma* formulations against stem bleeding disease-causing pathogen *Thielaviopsis paradoxa* under natural field conditions.

Treatments		* Stem Bleeding Disease Index (PDI)					
		2018-19			2019-20		
		Pre-treatment	3 MAT	Reduction over initial %	Pre-treatment	3 MAT	Reduction over initial %
T1.	*T. harzianum* cake application	6.20	0.00	100	7.96 (17.21)	0.00* (0.00)	100.00
T2.	*T. reesei* paste application	6.59	5.35	18.81	6.95 (15.81)	0.00 (0.00)	100.00
T3.	Copper oxychloride paste application	6.90	4.68	32.17	6.82 (15.43)	5.42 (13.80)	20.52
T4.	Control	8.35	15.61	-	11.70 (21.07)	15.70 (23.79)	-
	S.Em.±	1.12	5.37	-	2.29	2.52	-
	C.D. @ 5%	3.33	15.45	-	6.76	7.46	-

*Values in the parentheses are angular transformed values; MAT- months after treatment

Table 3 : Field evaluation of bio-control agents against stem bleeding disease in coconut.

Treatments		* Stem bleeding disease Index (PDI)					
		2020-21			2021-22		
		Pre-treatment	3 MAT	Reduction over initial	Pre-treatment	3 MAT	Red over. initial %
T1.	*T. reesei* cake application	15.07 (21.35)	0.00 (0.00)	100	11.28 (19.52)*	0.00 (0.00)	100.00
T2.	*T. reesei* paste application	19.50 (25.88)	1.66 (6.39)	91.48	10.14 (18.52)	1.64 (7.31)	83.82
T3.	Copper oxychloride paste application	22.17 (27.97)	7.33 (15.49)	66.93	12.16 (20.58)	3.34 (10.05)	72.53
T4.	Control	15.63 (22.18)	21.16 (27.79)	-	11.09 (19.38)	12.76 (20.87)	-
	S.Em.±	NS	2.28	-	NS	0.83	-
	C.D. at 5%	NS	6.90	-	NS	2.52	-

*Values in the parenthesis are angular transformed values; MAT- months after treatment

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phloroglucinol and lytic enzymes by *P. fluorescens* against fungal pathogens were reported by many workers (Ramamoorthy *et al*., 2002; Saravanakumar *et al*., 2008).

CONCLUSION

It is concluded that the cake application of *T. harzianum* and *T. reesei* formulation completely brought down the disease index by 100 per cent within 50 days of cake application. Paste application of bioagents *viz*., *T. harzianum*, *T. reesei*, and chemical fungicide copper oxychloride application also substantially reduced the disease.

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Research Article

Occurrence, characterization and management of Sri Lankan Cassava Mosaic Virus (SLCMV) in cassava growing Namakkal district of Tamil Nadu, India

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ABSTRACT

Cassava mosaic disease caused by begomoviruses of the Geminiviridae family is one of the major constraints for Cassava production in many Cassava growing states of India. The disease is known to be transmitted by whiteflies *Bemisia tabaci* and spread through the infected Cassava planting material. In India, the Cassava mosaic disease (CMD) is caused by both the Indian cassava mosaic virus (ICMV) and Sri Lankan cassava mosaic virus (SLCMV). However, Sri Lankan cassava mosaic virus was the most predominant virus species in the study area. To assess the severity of CMD, a survey was conducted during 2021-2022 in different blocks of the Namakkal district, Tamil Nadu state. The incidence ranged from 34-95 per cent, the highest 95 per cent was recorded in Venandur and the lowest 34% was recorded in Elachipalayam block. The disease prevalence ranged from 36-85 per cent, the highest 85% was recorded in Paramathi, and the lowest 36 per cent was recorded in the Elachipalayam block, respectively. Variety Sree Padmanabha was found to be resistant to the Cassava mosaic virus in a trial conducted in a farmers' field in Venandur block. Growing CMD-resistant varieties and use of disease-free Cassava planting material are the most important and economically feasible practices in the management of Cassava mosaic Disease.

***Key words*:** Cassava mosaic disease, cassava mosaic virus, ICMV, SLCMV, management.

Cassava (*Manihot esculenta* Crantz) is a woody shrub of the spurge family, Euphorbiaceae, native to South America. Although a perennial plant, cassava is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates. Cassava is one of the most important staple food crops for more than 700 million subsistence farmers in tropical and sub-tropical Africa, Asia, and Latin America (IFAD and FAO, 2000) and is grown widely under diverse environmental conditions (El Sharkawy, 2007). It is the third-largest source of food carbohydrates in the tropics, after rice and maize. It is one of the most drought-tolerant crops, capable of growing on marginal soils. Nigeria is the world's largest producer of cassava, while Thailand is the largest exporter of Cassava starch (Anonymous, 1990).

In India, Cassava production is 6.7 million tonnes. Kerela, ranks first in Cassava production and is the largest producer with 50 per cent of the area and Tamil Nadu accounts for 32 per cent of the area and 9 per cent area is in Andhra Pradesh (Srinivas and Anantharaman, 2005).

In India, dried cassava roots are sold in the name of tapioca chips, and the meal is in the name of tapioca flour. Cassava is predominantly consumed in boiled form, but substantial quantities are used to extract cassava starch, called tapioca, which is used for food, animal feed, and industrial purposes. It is a very good source of energy and rich in carbohydrates (Nitrogen free extract (NFE) 92%). The crude protein content is 2.4 per cent and the total digestible nutrients (TDN) content is 67 per cent (Anonymous, 2012).

The major constraint in Cassava production is mainly diseases like Anthracnose (*Colletotrichum gloeosporioides*), Brown leaf spot (*Cercosporidium henningsii*), Bacterial Blight (*Xanthomonas manihotis*) *etc.* but major disease which causes maximum yield loss is Cassava Mosaic Disease (CMD) caused by Cassava infecting Gemini viruses (CIGs) belonging to the family of Geminiviridae and Genus Begomovirus, which is transmitted by whitefly, *Bemisia tabaci* Genn. The CMD was first described in 1894 and there are 10 species of Cassava mosaic begomoviruses known in Africa and Asia. However, the production of cassava on the Indian subcontinent has been severely affected by the Indian cassava mosaic virus (ICMV) and Sri Lankan cassava mosaic virus

(SLCMV) (Uke *et al*., 2022). Compromised leaf development and photosynthetic capacity cause reduced storage root yields. Uke *et al*. (2022) also found that cassava root yield and starch content declined to 16–33 per cent and 22–38 per cent, respectively, when (SLCMV-infected cuttings were used at planting time, compared to the use of healthy disease-free cuttings. It has since received considerable attention in the southern states of Kerala and Tamil Nadu, which are the main Cassava growing areas of India.

The infected plants are stunted by means of a reduction in plant height, petiole length, mid-leaflet length, and width (Thankappan and Chacko, 1976). Initially, the leaves show faint greenish-yellow specks surrounded by normal green areas, which were mostly confined to the lower portion of the lamina. In case of severe infection, the leaf gets reduced in size, misshapen, and twisted with bright yellow areas separated normally by green areas (Jos *et al*., 1984; Mathew, 1989).

The current investigation presents the outcome of a CMD survey conducted across cassava plantations in major growing areas of Tamil Nadu state from November 2020 to November 2021. The disease is common in cassava-growing areas with varied degrees of incidence in different areas. Though Indian Cassava mosaic virus (Harrison *et al*., 1991) and Sri Lankan Cassava mosaic virus (Saunders *et al*., 2002) are prevalent in Tamil Nadu there is no information on which strain of virus is present in the surveyed areas. The study complies with the outcome of CMD distribution in the surveyed as well as the virus strain associated with the disease.

MATERIALS AND METHODS

Survey sample collection

The survey was conducted for two years 2021 and 2022 in major Cassava growing areas of Namakkal district in Tamil Nadu state. The surveyed areas include Erumapatti, Namakkal, Mohanur, Paramathi, Thiruchengode, Rasipuram, Venandur, Mallasamudram, Senthamangalam, Elachipalayam and Namagiriblocks of Namakkal. An area of 200 ha was covered during the survey and the age of the plants ranged from 2 to 9 months old. Cassava plants were randomly sampled from all the surveyed areas and leaf samples were collected for PCR identification. During the survey, information on Cassava variety, age of the plant, previous crop, and exact location of the surveyed area was recorded.

The CMD prevalence, incidence, and symptom severity

The severity of Cassava mosaic disease was calculated as the disease prevalence and percent incidence using the following formulae: Disease prevalence rate (%) was calculated using the following equation (Rabindran, 2011),

$$\text{Disease prevalence} = \frac{\text{Number of fields with visible symptoms}}{\text{Total number of fields observed}} \text{ X100}$$

Disease incidence (%) was calculated using the following equation:

$$\text{Percent Disease Incidence} = \frac{(N-n)}{N} \text{ X 100}$$

Where, *N* is the total number of observations, and

n is the total number of plants with disease symptoms

The disease severity was recorded using the standard 1-5 scale as described by Hahn *et al*. (1980) in which grade 1 represents Cassava mosaic symptomless plants; 2 - Mild chlorosis, mild distortions at bases of most leaves, 3 - mosaic pattern on most leaves, narrowing and distortion of the lower one-third of the leaflets; 4 - Severe mosaic distortion of two-thirds of most leaves and general reduction of leaf size and stunting of shoots and 5 - very severe mosaic symptoms on all leaves, distortion, twisting, misshapen and severe leaf reduction of most leaves accompanied by severe stunting of plants.

To identify the virus, and strain the samples were preserved in refrigerated condition with neatly labelled and proper care till the samples were sent to the designated laboratory. To confirm, the infected Cassava plants, leaf samples were brought to the Central Integrated Pest Management Centre, Trichy laboratory for identification. For further identification and characterization, the samples were sent to the Division of Crop Protection, ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India.

The DNA extraction and SLCMV detection

The DNA was extracted from cassava leaves using the (CTAB) method (Doyle and Doyle, 1990). The quality and concentration of the DNA were assessed using Nano Drop™ 1000 Spectrophotometer (Thermo Fisher Scientific). The DNA was resuspended in water containing 100 µg ml^{-1} RNase and stored at –20 °C. The quality and quantity of the isolated DNA were assessed by agarose gel electrophoresis.

To detect SLCMV, the AV1 gene (encoding coat protein) was amplified from SLCMV-infected cassava leaf samples by PCR using sequence-specific primers (forward: 5′ - GTTGAAGGTACTTAT TCC C-3′ and reverse: 3′ - TATTAATACGGTTGTAAA CGC-5′). The PCR reactions

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were carried out in a Gene Amp PCR system 9700 (PE Applied Biosystems, Foster City, CA) thermocycler. All amplifications were performed in volumes of 25 µL PCR mix containing 2µL DNA templates, 1.5 U Taq DNA polymerase, 25 mM $MgCl_2$, 2 mM dNTPs and 20 pmol of each primer. The PCR was programmed with the initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing temperature 55 °C for 40 seconds, and the final extension of 72 °C for 90 seconds. The PCR products were electrophoresed (1 h at 80V) in 0.8 per cent agarose gels in Tris-Borate-EDTA buffer, pH 8. Gels were stained with ethidium bromide (10 mg/mL) and viewed in a gel documentation system (Alpha Inno Tech, USA).

The amplified PCR products of Cassava mosaic virus sample were purified from agarose gels using the QIA quick gel extraction kit (Qiagen, Hilder, Germany) and sequenced using an automated DNA sequencing facility at Eurofins Genomics India Pvt. Ltd., Bengaluru, India.

RESULTS AND DISCUSSION

A systematic random survey was undertaken to record the incidence and prevalence of the Cassava mosaic virus in the Namakkal districts of Tamil Nadu. Samples were also collected to identify the prevalence of the Indian Cassava Mosaic Virus and Sri Lankan Cassava Mosaic Virus. During the survey, it was observed that 80 per cent of surveyed fields were infected with the Cassava mosaic virus.

Major symptoms recorded in the field were yellow mosaic (Plate 1. A, B), leaf curling, leaf mottling, twisted leaves (Plate 1, C), chlorotic patches, and deformed leaves. The disease usually spread through the infected cuttings and whitefly *Bemisia tabaci* transmission. Young plants were having severe infections compared to older ones. The disease is severe if it was transmitted through cuttings compared to white fly transmission. Two varieties were majorly grown in this area *i.e.* Mulluvadi and White Thailand, both varieties were found to be susceptible.

The incidence was severe in all the fields ranging from 34-95 per cent. The highest incidence (95%) was recorded in Venandur and the lowest (34%) was in Elachipalayam block. Whereas, Erumapatti, Namakkal, Mohanur, Paramathi, Thiruchengode, Rasipuram, Venandur, Mallasamudram, Senthamangalam, and Namagiri blocks of Namakkal district recorded 60, 50, 85, 90, 70, 50, 95, 80, 90, and 48 per cent, respectively.

The disease prevalence ranged from 36 to 85 per cent. The highest of 85 per cent was recorded in Paramathi and the lowest of 36 per cent was recorded in Elachipalayam block. Similarly, Erumapatti recorded 60 per cent, Namakkal 50 per cent, Mohanur 55 per cent, Thiruchengode 60 per cent, Rasipuram 55 per cent, Venandur 80 per cent, Mallasamudram 50 per cent, Senthamangalam 75 per cent, and Namagiri 40 per cent, respectively (Table 1). During 2005-2006, Rajinimala *et al.* (2011) also observed more than 90 per cent of incidence throughout the Tamil Nadu state. This high incidence could be due to indiscriminate use of infected planting material as a result of which there was a high percentage of cutting-borne infection than the whitefly-transmitted infection in the surveyed area.

To confirm the presence of virus associated with mosaic symptoms the representative samples were collected from each infected field of different blocks of Namakkal district. The DNA was isolated using CTAB method (Doyle and Doyle, 1990). The DNA samples were subjected to a polymerase chain reaction (PCR) using cassava mosaic virus-specific primers, and samples from all twelve blocks were found to be infected with Sri Lankan cassava mosaic virus

Table 1: Prevalence and percent incidence of *Cassava mosaic virus* in different blocks of Namakkal district.

Place	No. of fields surveyed	Disease prevalence	Incidence (%)	Samples collected	PCR positive samples	Variety
Erumapatti	24	60	60	12	12	Mulluvadi
Namakkal	50	50	50	15	15	Mulluvadi
Mohanur	35	55	85	20	20	Mulluvadi
Paramathi	10	85	90	10	8	Mulluvadi
Thiruchengode	8	60	70	8	8	White Thailand
Rasipuram	16	55	50	16	15	Mulluvadi
Venandur	14	80	95	14	14	Mulluvadi
			95			White Thailand
Mallasamudram	10	50	80	15	15	Mulluvadi
Senthamangalam	20	75	90	20	20	Mulluvadi
Elachipalayam	15	36	34	15	15	Mulluvadi
Namagiripettai	18	40	48	16	16	Mulluvadi
Sendhamangalam	12	45	50	15	15	White Thailand

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(Fig. 1). The partially amplified product of AV1 gene 1400bp was cloned and two samples were sequenced. In the blast analysis using NCBI blast the sequenced samples showed close 88.78 per cent sequence similarity with Sri Lankan Cassava mosaic virus (SLCMV).

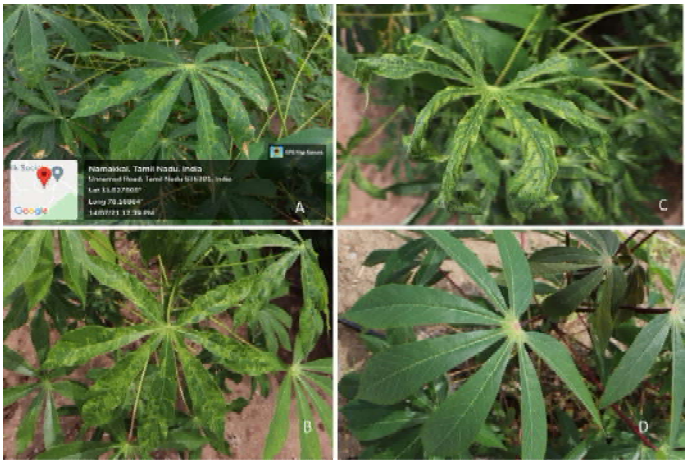

Plate 1: A & B: Cassava mosaic virus-infected leaves showing yellow mosaic with chlorotic patches, C: leaf mottling and twisted leaves, D: Healthy leaf without any mosaic symptom.

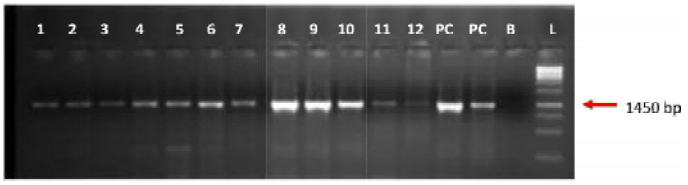

Fig. 1. The polymerase chain reaction of Cassava mosaic infected samples. lane 1-12 CMV samples from different blocks, PC= Positive control, B = Blank and L = ladder

Plate 2: Picture showing the trial conducted in farmers' field in Venandur block of Tamil Nadu, (R– CMD Resistant Sree Padmanabha and S-Susceptible Mulluvadi Variety).

In Venanadur block, one of the farmers had grown the Sree Padmanabha variety for trial purposes which was supplied by Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, Kerala through Shri Varalakshmi Company, manufacturer of Sago and Sabudana, Salem, Tamil Nadu. In the trial, farmers had grown one row of Mulluvadi variety of Cassava after every four rows of Sree Padmanabha. Interestingly, it was observed that the Sree Padmanabha variety recorded only 5 per cent of Cassava mosaic infection (grade 1 on 1-5 rating scale) when compared to the 95 per cent CMV infection (grade 5 on 1-5 scale) on the Mulluvadi variety.

This clearly indicates that the Sree Padmanabha variety is resistant to the Cassava mosaic virus in this region (Plate 2). It is also learned that CTCRI also had recommended this particular Sree Padmanabha (first CMD resistant) variety for the irrigated plains of the Tamil Nadu State.

Integrated disease management is a Crop protection strategy that combines biological, cultural, physical, and chemical control practices to safeguard the crop. Many authors reported the importance of integrated management options that have advantages from different points of view. One important advantage of integrated disease management is that it is utilized in a way that minimizes economic, health, and environmental risks (Banjaw and Regessa, 2017). In many crop protection reports integrated disease management includes the use of resistant variety, applying cultural practices, biological control as well as chemical control as major components.

Cultural cassava mosaic disease management includes the use of healthy disease planting material, rouging infected plants, adjusting planting time, and cropping system. The importance of sanitation such as management of plant left over after harvest, removing crop debris, cleaning farm tools, and rouging in reducing the sources of the pathogens that cause cassava diseases in farms stated (IITA, 2000).

Biological control of cassava whitefly depends on natural enemies such as fungi, predators, and parasitoids (Horowitz *et al*., 2011). However, because of the harmful effects of chemicals on humans and the ecosystem and because of resistance development, applications of alternative plant extracts have been reported. For instance, essential oils distilled from Geranium and Artemisia could be applied to control *B. tabaci* in greenhouse cucumber at V/V 12 ppm (Yarahmadi *et al*., 2013). Besides, insecticides are recommended as the last option in whitefly management while the importance of insect growth regulators have been emphasized and reviewed (Horowitz *et al*., 2011; Banjaw and Regessa, 2017).

CONCLUSION

It is concluded that Sri Lankan Cassava mosaic virus is the most predominant in the study area. However, with regard to the integrated management of this disease, growing resistant varieties like Sree Padmanabha is going to be economically feasible and it reduces the production cost. Apart from this use of CMD disease-free planting material is

most important in the management of disease as it reduces the initial inoculum and delays the disease development till the viruliferous whiteflies feed on the healthy cassava plant.

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Research Article

Sustainable management of sheath blight in rice (*Rhizoctonia solani* Kuhn)

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ABSTRACT

A study was done to explore the various strategies to manage the sheath blight of rice. During *kharif* 2018 and 2019, different treatments *viz*., foliar and soil application of *Trichoderma viride*, *Pseudomonas fluorescens*, mustard, and radish leaf and their combination were applied at 30 and 60 days after transplanting (DAT). The parameters such as disease severity, grain chaffiness, plant height, and yield were evaluated at 45 and 75 DAT and at harvest. Foliar application of propiconazole 25 per cent EC @ 0.1 per cent was most effective (50-75.01%) followed by soil application of mustard leaf @ 5 kg plot^{-1} before transplanting and foliar application of *P. fluorescens* @ 5 g l^{-1}, whereas foliar application of *T. viride* @ 5 g l^{-1} (9.64% and 46.64%) was least effective. The foliar application of propiconazole 25 per cent EC @ 0.1 per cent recorded the lowest percent of chaffiness (11-15%), the highest yield of 4941.7-4991.7 kg ha^{-1}, whereas the foliar application of *T. viride* @ 5 g l^{-1} showed highest percent of chaffiness (28.67-36.67 per cent) with a yield of 3516.7-3475 kg/ha^{-1}.

***Keywords*:** *Kharif*, chaffiness, rice, sheath blight, bioagents, biofumigants.

Rice (*Oryza sativa* L.) is the second most important cereal and the staple food for more than half of the world's population. Rice is the most prominent crop of India as it is the staple food for most of the people of the country. In India, rice crop is being cultivated in an area of 45.77 million ha, with a production of 124.37 million tonnes and productivity of 2717 kg ha^{-1} and in Karnataka, it occupies an area of 1.40 million ha, production of 4.29 million tonnes and productivity of 3072 kg ha^{-1} (Anonymous, 2022).

Rice is attacked by a number of fungal, bacterial, viral, and nematode diseases. Among all pathogenic organisms, fungal pathogens are limiting rice productivity to a great extent. Several outbreaks of diseases such as blast, sheath blight, and bacterial blight have been reported in many rice-growing areas of India. Worldwide the annual losses due to rice diseases are estimated to be 10-15 per cent, depending upon the age of the plant, time of infection, and severity, diseases caused yield loss to the extent of 5.9 to 69 per cent (Venkatrao *et al*., 1990; Naidu, 1992).

Sheath blight is one of the major biotic constraints that affect rice production in India and is considered an economically important disease of rice in the world (Lee and Rush 1983; Webster and Gunnell 1992). The disease is caused by *Rhizoctonia solani* Kuhn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk). The Sheath blight is becoming most destructive, being second only to rice blast disease among the rice diseases constraining rice productivity (Ou, 1985). The disease is endemic to areas where temperature and relative humidity are high and cultivation is intensive. The pathogen is a polyphagous competitive saprophyte and has a wide host range. Continuous rice cropping, high density, and heavy canopy associated with high nitrogen management favours disease build-up from tillering to panicle initiation (Biswas, 2001).

The incidence of rice sheath blight disease has increased in recent years, because of the unavailability of resistant cultivars or any other suitable economic disease management measures. The yield losses due to this disease are reported to range from 5.2 to 50 per cent, depending on environmental conditions, crop stages at which the disease appears, cultivation practices, and cultivars in India (Rajan, 1987; Sharma and Teng, 1996).

The management of sheath blight through fungicide application is the most common approach among farmers. Because of the disadvantages of using fungicides, it has become necessary to adopt eco-friendly approaches for enhancing crop yield and better crop health. The use of biological methods for the management of this disease is

scarce. It is necessary to evaluate the biological methods including the use of bioagents, bio fumigants, botanicals, *etc*., to manage the disease effectively, to avoid resistance developments in pathogens, and to minimize the fungicidal residues for ecological sustainability. In view of the importance of the crop and the seriousness of the sheath blight disease the present work was undertaken with the objective of managing the disease with a non-chemical sustainable strategy.

MATERIALS AND METHODS

The investigations were conducted during *kharif* 2018 and 2019 in the Department of Plant Pathology, College of Agriculture, V.C. Farm, Mandya.

Field evaluation of bioagents and bio fumigants against sheath blight of rice

A field trial was conducted during *kharif* 2018 and 2019 to study the effect of bio-agents and bio-fumigants on the severity of sheath blight and the yield of rice. The field selected for the experiment had a previous history of sheath blight which was ploughed twice and soil was brought to a fine tilth. The experiment was laid out in a randomized block design with 12 treatments and one untreated control where propiconazole 25% EC was used for the comparison (Table 1). All the treatments were replicated three times in mini-plots of size 5 × 4 m (20 m^2). The inoculum of *R. solani* was applied to each plot uniformly @ 1 kg of culture per plot. Two weeks after amending the plots, 25 days old Jyothi seedlings were transplanted at a spacing of 20 × 20 cm. Except for treatments, all other recommended packages of practices were followed for growing the crop. The assessment of disease severity was made by following the SES scale (IRRI, 1996) on 45 and 75 DAT. Percent chaffiness of grain, plant height, and yield were recorded at harvest, expressed per plot and hectare.

Sheath blight disease scoring was done on 0-9 scale where, 0= No infection, 1= Vertical spread of the lesions up to 20 per cent of plant height, 3= Vertical spread of the lesions up to 21-30 per cent of plant height, 5= Vertical spread of the lesions up to 31-45 per cent of plant height, 7= Vertical spread of the lesions up to 45-65 per cent of plant height, 9= Vertical spread of the lesions up to 66-100 per cent of plant height. The percent disease index was calculated according to the following equation:

$$\text{Percent Disease Index} = \frac{\text{Sum of numerical ratings}}{\text{Total no. of tillers observed X Maximum grade}} \times 100$$

Statistical analysis

The data obtained in different experiments were statistically analyzed by following Randomized Block Design, as per the procedures suggested by Snedecor and Cochran (1967) and Panse and Sukhatme (1978). The data pertaining to percentage were transformed into arc sin transformation, as is required before statistical analysis.

Table 1: Details of the field trial treatments evaluated during *kharif* 2018 and 2019.

T_1	Foliar application of *Trichoderma viride* (Tv) @ 5 g l^{-1} at 30 Days after transplanting (DAT)
T_2	Foliar application of Tv @ 5 g l^{-1} at 30 DAT and 60 DAT
T_3	Foliar application of *Pseudomonas fluorescens* (Pf) @ 5 g l^{-1} at 30 DAT
T_4	Foliar application of Pf @ 5 g l^{-1} at 30 DAT and 60 DAT
T_5	Soil application of Pf (20 g) at the time of transplanting
T_6	Soil application of Pf (20 g) and foliar application of Pf (5 g l^{-1}) at 30 DAT
T_7	Soil application of mustard leaf @ 5 kg $plot^{-1}$ before transplanting
T_8	Soil application of radish leaf @ 5 kg $plot^{-1}$ before transplanting
T_9	Soil application of mustard leaf @ 5 kg $plot^{-1}$ before transplanting and foliar application of Tv @ 5 g/l at 30 DAT
T_{10}	Soil application of mustard leaf @5 kg $plot^{-1}$ before transplanting and foliar application of Pf @ 5 g l^{-1} at 30 DAT
T_{11}	Foliar application of propiconazole 25 % EC @ 0.1 per cent at 30 DAT
T_{12}	Foliar application of propiconazole 25 % EC @ 0.1 per cent at 30 DAT and 60 DAT
T_{13}	Untreated control

RESULTS AND DISCUSSION

Field evaluation of bioagents and bio-fumigants against sheath blight of rice disease severity

The field trial was conducted during *kharif* 2018 and 2019 to study the effect of bioagents and bio-fumigants on the severity of sheath blight and yield in rice.

Effect on disease severity

The bioagents, bio-fumigants and their combination were evaluated for their effect on the disease severity of rice sheath blight during *kharif* 2018 and 2019. The result of the experiment conducted during *kharif* 2018 is shown in Table 2. On the basis of mean disease severity and per cent reduction over control, treatment foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT and 60 DAT (T12) was most effective (12.59% and 70.18%) followed by (T10) soil application of mustard leaf @ 5 kg $plot^{-1}$ before transplanting and foliar application of *P. fluorescens* @ 5 g l^{-1} at 30 DAT (17.77% and 57.91%). The least effective treatment was (T1) foliar application of *T. viride* @ 5 g l^{-1} at 30 DAT (38.15% and 9.64%) followed by (T8) soil application of radish leaf @ 5 kg $plot^{-1}$ before transplanting (36.29% and 14.05%) whereas in T13 (control) the mean disease severity was observed to be 42.22 per cent. The remaining treatments

showed the main disease severity ranging from 19.25 per cent to 36.29 per cent and 19.25 per cent to 34.81 per cent reduction over control. The same trend was also observed during *kharif* 2019 (Table 3) wherein treatment (T12) foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT and 60 DAT was most effective with mean disease severity and percent reduction over control (11.29% and 75.01%) followed by (T10) soil application of mustard leaf @ 5 kg plot^{-1} before transplanting and foliar application of *P. fluorescens* @ 5 g l^{-1} at 30 DAT (13.33 per cent and 70.50 per cent). Among the treatments, foliar application of *T. viride* @ 5 g l^{-1} at 30 DAT (T1) was least effective with mean disease severity of 39.26 per cent and per cent reduction over control 13.10 per cent.

Table 2: Effect of bioagents and bio-fumigants tested against sheath blight of rice on disease severity during *kharif* 2018.

Treatment	Disease severity (%)			
	45 DAT	75 DAT	Mean	Reduction over control (%)
T_1	31.85 (34.35)	44.44 (41.80)	38.15 (38.13)	9.64
T_2	20.74 (27.08)	37.03 (37.44)	28.89 (32.49)	31.57
T_3	27.40 (31.56)	42.22 (40.52)	34.81 (36.15)	17.55
T_4	18.51 (25.47)	33.33 (35.25)	25.92 (30.59)	38.61
T_5	24.44 (29.61)	41.48 (40.06)	32.96 (35.01)	21.93
T_6	15.55 (23.19)	32.59 (34.75)	24.07 (29.33)	42.99
T_7	22.96 (28.62)	37.77 (37.91)	30.37 (33.43)	28.07
T_8	28.88 (32.49)	43.70 (41.37)	36.29 (37.04)	14.05
T_9	11.85 (20.01)	26.66 (31.07)	19.25 (25.99)	54.41
T_{10}	9.62 (17.90)	25.92 (30.48)	17.77 (24.79)	57.91
T_{11}	13.33 (21.22)	28.88 (32.49)	21.11 (27.34)	50.00
T_{12}	8.14 (16.54)	17.03 (24.30)	12.59 (20.72)	70.18
T_{13}	33.33 (35.25)	51.11 (45.63)	42.22 (40.51)	0.00
S.Em.±	1.11	1.26	1.00	-
C.D. (0.05)	3.23	3.67	2.91	-
C.V. (%)	7.10	5.87	5.35	-

DAT = Days after transplanting; Figures in parentheses are arcsine transformed values

Table 3: Effect of bioagents and bio-fumigants tested against sheath blight of rice on disease severity during *kharif* 2019.

Treatment	Disease severity (%)			
	45 DAT	75 DAT	Mean	Reduction over control (%)
T_1	31.11 (33.89)	47.40 (43.50)	39.26 (38.79)	13.10
T_2	16.29 (23.73)	31.92 (34.34)	24.11 (29.35)	46.64
T_3	24.44 (29.61)	40.00 (39.22)	32.22 (34.57)	28.69
T_4	14.44 (22.32)	29.62 (32.95)	22.03 (27.97)	51.24
T_5	20.74 (27.04)	36.29 (37.03)	28.52 (32.25)	36.87
T_6	12.22 (20.45)	33.40 (35.30)	22.81 (28.52)	49.51
T_7	18.51 (25.42)	34.14 (35.74)	26.33 (30.84)	41.72
T_8	27.40 (31.56)	44.44 (41.80)	35.92 (36.81)	20.50
T_9	9.25 (17.70)	22.22 (28.04)	15.74 (23.32)	65.16
T_{10}	8.14 (16.52)	18.51 (25.42)	13.33 (21.36)	70.50
T_{11}	10.74 (19.12)	28.96 (32.54)	19.85 (26.44)	56.06
T_{12}	7.03 (15.36)	15.55 (23.19)	11.29 (19.61)	75.01
T_{13}	35.55 (36.57)	54.81 (47.76)	45.18 (42.23)	0.00
S.Em ±	1.00	1.22	0.99	
C.D. (0.05)	2.93	3.56	2.90	
C.V. %	7.00	5.98	5.66	

DAT = Days after transplanting; Figures in parenthesis are arcsine transformed values

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Effect on plant height

The plant height was significantly influenced by the treatments during *kharif* 2018 and 2019. The mean plant height in different treatments ranged from 73.4 cm to 92.5 cm (Table 4). The foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT and 60 DAT (T12) had a significant effect on plant height which recorded the highest mean plant height (92.5 cm) and mean percent increase over control (26%) compared to other treatments. It was followed by (T10) soil application of mustard leaf @ 5 kg plot^{-1} before transplanting and foliar application of *P. fluorescens* @ 5 g l^{-1} at 30 DAT (90.1 cm and 22.8%), (T9) Soil application of mustard leaf @ 5 kg plot^{-1} before transplanting and foliar application of *T. viride* @ 5 g/l at 30 DAT (88.8 cm and 21%) and (T11) foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT (87 cm and 18.5%). Soil application of mustard leaf @ 5 kg/plot before transplanting and foliar application of *P. fluorescens* @ 5 g l^{-1} at 30 DAT (T10) was on par with foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT and 60 DAT (T12) and the least mean plant height and mean per cent increase over control was observed in (T1) foliar application of *T. viride* @ 5 g l^{-1} at 30 DAT (76.4 cm and 4.1%) which was followed by (T8) soil application of radish leaf @ 5 kg/plot before transplanting (78.7 cm and 7.2%) and (T3) foliar application of *P. fluorescens* @ 5 g l^{-1} at 30 DAT (81.2 cm and 10.6%).

Effect on grain chaffiness

The percent of grain chaffiness was significantly influenced by different treatments (Fig. 1). The mean chaffiness percentage observed in different treatments ranged from 11 to 30.83%. The treatment, foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT and 60 DAT (T12) was superior to all other treatments which recorded the lowest per cent of chaffiness and highest percent decrease over control (11% and 64.32%) followed by (T10) soil application of mustard leaf @ 5 kg plot^{-1} before transplanting and foliar application of *P. fluorescens* @ 5 g l^{-1} at 30 DAT (11.33% and 63.25%), (T9) soil application of mustard leaf @ 5 kg plot^{-1} before transplanting and foliar application of *T. viride* @ 5 g l^{-1} at 30 DAT (12.67% and 58.9%) and (T11) foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT (14% and 54.59%). The highest percent of chaffiness and lowest percent decrease over control was observed in (T1) foliar application of *T. viride* @ 5 g l^{-1} at 30 DAT (28.67% and 7%), which was followed by (T8) soil application of radish leaf @ 5 kg plot^{-1} before transplanting (27% and 12.42%) and (T3) foliar application of *P. fluorescens* @ 5 g l^{-1} at 30 DAT (25% and 18.91%).

Effect on grain yield

The data for perusal on grain yield is presented in Table 5. There was a significant difference in grain yield among treatments. During *kharif* 2018, the highest yield per plot and yield per hectare was recorded in (T12) foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT and 60 DAT (9.98 kg and 4991.7 kg) with 44.3 per cent increase over control, followed by (T10) soil application of mustard leaf @ 5 kg plot^{-1} before transplanting and foliar application of *P. fluorescens* @ 5 g l^{-1} at 30 DAT (9 kg and 4433.3 kg) with 24.3 per cent increase over control. The lowest yield per plot and hectare was recorded in (T1) foliar application of *T. viride*

Table 4: Effect of bioagents and bio-fumigants tested against sheath blight of rice on plant height.

Treatment	*Kharif* 2018		*Kharif* 2019		Mean	
	Plant height (cm)	Increase over control (%)	Plant height (cm)	Increase over control (%)	Plant height (cm)	Increase over control (%)
T_1	75.9	3.8	76.8	4.2	76.4	4.1
T_2	83.5	14.2	82.6	12.1	83.1	13.2
T_3	82.3	12.6	80.0	8.5	81.2	10.6
T_4	84.5	15.6	83.4	13.2	83.9	14.3
T_5	82.8	13.3	80.4	9.1	81.6	11.2
T_6	86.5	18.3	84.8	15.1	85.7	16.8
T_7	82.9	13.4	80.8	9.6	81.9	11.6
T_8	79.1	8.2	78.3	6.2	78.7	7.2
T_9	89.5	22.4	88.0	19.4	88.8	21.0
T_{10}	91.0	24.5	89.2	21.0	90.1	22.8
T_{11}	87.5	19.7	86.4	17.2	87.0	18.5
T_{12}	93.5	27.9	91.5	24.2	92.5	26.0
T_{13}	73.1	0.0	73.7	0.0	73.4	0.0
S.Em ±	1.10	-	1.09	-	0.66	-
C.D. (0.05)	3.20	-	3.19	-	1.92	-
C.V. %	2.27	-	2.30	-	1.37	-

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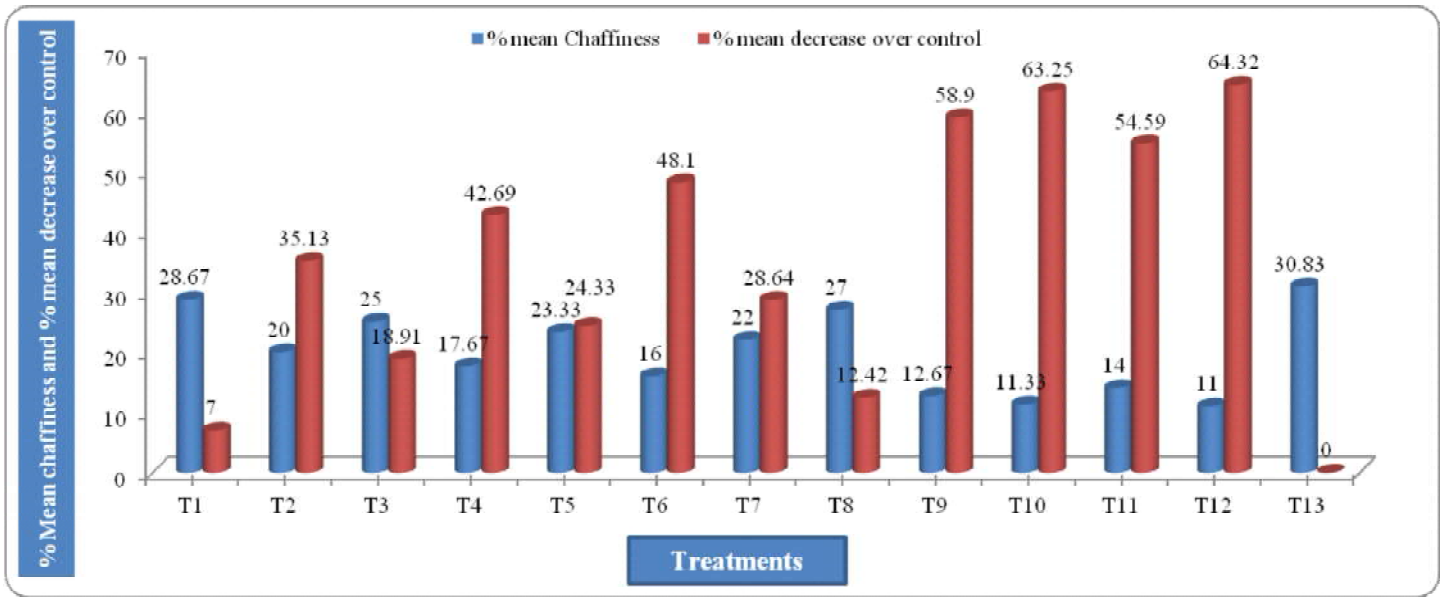

Fig. 1c: Effect of bioagents and bio-fumigants against sheath blight of rice on average grain chaffiness in field during Kharif 2018 and 2019

Treatment details:			
T1 :	Foliar application of *Trichoderma viride* (Tv) @ 5 g/L at 30 DAT	T8 :	Soil application of radish leaf @5 kg/plot before transplanting
T2 :	Foliar application of Tv @ 5 g/L at 30 DAT and 60 DAT	T9 :	Soil application of mustard leaf @5 kg/plot before transplanting and foliar application of Tv @ 5 g/L at 30 DAT
T3 :	Foliar application of *Pseudomonas fluorescens* (Pf) @ 5 g/L at 30 DAT	T10 :	Soil application of mustard leaf @ 5 kg/plot before transplanting and foliar application of Pf @ 5 g/L at 30 DAT
T4 :	Foliar application of Pf @ 5 g/L at 30 DAT and 60 DAT	T11 :	Foliar application of propiconazole 25 EC @ 0.1 % at 30 DAT
T5 :	Soil application of Pf (20 g) at the time of transplanting	T12 :	Foliar application of propiconazole 25 EC @ 0.1 % at 30 DAT and 60 DAT
T6 :	Soil application of Pf(20 g) and foliar application of Pf (5 g/L) at 30 DAT	T13 :	Untreated control
T7 :	Soil application of mustard leaf @5 k g/plot before transplanting		

(Tv) @ 5 g l^{-1} at 30 DAT (7.03 kg and 3516.7 kg) with 1.7 per cent increase over control. A similar trend was noticed during *Kharif* 2019, where the highest yield per plot and yield per hectare was recorded in (T12) foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT and 60 DAT (9.9 kg and 4941.7 kg) with 46.1 per cent increase over control and the lowest was recorded in (T1) foliar application of *T. viride* (Tv) @ 5 g l^{-1} at 30 DAT (7 kg and 3475 kg) with 2.7 per cent increase over control.

A similar trend was noticed in field studies where the (T12) foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT and 60 DAT was most effective with reduced disease severity and increased yield compared to (T10) soil application of mustard leaf @ 5 kg $plot^{-1}$ before transplanting and foliar application of *P. fluorescens* @ 5 g l^{-1} at 30 DAT which was on par with foliar application of propiconazole 25 EC @ 0.1 per cent at 30 DAT and 60 DAT (T12).

The fungicide propiconazole 25% EC which was used for comparison showed effectiveness in reducing disease severity, chaffiness of grains, and increasing plant height and yield but exploration of various strategies comprising bioagents and botanicals has become essential for the sustainable management of sheath blight disease.

Sheath blight disease has become a serious problem over the years due to an intensive cultivation system of rice. The present-day agricultural practices such as poor sanitation, reuse of irrigation water, excessive application of nitrogenous fertilizers, and high plant density have aggravated the incidence of sheath blight disease in rice leading to severe yield losses (Webster and Gunnell, 1992; Brooks, 2007; Pramesh *et al*., 2016). The use of plant products has been reported for the control of the most destructive plant diseases. The presence of phytochemical compounds such as steroids, tannins, flavonoids, alkaloids, and saponins was

Table 5: Effect of bioagents and bio-fumigants tested against sheath blight of rice on grain yield.

Treatments	Grain yield/plot (kg)		Grain yield ha^{-1} (kg)		Increase over control (%)	
	2018	2019	2018	2019	2018	2019
T_1	7.03	7.0	3516.7	3475.0	1.7	2.7
T_2	7.62	7.5	3808.3	3758.3	10.1	11.1
T_3	7.30	7.2	3650.0	3575.0	5.5	5.7
T_4	7.88	7.8	3941.7	3891.7	14.0	15.0
T_5	7.38	7.2	3691.7	3616.7	6.7	6.9
T_6	8.10	8.0	4050.0	4000.0	17.1	18.2
T_7	7.50	7.4	3750.0	3675.0	8.4	8.6
T_8	7.22	7.1	3608.3	3533.3	4.3	4.4
T_9	8.60	8.5	4300.0	4250.0	24.3	25.6
T_{10}	9.00	8.8	4433.3	4383.3	28.2	29.6
T_{11}	8.42	8.3	4208.3	4158.3	21.7	22.9
T_{12}	9.98	9.9	4991.7	4941.7	44.3	46.1
T_{13}	6.92	6.8	3458.3	3383.3	0.0	0.0
S.Em ±	0.30	0.28	145.92	139.62	-	-
C.D. (0.05)	0.88	0.82	425.91	407.53	-	-
C.V. %	6.75	6.34	6.52	6.34	-	-

reported for antimicrobial activity in plant extracts (Oloumi, 2014; Yogi *et al*., 2016). Likewise, the utility of biocontrol agents has received great attention in the management of plant diseases over the years. Several researchers have reported the successful evaluation and identification of bioagents for the control of plant diseases (Saravanakumar *et al*., 2007; Manikandan *et al*., 2010; Nagendran *et al*., 2013). The growth promotion, antagonism, lysis, and induction of defense enzymes have been reported as mechanisms of biological control of plant diseases (Harish *et al*., 2008; Saravanakumar *et al*., 2009; Karthiba *et al*., 2010). Lenka and Pun (2014) assessed six bio-control agent formulations from different sources under field conditions with the fungicide validamycn 3% for comparison. The biocontrol agent *T. viride* (PCI isolate) showed a maximum sheath blight reduction of 53.8 per cent and maximum grain yield of 4.94 t/ha followed by treatment of *T. viride* (Bhubaneswar isolate) showing 45.8 per cent sheath blight reduction and grain yield of 4.33 t ha^{-1}.

Handiseni *et al*. (2015) used *Brassica juncea* plants alone for soil fumigation to manage sheath blight in a field study as a cover crop. The *B. juncea* cover crop significantly reduced sheath blight severity and led to a significantly higher grain yield over control. Manibhushan Rao and Baby (1991) studied the effect of organic manures (glyricidia and neem cake) alone and combined with *T. longibrachiatum* and *Gliocladium virens* against *R. solani* causing rice sheath blight and found the combined treatments to be more effective in suppressing the disease.

Chhabra *et al*. (2023) reported that botanical extract (*A. indica*) induces the early activation of disease resistance, mediates the expression of defense mechanisms, and reduced the disease severity. Kabdwal *et al*. (2023) reported that soil drenching and three foliar sprays with a combination of *T. harzianum*, *P. fuorescens* and *Herbal Kunapajala* was found very effective in reducing the disease incidence, disease severity of *R. solani* and increasing growth promotion activity of paddy plants. The current study also revealed the combined treatment of bio-fumigant plant and bioagent was more effective than the individual treatments and on par with the fungicide treatment in decreasing the disease severity and increasing the yield over control.

CONCLUSION

Currently, there are no resistant rice cultivars against sheath blight disease, which warrants the use of chemical fungicides for the management of the disease. Development of alternative eco-friendly strategies like identifying suitable strains of bioagent and employing them and using bio-fumigants needs to be explored and adopted for sustainable management of the disease. Hence, the combination of chemical fungicides, plant products, and biocontrol agents identified in the present study can be used for the integrated management of rice sheath blight disease.

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Research Article

Cultural and morphological variability of *Botrytis* sp. isolates causing grey mould of Gladiolus in India

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ABSTRACT

The gladiolus is one of the important cut flowers with the utmost commercial value in India. Gladiolus has about 180 species and over 30,000 cultivars, of which over 20 are produced in India for the production of flowers commercially. Botrytis grey mould (BGM) caused by the fungus *Botrytis cinerea* is one of the most destructive diseases of the gladiolus. In a present study, a total of 35 isolates of *Botrytis* were obtained from the gladiolus surveyed area. Colony morphological studies (texture, form, colour, production, and degree of sporulation) have been conducted using three types of solid media *i.e.*, PDA (Potato Dextrose Agar), Czapeck's Dox Agar (CDA), Kritzmen and Netzer Agar medium. All *B. cinerea* strains gave maximum fungal mycelium growth on PDA (90.0 mm to 87.5 mm) and CDA than KN Agar medium. Spores of *B. cinerea* were also observed under Scanning Electron Microscope (SEM) and Phase Contrast Microscope (PCM) for conidial size and number of conidia. The maximum conidial size was recorded on PDA in comparison to CDA and KN Agar media.

Keywords: *Botrytis cinerea,* BGM, Gladiolus, SEM and PCM

Botrytis spp. and *Botrytis cinerea* Pers. Fr. (teleomorph, *Botryotinia fuckeliana* (de Bary) Whetzel) pathogens are necrotrophic in nature and attack every stage of flowers of gladiolus, mainly the new tissues that are tender and senescent and are susceptible exceptionally at proper conditions in fields, greenhouses or during shipping at any time (Hala Abdel *et al.,* 2020). It also has an impact on 200 other crops of agricultural importance (Zhou *et al.,* 2014). In recent studies, it is identified that *B. cinerea* has multiple complex systems of genealogies (Fournier *et al*., 2005; Fekete *et al*., 2012). This disease has also proved devastating in several parts of India. The occurrence of Botrytis grey mould was first reported in India in gladiolus flowers (Kaur and Chandel, 2016) and it is more devastating in windy, wet, and moderately cool weather conditions. *Botrytis* blight is the most serious disease produced by *Botrytis gladiolorum* in the gladiolus crop and is the major danger to its production or cultivation (Dimock, 1940).

Botrytis cinerea fungus is common and damages flowers, stems, leaves, fruits, and other parts of many other plants (Ellis, 1971) comprising the gladiolus crop. Ahmad (2007), Hosen *et al*. (2010), and Hosen (2011) also found that the temperature of 20±1°C was most favourable for the development of *B. cinerea.* Fernández *et al.* (2014) described in their study that pathogenicity proved the destructive or virulent potentials of *B. cinerea* that may be altered by the temperature of the environment of their growth. There have been many researches on the pathogenic, morphological, and genetic varieties of *B. cinerea* in the world's different parts (Fekete *et al*., 2012; Zhou *et al*., 2014; Vercesi *et al.,* 2014), there are no reports that exhibited the present status of this pathogen in India. However, on the basis of pathogenic and morphological characteristics that allow for increased awareness of this pathogen in India.

MATERIALS AND METHODS

Symptomology

The botrytis grey mould (BGM) indications as seemed in the field during the investigation were observed on gladiolus plants and collected for further studies.

Isolation, purification, and identification of the *Botrytis* isolates from a different host(s)

The isolates of *B. cinerea* were identified based on their morphological and cultural features, such as coloration and appearance of the mycelium of fungus along with conidiophores and conidia. The organism that causes grey mould disease in gladiolus was aseptically isolated from diseased gladiolus parts of the plant var. Nova-Lux was collected during the process of the survey. The diseased plant

pieces were aseptically employed on disinfected and solidified Potato Dextrose Agar (PDA) medium in disinfected glass petri plates of diameter 9 cm and set for incubation at 25±1°C. The fungal culture thus obtained was purified by single spore isolation, and PDA slants were used to maintain the culture at 4°C in the refrigerator, and at every 15-day interval, the sub-culturing was done. The fungal cultures obtained from diseased leaves and flowers of the gladiolus plant were identified as per the morphological characteristics given and issued in the book (*Botrytis*: Biology, Pathology and Control) by Elad *et al.*, 2007; Jarvis, 1997 and Hennebert, 1973).

Cultural and morphological characteristics variation of *Botrytis cinerea* isolates on different solid media

In order to understand the *in vitro* variation in *B. cinerea* isolates in terms of sporulation, growth characters, and formation of sclerotia, if any, three media, Potato Dextrose Agar (PDA), Kritzman, Czapeck's Dox Agar (CDA), and Kritzmen and Netzer Agar medium (KN) and were taken in this study. The sporophytes or facultative parasites do not exist in nature in a pure culture state. Therefore, in pure culture, the isolation of these fungi becomes problematic. For such pathogens, it is cautious to use selective media. Due to this, a selective media (Kritzman and Netzer, 1978) improved with fungicides, tannic acid, Cu^{++} ions, and antibiotics was also used in addition to PDA and CDA in the current research. After eight days of inoculation, the diameter of the colony was measured and other characteristics including texture and colour of the colony, size of spores, sporulation, and presence or absence of sclerotium, were also documented.

Scanning electron microscopy (SEM) and Phase contrast microscopic (PCM) studies of *Botrytis cinerea* isolates

We observed and photographed conidiophores on a semi-adhesive dual-sided cement carbon tape that was attached to an aluminium scanning electron microscopy (SEM) tub. A few *B. cinerea* spores were extracted from pure culture (sporulating plate, 15 days old culture) using a tungsten loop that was carefully plated onto the adhesive cement carbon tape. To make the test samples conductive, the aluminium tub was gently employed in the coater (Carbon Coating Unit, HITACHI-03E-0635) and subjected to a 10 mA current flow for 10 seconds. After the coating of test samples, they were photographed using an SEM (Scanning Electron Microscope, HITACHI-0S-3400N). The isolated *B. cinerea* was also examined using a Phase Contrast Microscope (Model-CX41RP, Olympus, Japan).

RESULTS AND DISCUSSION

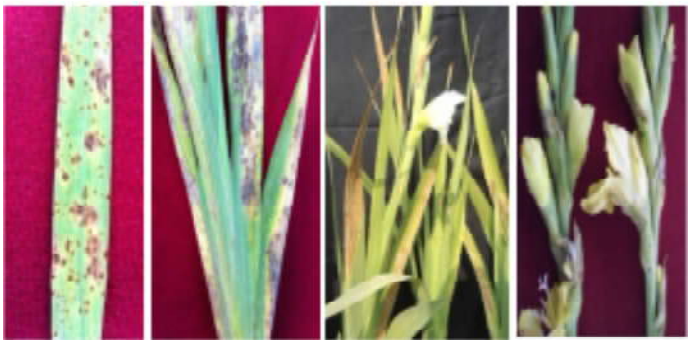
Symptomology

A total of 35 isolates of *Botrytis* were obtained from surveyed fields of gladiolus, out of which 15 of them were confirmed as *B. cinerea* in India. *Botrytis* infection appeared first as a water-soaked, brown lesion irrespective of the affected tissue on the surface of the leaf. A visible, tan to grey fuzzy mould (comprised of thousands of spores borne in grape-like clusters) developed on decayed tissues under moist conditions. The fungal black resting bodies (sclerotia), flag to roundish, were detected on affected and sporulating tissues of the plant. The petals of diseased flowers frequently become matted and stuck together. (Fig. 1).

Isolation and Identification of *Botrytis* isolates from different host(s)

In the culture, the fungal mycelium appeared thin, septate, branching, and hyaline to brown. The shape of the conidia observed was ellipsoidal, ovate, narrowly ellipsoidal, pyriform, or sometimes globose to subglobose.

A) Water-soaked, brown lesions/flecks on floret spike.



B) Leaf spots and sporulation of grey mould fungus.

Fig. 1. Symptoms of *Botrytis* grey mould on gladiolus.

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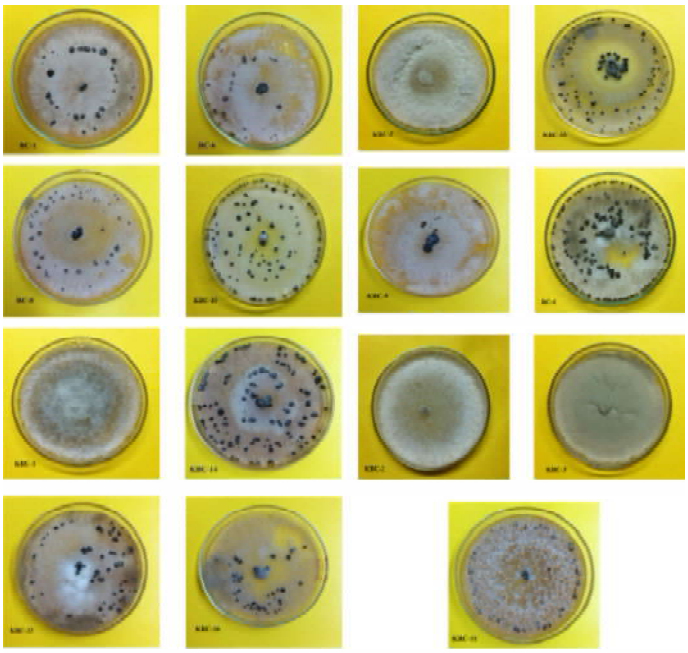

Fig. 2. Cultural characteristics of *Botrytis* isolates on Potato Dextrose Agar.

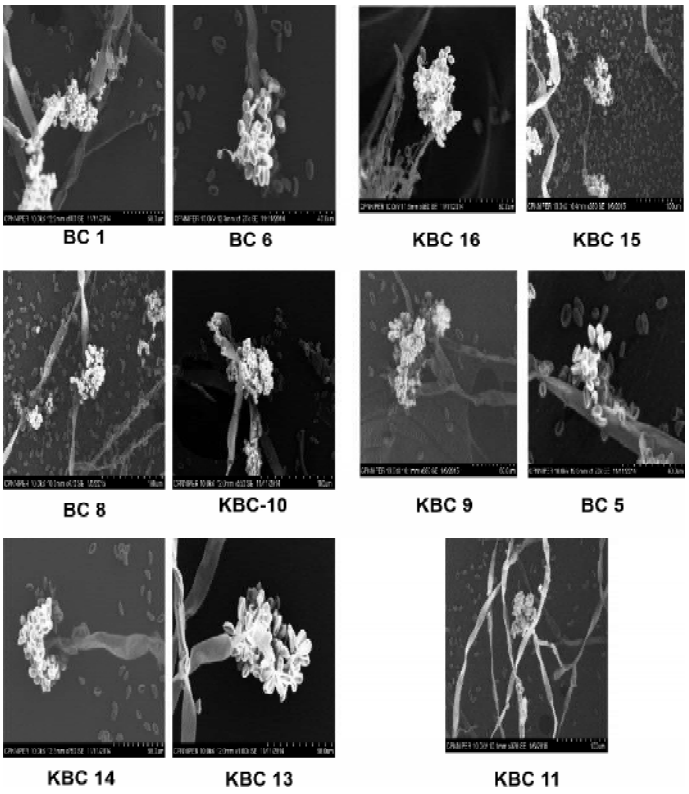

Fig. 3. Scanning Electron Microscopy of *Botrytis* isolates containing conidiophores with botryose conidia.

The average size of the conidia ranges from 7.30-9.10 × 4.58-6.30 µm. Sclerotia quantity and distribution also differed. Sclerotia were abundant in some isolates but rare or absent in others (Fig. 2). On the basis of their morphological characteristics all the isolates concluded as *Botrytis cinerea* and the identification provided by Hennebert (1973) and

Table 1: Colony characteristics of *Botrytis cinerea* isolates on Potato Dextrose Agar.

	Colony characteristics						
Isolates	**Colour**	**Texture**	**Form**	**Elevation**	**Margins**	**Production of Sclerotia***	**Degree of Sporulation****
BC - 1	off white	velvety with radial growth	circular	raised	filiform	medium to large, concentric, black in colour	+ + + +
BC - 6	off white	Velvety	circular	flat	entire	medium, irregular, black in colour	+ + + +
BC - 8	white	Velvety	Warty	flat	undulate	medium, on the edge of the plate, grey in colour	+ + + +
KBC - 10	light grey	Fluffy	circular	raised	undulate	medium to large, periphery or on the edge of the plate, black	+ + + +
KBC - 1	off white	Cottony	circular	raised	undulate	NP	NS
KBC - 14	ashy off white	Cottony	circular	raised	entire	large, irregular, black in colour	+ + + +
KBC - 13	ashy off white	Cottony	circular	raised	undulate	medium to large, irregular, black in colour	+ + + +
KBC - 16	off white	Velvety	Warty	flat	undulate	medium to large, irregular, black in colour	+ + + +
KBC - 5	off white	Cottony	circular	raised	entire	NP	NS
KBC - 15	dark grey	Fluffy	circular	raised	entire	medium, centre or on edge of the plate, black in colour	+ + +
KBC - 9	off white	Fluffy	Warty	flat	entire	large, centre of the plate, black in colour	+ + + +
BC - 5	off white	Velvety	circular	flat	entire	medium to large, irregular, black in colour	+ + +
KBC - 2	off white	Velvety	circular	flat	entire	NP	NS
KBC - 3	off white	Velvety	circular	Flat	undulate	NP	NS
KBC - 11	off white	Fluffy	circular	Raised	undulate	medium, centre or at periphery, black in colour	+ + +

*Sclerotia Non-producing = NP, ** + + + + = Excellent, + + + = Very Good, + + = Good, + = Poor and NS = Non-sporulating

Jarvis (1977) also verified this. Table 1 lists the names of the isolates as well as the location of their collection. The highest sclerotia formation occurred at temperatures of 10°C and 15°C, respectively, whereas minimum sclerotia production occurred at 25°C. While at 30°C, no sclerotia formation occurred in all the tested isolates. Martinez *et al.* (2008) described the distribution of scattered sclerotia in the circles or on the edges of the Petri plates of PDA, whereas Tanovic *et al.* (2009) observed different sclerotial and mycelial growth rates.

Cultural and morphological variation of fifteen *Botrytis cinerea* isolates

The characteristics of *B. cinerea* were studied using a Phase Contrast and Scanning Electron Microscope. Conidiophore evolved immediately from the mycelium that bears the cluster of conidia. They were septate, more or less straight, and branched often dichotomously near the apex. The conidia of the fungus were single-celled, oval, and often spherical shaped with sizes ranging between 7.30 × 4.50 to 9.00 × 6.30 µm (Fig. 2 & 3).

Cultural variation of *Botrytis cinerea* isolates on solid media (Potato Dextrose Agar (PDA), Czapeck's Dox Agar (CDA) and Kritzmen and Netzer (KN)

The isolates KBC-13 and KBC-14 favoured ashy off-white colonies on the PDA medium, but most isolates favoured velvety colonies. BC-8, KBC-16, and KBC-9 had undulating colonies. In CDA, Kritzmen, and Netzer media, *Botrytis* colonies have different features. Form (circular to irregular), texture (fluffy to cottony), elevation (flat to elevated), and margin smoothness (entire to undulating) were seen in most *Botrytis* isolates, although spore production was not observed in KBC-1, KBC-2, KBC-3, and KBC-5. All *B cinerea* isolates grew better on Potato Dextrose Agar and Czapeck's Dox Agar than K and N. Grey to brown different colours showed by *B. cinerea* isolates on the above three media (Tables 1, 2 & 3). *Botrytis cinerea* isolates showed whitish colour initially but after that, various shades of brown and grey were also displayed (Khazaeli *et al*., 2010; Xie *et al*., 2016).

Comparative growth of *Botrytis cinerea* on solid media *viz*., PDA, CDA and K and N media

All *B. cinerea* isolates were variable in their cultural characteristics. The mycelial growth observed was significantly variable on three solid media (PDA, CDA and KN). After seven days of incubation, all *B. cinerea* isolates showed significantly greater growth of fungus on PDA and CDA medium than on KN media. Several researchers, like Chardonnet *et al*. (2000) and Mirzaei *et al*. (2009), also discovered heterogeneities in the growth rates of *B. cinerea*. PDA, CDA, and KN had the fastest-growing colonies after seven days. PDA had the fastest (90 mm) and KBC-3 the slowest mycelial colony diameter expansion (Tables 4 & 5).

Table 2: Colony characteristics of *Botrytis cinerea* isolates on Czapeck's Dox Agar.

	Morphology characteristics						
Isolates	**Colour**	**Texture**	**Form**	**Elevation**	**Margins**	**Production of sclerotia***	**Degree of sporulation****
BC - 1	dark grey	fluffy	circular	flat	entire	NP	+ + + +
BC - 6	off white	fluffy with radial growth	circular	flat	entire	NP	+ +
BC - 8	light grey	fluffy with radial growth	circular	raised	entire	NP	+ +
KBC - 10	light brown with white mycelium	cottony	irregular	raised	undulate	NP	+
KBC - 1	light brown	cottony	irregular	raised	undulate	NP	NS
KBC - 14	light brown	cottony	irregular	raised	undulate	NP	+ +
KBC - 13	dark grey	cottony	irregular	raised	undulate	NP	+ +
KBC - 16	dark grey	cottony	irregular	raised	undulate	NP	+ +
KBC - 5	off white	fluffy	circular	flat	undulate	NP	NS
KBC - 15	dark grey	fluffy	circular	flat	circular	NP	+ +
KBC - 9	off white	cottony	circular	raised	circular	NP	+ + + +
BC - 5	light grey	fluffy	circular	raised	circular	NP	+ +
KBC - 2	off white	velvety	irregular	flat	undulate	NP	NS
KBC - 3	off white	velvety	irregular	flat	undulate	NP	NS
KBC - 11	light brown	fluffy	circular	raised	undulate	NP	+ +

* Sclerotia Non-producing = NP, ** + + + + = Excellent, + + + = Very Good, + + = Good, + = Poor and NS = Non-sporulating

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Table 3: Colony characteristics of *Botrytis cinerea* isolates on Kritzmen and Netzer Agar medium.

Morphology characteristics							
Isolates	**Colour**	**Texture**	**Form**	**Elevation**	**Margins**	**Production of sclerotia***	**Degree of sporulation****
BC – 1	dark grey	fluffy	irregular	raised	undulate	NP	+ + +
BC – 6	off white	cottony	circular	raised	entire	NP	+ + + +
BC – 8	dark grey	fluffy	irregular	raised	undulate	NP	+ +
KBC – 10	dark grey	fluffy	circular	flat	entire	NP	+ + + +
KBC – 1	dark grey	fluffy	circular	umbonate	circular	NP	NS
KBC – 14	dark grey	fluffy	irregular	raised	undulate	NP	+ + +
KBC -13	dark grey	fluffy	irregular	raised	undulate	NP	+ +
KBC -16	light brownish	cottony	circular	raised	entire	NP	+ + + +
KBC -5	off white	fluffy	circular	raised	entire	NP	NS
KBC -15	dark grey	fluffy	irregular	raised	undulate	NP	+ + +
KBC -9	dark grey	fluffy	circular	flat	entire	NP	+ +
BC- 5	dark grey	fluffy	irregular	flat	undulate	NP	+ +
KBC -2	dark grey	fluffy	irregular	raised	undulate	NP	NS
KBC-3	light brown in centre with off white margins	cottony	circular	raised	entire	NP	NS
KBC-11	dark grey	fluffy	irregular	raised	undulate	NP	+ + +

*Sclerotia Non-producing = NP, ** + + + + = Excellent, + + + = Very Good, + + = Good, + = Poor and NS = Non sporulating

Table 4: Comparative spore size of different isolates of *Botrytis cinerea* on solid media *viz*., PDA, CDA and KN.

Isolates	**Conidia size (µm)***		
	PDA	**CDA**	**KN**
BC - 1	9.10 x 4.48	8.10 x 3.57	7.22 x 2.55
BC - 6	7.80 x 4.46	6.81 x 3.87	5.75 x 2.56
BC - 8	7.39 x 5.04	6.45 x 4.08	5.44 x 2.65
KBC - 10	7.46 x 4.84	6.33 x 3.87	4.99 x 2.09
KBC - 1	NS	NS	NS
KBC - 14	8.28 x 5.09	7.26 x 4.09	6.22 x 2.22
KBC -13	8.82 x 4.66	7.23 x 3.44	6.12 x 2.12
KBC -16	9.00 x 6.30	8.00 x 5.40	6.44 x 2.45
KBC -5	NS	NS	NS
KBC -15	8.09 x 4.96	7.04 x 3.67	5.66 x 2.56
KBC - 9	8.16 x 4.29	7.16 x 3.22	5.11 x 2.22
BC- 5	7.30 x 4.50	6.30 x 3.55	5.22 x 2.09
KBC - 2	NS	NS	NS
KBC- 3	NS	NS	NS
KBC-11	7.44 x 4.15	6.45 x 3.11	5.45 x 2.11

* NS= Non sporulating

Table 5: Comparative growth of different isolates of *Botrytis cinerea* on solid media *viz*., PDA, CDA and KN.

Isolates	**Mycelial growth (mm)**			
	PDA	**CDA**	**KN**	**Mean**
BC - 1	90.0	90.0	85.7	88.5
BC - 6	90.0	85.7	88.7	88.1
BC - 8	90.0	90.0	66.7	82.2
KBC - 10	90.0	88.3	88.7	89.0
KBC - 1	90.0	73.7	27.7	63.8
KBC -14	90.0	82.7	47.0	76.5
KBC -13	90.0	90.0	53.0	77.6
KBC -16	90.0	89.3	90.0	89.7
KBC -5	90.0	90.0	37.7	72.5
KBC -15	90.0	89.3	76.7	85.3
KBC -9	90.0	87.7	62.0	79.9
BC- 5	90.0	88.3	63.0	80.4
KBC -2	89.0	64.7	21.0	58.2
KBC-3	87.3	53.7	87.3	76.1
KBC-11	90.0	87.0	63.3	80.1
Mean	89.8	84.0	63.9	79.2
CD $_{(0.05)}$	Isolate = 1.05; Media = 0.47; I X M = 1.82			

CONCLUSION

The present study was conducted on cultural and morphological variability recorded among the various isolates of *B. cinerea*. *Botrytis cinerea* is a phytopathogenic fungus that causes grey mould in gladiolus and is a major threat to the floriculture industry. There is a great need to research the variability of fungi in the cultural and morphological pattern to solve this problem and control the development of the infected crop. The results obtained from this research can be used as informative and visual materials for further studies of *Botrytis* isolates on different crops.

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Research Article

Effect of botanicals and bioagents on disease intensity and cost-benefit ratio of Sponge Gourd

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ABSTRACT

As the population grows, it is crucial to create a yield that will feed the expanding population. To do this, it is required to control diseases in crops, vegetables, and fruits because they are a major cause of production losses. Different illnesses harm sponge gourd, which reduces output. Bioagents and botanicals are secure alternative methods for the management of diseases that can be used to solve this problem. At 90 days after sowing also the lowest disease intensity was recorded by the treatment with Neem oil+ *Trichoderma viride* (2.5%+2.5%) followed by Castor oil + *Trichoderma viride* (2.5%+2.5%) and *Trichoderma viride* (5%) with 11.17, 13.96 and 17.26 per cent, respectively, while the control recorded the highest disease intensity of 40.85 per cent. The highest fruit yield was recorded by the treatment with Neem oil + *Trichoderma viride* (2.5%+2.5%) followed by Castor oil + *Trichoderma viride* (2.5%+2.5%) and *Trichoderma viride* (5%) with 258.4, 220.8 and 227.2 q/ha, respectively. The treatments Neem oil + *Trichoderma viride* (2.5%+2.5%) and Castor oil + *Trichoderma viride* (2.5%+2.5%) recorded higher net returns of Rs. 102845.5 and Rs. 84590 with the cost-benefit ratio of 1:1.97 and 1:1.62, respectively

Key words: Botanicals, bioagents, disease management, neem oil, Trichoderma.

Sponge gourd belongs to the family Cucurbitaceae and is also known as towel gourd, smooth loofah, vegetable sponge, and dishcloth gourd. In India, the largest number of vegetable crops are grown as compared to any other country in the world (Maheshwari *et al.*, 2007). China, India, Korea, Japan, and Central America are the major regions of commercial cultivation of Luffa. Sponge gourd is fast becoming an indispensable crop due to its wide industrial applications. The potential and industrial applications of sponge gourd are highlighted by Boynard and D'Almeida (1999), Bal *et al.* (2004), and Demir *et al.* (2008), and now it is becoming a highly demanding natural fiber worldwide. As the population is increasing day by day, it is important to produce a yield that is sufficient to feed the increasing population for which it is necessary to manage the diseases in crops, vegetables, and fruits as diseases are responsible for a lot of losses in production. Sponge gourd is affected by various diseases which results in loss of production. To overcome this issue a lot of pesticides are being used which is deteriorating the quality of the soil. These chemicals are also responsible for soil pollution as well as environmental pollution. The use of certain fungicides has led to the appearance of resistance in pathogens. Synthetic pesticides have hazardous impacts on human health that range from the bioaccumulation of pollutants in food chains to their widespread use in land and aquatic ecosystems. To overcome this issue bioagents and botanicals are safe alternative approaches in the management of diseases. These bioagents and botanicals are environment friendly as well as don't deteriorate the quality of the soil. Botanicals include all the parts of plants that are used for the management of diseases, whereas, bioagents include mostly fungi and bacteria that are used for the management of diseases in plants. Biological control works in various ways which is demonstrated in Figure 1.

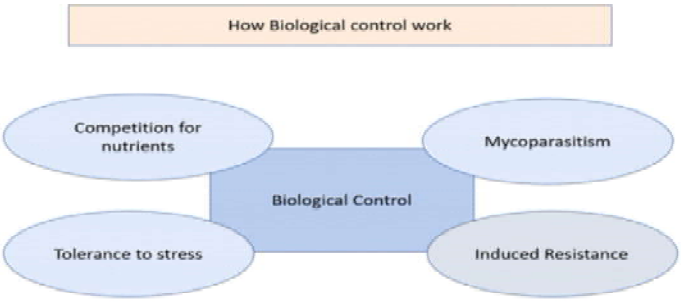

Figure 1. How biological control works.

MATERIALS AND METHODS

The present investigation was carried out during the Zaid season 2021 at the central research field of the Department of Plant Pathology, the Sam Higginbottom University of Agriculture, Technology, and Science,

Prayagraj. A field experiment was laid out in a Randomized block design with three replications. The treatments were imposed as per Kanna Reddy *et al.* (2021). The disease intensity was recorded at 30, 60, and 90 days after sowing. The disease symptom is shown in Figure 2. Fruit yield was recorded at different pickings and pooled to convert to per hectare. The cost economics of the treatments were also worked out.

The analysis of variance (ANOVA) technique was applied for drawing conclusions from the data. The calculated values were compared to the tabulated values at a 5% level of probability for the appropriate degree of freedom. Details of treatment are given in Table 1.

Table 1. Details of Treatments

Tr. No.	Treatment	Concentration (% w/w)	Reference
T_0	Control (Untreated check)	-	-
T_1	Neem oil	5%	Maheshwari *et al.* (2017)
T_2	Clove oil	5%	Ragupathi *et al.* (2020)
T_3	Castor oil	5%	Subhadarshini *et al.* (2020)
T_4	*Trichoderma viride*	5%	Sriraj *et al.* (2014)
T_5	Neem oil+ *Trichoderma viride*	2.5%+2.5%	Subhadarshini *et al.* (2020)
T_6	Castor oil + *Trichoderma viride*	2.5%+2.5%	Madhuri *et al.* (2021)

Figure 2. Disease symptom.

RESULTS AND DISCUSSION

At 30 days after transplanting (DAT), the lowest disease intensity was recorded by the treatment with Neem oil+ *Trichoderma viride* (2.5%+2.5%) followed by Castor oil + *Trichoderma viride* (2.5%+2.5%) and *Trichoderma viride* (5%) with 5.15, 8.02 and 9.82 per cent, respectively, while the control recorded the highest disease intensity of 25.33 per cent (Table 2). At 60 DAT, the lowest disease intensity was recorded by the treatment with Neem oil + *T. viride* (2.5%+2.5%) followed by Castor oil + *T. viride* (2.5%+2.5%) and *T. viride* (5%) with 8.64, 11.80 and 14.18 per cent, respectively, while the control recorded the highest disease intensity of 29.85 per cent (Table 2). At 90 DAT also the lowest disease intensity was recorded by the treatment with Neem oil + *T. viride* (2.5%+2.5%) followed by Castor oil + *T. viride* (2.5%+2.5%) and *T. viride* (5%) with 11.17, 13.96 and 17.26 per cent, respectively, while the control recorded the highest disease intensity of 40.85 per cent (Table 2). This is also in agreement with results obtained from the findings of Reddy *et al.* (2020) who reported that minimum disease intensity was found in the treatment Neem oil+ *T. viride* (2.5%+2.5%). Gayathri and Rao (2018) reported that carbendazim was found to be most effective and showed maximum inhibition of mycelial growth (43.33%) followed by *Neem* oil (30.53%). Kanna Reddy *et al.* (2021) also observed that seedling treatment with neem oil @ 2.5% + *T. viride* @ 2.5% was most effective against Alternaria leaf spot disease in brinjal.

Table 2. Effect of Treatments on disease intensity.

Tr. No.	Treatment	Concentration	Disease intensity		
			30 DAT	60 DAT	90 DAT
T_0	Control	-	25.33	29.85	40.85
T_1	Neem oil	5%	12.06	16.76	20.47
T_2	Clove oil	5%	15.01	19.80	22.96
T_3	Castor oil	5%	19.04	23.03	26.66
T_4	*Trichoderma viride*	5%	9.82	14.18	17.26
T_5	Neem oil+ *T. viride*	2.5%+2.5%	5.15	8.64	11.17
T_6	Castor oil + *T.viride*	2.5%+2.5%	8.02	11.80	13.96
	F-Test	-	S	S	S
	S.Ed.±	-	0.684	0.505	0.290
	C.D. (0.5%)	-	1.507	1.111	0.638

DAT: Days after transplanting

The highest fruit yield was recorded by the treatment with Neem oil + *T. viride* (2.5%+2.5%) followed by Castor oil + *T. viride* (2.5%+2.5%) and *T. viride* (5%) with 258.4, 220.8 and 227.2 q/ha, respectively. The control recorded the lowest fruit yield of 154.7 q/ha (Table 3). This is in agreement with results obtained from the findings of Reddy *et al.* (2020) who reported that maximum yield was found in treatment with Neem oil + *T. viride* (2.5%+2.5%).

The treatments Neem oil + *T. viride* (2.5%+2.5%) and Castor oil + *T. viride* (2.5%+2.5%) recorded higher net returns of Rs. 102845.5 and Rs. 84590 with the cost-benefit ratio of 1:1.97 and 1:1.62, respectively (Table 4).

Table 3. Effect of different treatments on fruit yield of Sponge gourd.

Tr. No.	Treatment	Concentration	Yield (q ha^{-1})
T_0	Control	-	154.7
T_1	Neem oil	5%	214.3
T_2	Clove oil	5%	196.4
T_3	Castor oil	5%	177.2
T_4	*Trichoderma viride*	5%	227.2
T_5	Neem oil + *Trichoderma viride*	2.5%+2.5%	258.4
T_6	Castor oil + *Trichoderma viride*	2.5%+2.5%	220.8
	F-Test	-	S
	S.Ed.±	-	0.653
	C.D. (0.5%)	-	1.439

Table 4. Effect of different treatments on Cost-benefit ratio.

Tr. No.	Yield (t ha^{-1})	Price of yield	Total price of yield	Common cost	Treatment cost	Total cost	Net return	CB ratio
T_0	15.47	6000	92820	51700	-	51700	41120	1:0.79
T_1	21.43	6000	128580	51700	550	52250	76330	1:1.46
T_2	19.64	6000	117840	51700	625	52325	65515	1:1.25
T_3	17.72	6000	106320	51700	662.5	52362.5	53957.5	1:1.03
T_4	22.72	6000	136320	51700	600	52300	84020	1:1.60
T_5	25.84	6000	155040	51700	494.5	52194.5	102845.5	1:1.97
T_6	22.80	6000	136800	51700	510	52210	84590	1:1.62

CONCLUSION

The quality of the soil is declining due to the usage of chemicals like pesticides and fertilizers. These substances are a significant contributor to soil pollution, dangerous to human life, and unfriendly to the environment. We should choose the biological method more frequently because it is affordable and friendly to the environment. It promotes plant health and development characteristics. As per the result obtained from this study, it was shown that neem oil + *Trichoderma viride* and Castor oil + *Trichoderma viride* (2.5%+2.5%) were found to be effective in reducing disease intensity, increasing the yield of sponge gourd as well as maintaining the higher cost-benefit ratio.

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Research Article

A comparative study of plant extracts and biocontrol agents for managing *Alternaria burnsii* in Cumin (*Cuminum cyminum* L.): An *in-vitro* assessment

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ABSTRACT

The objective of the current study was to assess the *in-vitro* efficacy of plant extracts and bio-agents in managing *Alternaria burnsii*, the causal agent of blight disease in Cumin. Eleven plants extract were selected based on their availability in the local region and evaluated for antifungal activity in the laboratory for their effectiveness against *A. burnsii*. Plants extract was tested at four different concentrations 5, 10, 15, and 20% each by food poisoning technique. Additionally, four bio-agents, namely *Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *A. burnsii*. The experiments were conducted from 2019 to 2021 at the Advanced Laboratory on Bioagents in the Plant Pathology Department, SKRAU, Bikaner. Among the plant extracts, Neem Seed Kernel Extract (NSKE) and Neem leaf extract were found to be the most effective, resulting in mean growth inhibition of 48.88 and 45.85 per cent, respectively. The least mean growth inhibition of 13.14 per cent was recorded with Eucalyptus (*Eucalyptus globulus*). Among the four different bio-agents, *Trichoderma harzianum* was found to be the most effective in suppressing the growth of the pathogen, achieving a growth inhibition of 69.63 per cent. This study provides valuable insights into the use of locally available plant extracts and bio-agents for the management of cumin blight, promoting eco-friendly approaches.

Key words: *Alternaria burnsii*, bio-agents, blight, *in-vitro,* management, plant extracts.

Cumin, scientifically known as *Cuminum cyminum* L., is a spice crop native to the eastern Mediterranean region and widely cultivated around the world, particularly in Asia, Africa, and Latin America. It belongs to the Apiaceae family, which also includes parsley, celery, and carrots. Cumin has a variety of uses, including culinary and medicinal purposes.

Cumin is affected by several diseases, and one of the major devastating diseases of cumin is Alternaria blight, caused by *Alternaria burnsii*. This disease severely affects the seed quality of the crop, leading to significant monetary losses.

Uppal *et al.* (1938) first reported the blight caused by *A. burnsii* in India, while Joshi (1955) reported it specifically in Rajasthan. This disease poses a constant challenge to the sustainable cultivation of cumin, causing both quantitative and qualitative losses in yield (Holliday, 1980).

While the management of Alternaria blight has previously relied on the use of fungicides, overuse of these chemicals can lead to environmental contamination and negative impacts on human health. As a result, there is a considerable requirement for environmentally friendly disease control strategies. Inspired by traditional Indian agricultural practices that utilized organic substances, such as cow dung and mustard oil, this study aims to evaluate the efficacy of plant extracts and bio-agents in managing Alternaria blight in cumin under *in-vitro* conditions. By exploring these traditional methods, we aim to provide sustainable and environmentally friendly alternatives for disease management in cumin cultivation.

MATERIALS AND METHODS

The laboratory experiments of *in vitro* evaluation of plant extracts and bio-agents against *A. burnsii* were carried out in the Advanced Laboratory on Bioagents in Plant Pathology Department in the years 2019 to 2021.

In vitro evaluation of plant extracts against *Alternaria burnsii*

The effectiveness of various plant extracts (Table 1) was examined *in vitro* against *A. burnsii* at doses of 5, 10, 15, and 20 per cent. For each treatment, the effect of extracts from readily accessible local plants was evaluated for their ability to suppress the growth of *A. burnsii*, and the percentage of fungal growth that was inhibited was estimated using the

poisoned food technique (Nene and Thapliyal, 1993). In order to achieve the appropriate plant extract concentrations of 5, 10, 15, and 20 per cent, the estimated amounts of plant extract were well mixed in the molten, almost-cool PDA medium before being poured into Petri plates.

Table 1. Details of plant extracts used in lab condition.

Sl. No.	Common name	Botanical name	Plant parts used
1.	Datura	*Datura stramonium* L.	Stem, flower, leaf
2.	Olive	*Olea europaea* L.	Leaves, fruits
3.	Ginger	*Zingiber officinale* Rosc.	Rhizome
4.	Kheep	*Leptadenia pyrotechnica* (Forssk.) Decne.	Twigs
5.	Neem leaf	*Azadirachta indica* A, Juss.	Leaves
6.	NSKE	*Azadirachta indica*	Seed kernel
7.	Onion	*Allium sativum* L.	Bulb
8	Giloy	*Tinospora cordifolia* (Willd. (Miers)	Stem
9.	Aloe vera	*Aloe barbadensis* Miller	Leaves
10.	Eucalyptus	*Eucalyptus globules* Labill.	Leaves
11.	Tumba	*Citrullus colocynthis* (L.) Schrad	Fruit
12.	-	Control	-

The plates were aseptically inoculated with a 5 mm disc cut from the periphery of 7 days old actively growing cultures of the three representative replications of each of *A. burnsii*, and controls without plant extracts were maintained for comparison. Three replications of each treatment were used in the completely randomized design of the experiment and the inoculated plates were incubated at 28 ± 2 °C. The colony diameter was measured after seven days when the control plates were full of fungal growth. Percent inhibition of mycelial growth was calculated by using the formula given by Bliss (1934).

$$\text{Percent inhibition (I)} = \frac{(C - T)}{C} \times 100$$

Where,

I = Percent inhibition

C = Colony diameter in control

T = Colony diameter in treatment

Method of extraction

Using sterile distilled water, the appropriate plant parts were carefully cleaned before being air-dried. One hundred grams of plant material were blended with 100 ml of distilled water for 10-15 minutes. The corresponding macerates were centrifuged at 10,000 rpm for 10 minutes after filtering through two layers of muslin cloth. Using a vacuum filtration unit, the supernatant was decanted and passed through a bacteria-proof filter. The filtered extracts were kept in the refrigerator for use in further laboratory experiments.

***In vitro* evaluation of bioagents**

Under laboratory conditions, the efficacy of four bioagents (*Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis*) against *A. burnsii* was evaluated.

Dual inoculation test

The antagonistic potential of *Trichoderma* sp. against *A. burnsii* was evaluated using a dual culture technique. Potato dextrose agar (PDA) medium was prepared and sterilized using an autoclave. A mycelial disc of *A. burnsii* was inoculated on the PDA plate, and a mycelial disc of *Trichoderma* sp. (either *T. harzianum* or *T. viride*) was placed on the opposite side of the plate, 4-5 cm away from the *A. burnsii* disc. The plate was then incubated at 28 ± 2°C for 7 days. The plates were observed for the presence of inhibition zones around the *Trichoderma* sp. disc, which indicated the antagonistic activity of *Trichoderma* sp. against *A. burnsii*. The diameter of the *A. burnsii* colony in the direction of *Trichoderma* sp. disc and the opposite direction was measured to determine the degree of inhibition. The experiment was replicated three times for each *Trichoderma* sp. (*T. harzianum* and *T. viride*).

The percentage of inhibition of *A. burnsii* by *Trichoderma* sp. was calculated using the formula:

$$\text{Percent inhibition (I)} = \frac{(C - T)}{C} \times 100$$

Where,

I = Percent inhibition

C = Mycelial growth of *A. burnsii* in control (mm)

T = Mycelial growth of *A. burnsii* in the presence of antagonist (mm)

Paper disc inoculation method

The stock culture of *B. subtilis* was streaked on Nutrient Agar Media, and the Stock culture of *P. fluorescens* was streaked on Pseudomonas Agar Fluorescens (PAF) slants and incubated at 28±2 °C for 48 hours. Ten milliliters of sterilized distilled water were added to each slant containing the fresh colony of the bacterial antagonist, and the suspension was prepared by scrapping the bacterial growth with the help of a sterilized inoculating needle. The suspension was then transferred to sterile Petri dishes. Sterilized filter paper discs (5 mm diameter) were dipped in the bacterial suspension. Four such inoculated discs were placed in opposite directions on the surface of Potato Dextrose Agar Media in Petri dishes. Mycelial discs (5 mm diameter) were taken from the periphery of the actively growing culture of *A. burnsii* raised on Potato Dextrose Agar Medium was placed at the center of Petri dishes containing the inoculated paper discs. In the control, Petri dishes were inoculated with the mycelial discs of the test pathogen only. Four replications were kept for each treatment. The inoculated Petri dishes having *P. fluorescens* and *B. subtilis,* discs were incubated at 28±2 °C. Mycelial growth was recorded after seven days of incubation. The inhibition of mycelial growth by the bacterial antagonist was calculated by using the following formula.

$$\text{Per cent inhibition (I)} = \frac{(C - T)}{C} \times 100$$

Where,

I = Percent inhibition

C = Mycelial growth of *A. burnsii* in control (mm)

T = Mycelial growth of *A. burnsii* in the presence of antagonist (mm)

RESULTS AND DISCUSSION

In vitro evaluation of bioagents and plant extracts against *Alternaria burnsii*

In vitro evaluation of bioagents

The antagonistic actions of two species of *Trichoderma* and two species of bacteria were evaluated against *A. burnsii* using the dual culture and paper disc technique, respectively. The radial growth of the bioagents and the test fungus were observed, and the percent inhibition was calculated. The results, demonstrated in Table 2, reveal that all the biocontrol agents were significantly superior in inhibiting the growth of *A. burnsii* compared to the control. The highest growth inhibition was recorded in *T. harzianum* (69.63%), followed by *T. viride*, while *Pseudomonas fluorescens* (60.74%) and *Bacillus subtilis* (57.41%) showed similar levels of inhibition.

Table 2. Effect of different bioagents on mycelial growth of *Alternaria burnsii* after seven days of incubation.

Bioagents	Percent growth inhibition
Trichoderma harzianum	69.63 (56.55) *
T. viride	64.07 (53.15)
Pseudomonas fluorescens	60.74 (51.18)
Bacillus subtilis	57.41 (49.24)
Control	(0.0)
S.Em.±	1.17
C.D. (P=0.05)	3.74
C.V. (%)	4.84

*Values in the parentheses are angular transformations

Trichoderma spp. was the most potent inhibitor overall in this experiment, probably due to its ability to produce a wide range of enzymes and secondary metabolites toxic to other fungi, such as chitinases, β-1,3-glucanases (Cherif and Benhamou, 1990) and proteases (Goldman *et al.*, 1994). These enzymes break down the cell wall of the fungal pathogen, leading to its death. *Pseudomonas fluorescens* showed the highest inhibition among the bacterial antagonists, likely due to its ability to produce several antibiotic metabolites, including DAPG, pyrrolnitrin, and phenazine (Raaijmakers and Mazzola, 2012). Overall, biological control may be an effective alternative to chemical-based methods for disease control in spice crops.

In vitro evaluation of plant extracts

The efficacy of ten plant extracts against *A. burnsii* was evaluated *in vitro* at four concentrations *viz*., 5, 10, 15, and 20 per cent on PDA medium by poison food technique. Data suggested that (Table 3) an increase in the concentration of plant extract resulted in higher inhibition of the mycelium growth of fungus. Among these plant extracts, NSKE was found superior in inhibiting the growth of *Alternaria burnsii* at all four concentrations with a mean inhibition of 48.89 per cent, followed by Neem leaf (45.83%). However, Eucalyptus (*Eucalyptus globules*) recorded the least percent inhibition of mycelial growth at all the concentrations, with a mean value of 13.14 per cent growth inhibition.

Data pertaining to the effect of plant extracts on the radial growth of *Alternaria burnsii* are given in Table 3. The findings suggested that significant differences were observed between the treatments. At 5 per cent concentration, NSKE showed maximum inhibition (41.85%) followed by Neem leaf (37.77%), and the least inhibition was recorded by

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Table 3. Effect of plant extracts on mycelial growth inhibition of *Alternaria burnsii* after seven days of incubation.

S. No.	Plant Extract	Radial Growth (mm)				Percent Growth Inhibition				
		5%	10%	15%	20%	5%	10%	15%	20%	Mean
1.	NSKE (*Azadirachta indica*)	52.34	49.34	44.34	38.00	41.85 (40.28)*	45.18 (42.22)	50.74 (45.40)	57.78 (50.31)	48.88
2.	Neem Leaf (*Azadirachta indica*)	56.00	53.00	46.00	40.00	37.77 (37.90)	41.12 (39.86)	48.89 (44.34)	55.56 (29.84)	45.83
3.	Datura (*Datura stramonium*)	59.00	57.00	49.34	44.34	34.44 (35.92)	36.67 (37.25)	45.18 (42.22)	50.74 (44.55)	41.75
4.	Ginger (*Zingiber officinale*)	61.34	58.00	52.00	48.34	31.85 (34.34)	35.56 (36.58)	42.24 (40.50)	46.29 (41.15)	38.98
5.	Onion (*Allium sativum*)	64.00	62.00	56.34	49.67	28.89 (32.49)	31.12 (33.88)	37.40 (37.68)	44.81 (33.42)	35.56
6.	Tumba (*Citrullus colocynthis*)	68.00	63.34	60.00	54.00	24.45 (29.61)	29.62 (32.95)	33.34 (35.24)	40.00 (53.60)	31.85
7.	Kheep (*Leptadenia pyrotechnica*)	71.00	67.00	64.00	58.00	21.12 (27.33)	25.56 (30.35)	28.89 (32.49)	35.56 (35.69)	27.77
8.	Aloe Vera (*Aloe barbadensis*)	76.00	73.67	69.00	63.33	15.56 (23.21)	18.14 (25.20)	23.33 (28.86)	29.62 (47.74)	21.66
9.	Giloy (*Tinospora cordifolia*)	81.00	76.67	72.00	67.00	10.00 (18.41)	14.81 (22.62)	20.00 (26.54)	25.56 (58.66)	17.59
10.	Olive (*Olea europaea*)	82.00	78.00	74.00	69.00	8.89 (17.27)	13.34 (21.39)	17.78 (24.92)	23.34 (62.69)	15.83
11.	Eucalyptus (*Eucalyptus globules*)	84.00	81.00	75.67	72.00	6.67 (14.92)	10.00 (18.29)	15.92 (23.43)	20.00 (34.11)	13.14
12.	Control	90.0	90.0	90.0	90.0	0	0	0	0	0
	S.Em.±	-	-	-	-	0.68	0.63	0.65	0.66	-
	C.D. (P=0.05)	-	-	-	-	2.02	1.86	1.92	1.94	-
	C.V. (%)	-	-	-	-	4.58	3.86	3.57	3.26	-

*values in parentheses are angular transformations.

Eucalyptus (*E. globules*) (6.67%). At 10 per cent, NSKE showed 45.18 per cent inhibition, followed by Neem leaf (41.12%), and the least inhibition was recorded by Eucalyptus (*E. globules*) (10.00%). At 15 per cent, NSKE showed 50.74 per cent inhibition followed by Neem leaf (48.89%), and the least inhibition was recorded by Eucalyptus (*E. globules*) (15.92%), and at 20 per cent, NSKE showed 57.78 per cent inhibition followed by Neem leaf (55.56%) and least was recorded by Eucalyptus (*E. globules*) (20.00%). It is evident from the data that the increase in the concentration resulted in increased percent growth inhibition of the pathogen.

The outcomes showed that there were notable variations among plant extracts tested for inhibiting the pathogen's mycelial growth. The results showed that, in comparison to the control, the tested extracts at various doses considerably decreased linear growth and increased inhibition percentage. The effectiveness of NSKE or Neem leaf could be because of the presence of the anti-fungal compound in seed kernels of neem and its leaves, respectively. According to Schmutterer (1990), Mahmoodin, nimolide, nimbic acid, α- and β-nimolactones are among the components of neem that have antimicrobial properties. Nimbin and its compounds are bactericidal and antifungal. Additionally, the effects of two locally grown weed plants, kheep (*Leptadenia pyrotechnica*) and tumba (*Citrullus colocynthis*), on the mycelial growth of *A. burnsii* were also investigated. They successfully inhibited the tested pathogen's growth with 31.85 and 27.77 per cent. Eucalyptus, however, was shown to be least efficient in inhibiting *A. burnsii*'s mycelial growth. Various plants' diverse anti-fungal chemical compositions may account for the disparity in extracts' anti-fungal efficacy.

CONCLUSION

The findings suggest that locally available plant extracts, particularly Neem Seed Kernel Extract (NSKE) and Neem leaf extract, showed promising antifungal activity against *Alternaria burnsii*, the causal agent of blight disease in cumin. These plant extracts exhibited significant growth inhibition of the pathogen, indicating their potential as eco-friendly alternatives for disease management. Additionally,

Trichoderma harzianum, among the tested bio-agents, showed strong efficacy in suppressing the growth of *A. burnsii*. Overall, this study highlights the potential use of these plant extracts and bio-agents for the sustainable management of Cumin blight.

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Research Article

Design of animal-drawn cereal grains no-till planter

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ABSTRACT

The development of the no-till system in Eritrea presents a significant opportunity to achieve earlier and more timely planting. Tillage wastes a lot of time, energy, and a lot of money in the form of fuel, labor, and equipment concerning the lifestyle of Eritrean farmers. Therefore, to solve this problem animal drawn no-till planter is designed to make the farming operation easy and efficient, simple to operate, it reduces the amount of work for tillage and combines several steps of tillage in the machine. No-till systems can be designed in different forms of tractor-mounted, manually operated, and animal-drawn planters. These mechanized systems would introduce additional barriers to adoption including the high cost of investment, availability and cost of fuel, and the need to use syndication ownership models. So due to the economic standard of living of local farmers animal-drawn no-till planter is more efficient for smallholder farmers. Animal-drawn no-till planter is designed with a pulling force of 73.5 kgf walking speed of 0.6m/s and with a frame dimension of 1000 mm x 450 mm x 50 mm. The equipment used in this design is made locally available, making the overall cost cheap and affordable to the farmers in contrast with no-till planters imported from overseas so that farmers do not face difficulties in obtaining these planters and providing a basic saving from budgeting a large amount of currency for importing a conventional tiller machine. To attain better accuracy, fabrication of the designed animal-drawn planter in the actual field with local materials is advisable to give major concern in future work. And also, testing and evaluation performance of the animal-drawn no-till planter is highly recommended.

***Keywords*:** Planter, animal drawn, tiller machine, tillage.

Tillage means the agricultural preparation of the soil by ploughing, ripping, or turning it. Tillage can also mean the land that is tilled (Brady and Weil, 2002). As large areas of the surface of the earth are subject to tillage, man has tried to ease the cumbersome and time-critical work of tillage and developed machines which allow in most places of the world to perform this task with ease and efficiency. Usually, tillage is limited to the arable layer of soil, which contains organic matter and where plant life actually can occur. Tillage has to be performed to clear virgin soils of plants and animals for agricultural use. Furthermore, it must be performed to bring the seedlings into the soil and procure them in a good environment for further development. Tillage operations for seedbed preparation are often classified as primary or secondary. A primary tillage operation constitutes the initial, major soil-working operation after the harvest of the previous crop; it is normally designed to reduce soil strength, cover plant materials, and rearrange aggregates. Secondary tillage operations are intended to create refined soil conditions following primary tillage. The final tillage operation prior to planting a crop is usually secondary tillage, but farmers may use more than one secondary tillage operation. In some situations, a tillage operation may fit the definition of both secondary and primary tillage (Srivastava *et al*., 2006)

The conservation tillage system is defined as a system that "provides means of profitable crop production while minimizing soil erosion caused by wind and/or water moisture, energy, labor, and even equipment conservation are additional benefits" (Conservation tillage systems and Management, 1992). Conservation tillage systems try to disturb the soil as little as possible to conserve its natural structure, leave the maximum vegetal residue next to the soil surface, and/or try to build a rough surface. Thus, the soil will be protected against wind and runoff erosion, and water evaporation can be limited, especially in arid areas. When considering water balance in rainfall agriculture, water losses are due essentially to runoff, evaporation, and transportation. The plant residues that are found on the surface protect the soil from sunbeams and reduce water losses due to evaporation (Willcocks, 1981; Al-Darby and Lowery, 1984). Willcocks has shown in the semiarid area of Botswana that the soil temperature can reach 70°C when the

soil is without cover and plant residues can reduce the temperature by 10% (Willcocks, 1981).

No-till farming systems have revolutionized agriculture by improving erosion control, soil water storage, soil quality, and in many instances, yield, and net farm income. No-till system also allows farmers to manage greater areas of land with reduced energy and machinery inputs (Triplett and Dick, 2008). No-till systems are defined as those that do not undertake any tillage operations and conduct seeding into untilled soil where the previous crop residues have been retained on the surface. Sowing and fertilizing equipment in no-till systems must be able to handle residue, penetrate hard soil, regulate the working depth, cover seeds, and give sufficient soil-seed contact. For these reasons, the no-till seeding and planting equipment must be heavier and stronger than the conventional tillage equipment (Stout and Cheze, 1999). In some cases, conventional equipment can be converted to a no-till machine, although specific machines are advisable.

The basic action which must be performed by a no-till machine consists of residue and soil cutting, furrow opening, seed and fertilizer placement, and covering. Weed and pest control have to be done chemically. Nowadays, several direct drills are combined with fertilizer and pesticide applicators. To avoid germination damage, fertilizers generally are applied beside or below the seed rows (Triplett and Dick, 2008). The general objective of this research is to design animal-drawn no-till farming equipment for smallholder Eritrean farmers in semiarid areas which leads to preserving and improving the quality of the soil.

MATERIALS AND METHODS

Preliminary design of animal-drawn cereal grains No-till Planter

Design consideration

This Animal-drawn no-till machine consists of a seedbox, fertilizer box, seed metering mechanism, fertilizer metering mechanism, seed tubes, furrow openers, soil compactor, and transport cum power transmitting wheel. The design of the equipment was patterned on animal-drawn no-till equipment from Brazil. Factors considered in this design are: Ease of fabrication of component parts, the safety of the operator and bullocks, operation of the machine should be simple for small scale or rural farmers, materials available locally were used in the fabrication of the components, availability, and cost of the materials, performance of the planter, ease of manufacture of the planter and low initial cost.

Secondary design of animal-drawn cereal grains No-till planter

Determination of the weight of seed:

The weight of the seed (W_s) can be calculated by

$$W_s = M_s * g$$

Where, M_s = mass of seed in the seed hopper, g = gravitational acceleration

Design analysis of seed and fertilizer hopper

The trapezoidal shape of seed and fertilizer boxes is generally used in the planter for the free flow of seed and fertilizer in the hopper bottoms.

Design analysis of seed hopper

As stated above the shape of the seed hopper is trapezoidal having a small rectangular shape at the upper portion. The calculation to determine the volume of the seed hopper is given by Sharma and Mukesh (2013).

$$V_b = 1.1\ V_S$$

Where, V_b = volume of seed box in, cm^3, V_S = volume of seed, cm^3

$$V_S = W_S/\gamma_S$$

Where, W_S = weight of seed in the box, γ_S = bulk density of seed g/cm^3

Volume of the trapezoidal section:-

$$V_{b1} = \frac{(a + a + l + l)}{2} * h * l_b$$

$l = h \cot \theta$, $V_{b1} = (a + h \cot \theta) * h * l_b$

Where, V_{b1} = volume of the trapezoidal section only, cm^3

a = bottom width of seed box, cm, b = top width of seed box, cm, l_b = length of seed box, cm

θ = angle of repose of seed, h= height of seed box, cm (only for the trapezoidal section)

Volume of the rectangular section:- $V_{b2} = b * h' * l_b$

Where, V_{b2} = volume of the rectangular section only, cm^3 h′ = height of the rectangular section,

$$V_b = V_{b1} + V_{b2}$$

Design analysis of fertilizer hopper

For easy construction, balanced operation of the animal-drawn planter, and symmetry in size, the same size

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and shape of the fertilizer box is selected as that of the seed box.

$M_f = V_f * \gamma_f$

Where, M_f = wegiht of fertilize γ_f = bulk density of fertilize g/cm V_f = volume of fertilizer inside the hopper cm^3

Design analysis of the planter size

The size of the animal-drawn planter can be calculated: (Sharma and Mukesh, 2013)

Z = D/d Where:- Z = number of furrow openers in the planter, D = draft of planter, kgf d= draft of each row, kgf

$$D = \frac{horse\ power\ of\ bullocks(w)}{speed\ of\ the\ bullocks\left(\frac{m}{s}\right) * g}$$

Then, the working width of the machine would be

W = Z * n Where:- W = working width of machine, cm, n = row to row distance, cm

Design analysis seed chute

The chute is a tube through which the seeds metered out by the cells travel before they are deposited into the furrow. It is made of rubber hose pipe 15 mm in diameter and 60 cm long.

Design analysis furrow opener

The furrow opener fits the planter which penetrates the soil at a constant depth. The design of furrow openers of seed planters varies to suit the soil conditions of a particular region. In this case, the shank-type opener was designed to provide a narrow opening which is ideal for smaller seeds. It was designed to have a block to facilitate seed placement from the hose (seed cute) (Khurmi and Gupta, 2005).

Selection of standard (tyne) of furrow opener

The draft force on a furrow opener is 20 kgf/tyne (for shallow depth draft force per furrow opener ranges from 10 kgf - 15 kgf (Sharma and Mukesh, 2013) and acting at a height of h/3 from the bottom of the furrow opener where the h is the total length of furrow opener and tyne. The standard will be in bending, so, it will be designed for easy operation and proper balancing of the machine.

To determine the size of the furrow opener these equations are used: (Sharma and Mukesh, 2010)

a = h/3

Moment on arm length = (h–a) and Bending moment in tyne = D * (h–a)

$$Z = {M_b}/{f_b} \qquad Z = \frac{1}{6}tb^2$$

Where, a = distance of draft application on furrow opener,

h = height of furrow opener, mm,

D = draft force per furrow opener, N

t = thickness of flat, mm,

Z = section modules of tyne,

M_b = bending moment kgm

Design analysis of furrow closer

The furrow closer is designed to allow for proper covering and compaction of the soil over the seeds in the furrows. Press wheels to close the slot in order to secure good contact between soil and seed. In this design, in order to manage the width of the planter v-shaped furrow closer is chosen (Murray *et al*., 2006).

Design of beam for the main frame

The size of the beam for the main frame which can tolerate the bending moment can be calculated: (Khurmi and Gupta, 2005).

$$\sigma_{allowable} = \frac{\sigma_y}{F.O.S}$$

$$M = \frac{W * L^2}{12}$$

$$Z = M/\sigma_{allowable}$$

$$Z = \frac{bh^2}{6}$$

Where, $\sigma_{allowable}$ = aloowable bending stress, M = bending moment of the beam Z = Module of inertia

Design analysis of the front wheel

According to Bharat and Sidharth (2014), wheels of larger diameters are to reduce rolling resistance, especially in the case of traction wheels. In this work, the front wheel is designed to be a traction wheel to enhance movement on loose soils. The front wheel provides the drive for the metering mechanism through a sprocket and chain system.

Design analysis of residue manager

The residue managers are designed to move the residue to each side of the furrow, creating a clean soil surface for the

furrow openers and keeping the residue from interfering with the seeding process. The residue managers are set at the front of the seeder units. In the case of this planter notched disc coulters are preferred, in which notched disc coulters are flat circular discs with a sharpened, notched circumference. They have similar characteristics to the plain disc coulter except they are arguably more suited for use in very hard soils and very heavy residue situations.

Design analysis of the shaft

A shaft is a rotating member, usually of a circular cross-section used to transmit power or motion. It provides an axis of rotation, or oscillation, of elements such as gears, pulleys, flywheels, cranks, and sprockets. In order to transmit power various members such as pulleys, sprockets, and gears are mounted.

The shaft considered for optimum performance is to be tough, wear resistance, and high strength. The need for a solid circular shaft was necessitated for analysis of its combined bending and torsional stresses. The calculation for the shaft diameter was determined using the Association of Mechanical Engineers (ASME) code equation for a solid shaft having little or no axial loadings (Khurmi and Gupta, 2005). In this design 1 axle and 2 main shafts (one for seed metering and another for fertilizer metering) are used:

We know that the power is transferred to the machine by the chain drive system so the measurement of the bending moment of the shaft or machine is measured by the theorem of the chain drive system. So load on the chain or chain load (Q) (Khurmi and Gupta 2005) is:

$Q = K_1 \times P_t$

Where, Q = chain load, K_1 = the coefficient of chain (1.15 for the mild steel), P_t = the push force of the chain.

The chain drive is working at an angle φ with the horizontal, therefore the equivalent chain load on the machine is:

$Q_v = Q \sin \varphi$ Where, Q_v = vertical chain load

Now Maximum bending moment on the shaft given by the chain drive system given by

M_b = (Weight on wheel × over hung) + (Q_v × over hung)

The torque (twisting moment) acting on the shaft is given by the formula:

$$T = \frac{\pi}{16} d^3 \tau_s$$

Where, T = Torque (twisting moment) acting on the shaft

τ = torsional shear stress, d = diameter of the shaft

We know that power transmitted (in watts) by the shaft is calculated as:

$$p = \frac{T * 2 * \pi * N_{seed}}{* 60}$$

Now we have,

$$T = \frac{P * 60}{2 * \pi * N_{seed}}$$

Where, T = twisting moment in N-m, N = shaft speed in rpm, P = power transmitted in watts

The bending moment acting on the shaft is calculated by the formula: $M = \frac{\pi}{32} * \sigma_b * d^3$

Where, M = bending moment acting on the shaft, σ_b = maximum bending stress of mild steel with key, d = diameter of the shaft

When the shaft is subjected to combined twisting and bending moment, then the shaft must be designed on the basis of the two moments simultaneously.

According to maximum shear stress theory, the maximum shear stress in the shaft is given by:

$$\tau_{max} = \frac{1}{2}\sqrt{(\sigma_b)^2 + 4\tau^2}$$

Now,

$$T_e = \frac{\pi}{16} * \tau_{max} * d^3 = \sqrt{M^2 + T^2}$$

Where, T_e = equivalent twisting moment

τ_{max} = maximum shear stress of mild steel with key

And also,

$$M_e = \frac{1}{2}\left[M + \sqrt{M^2 + T^2}\right] = \frac{\pi}{32} * \sigma_{b_{max}} * d^3$$

Where, M_e = equivalent bending moment

$\sigma_{b_{max}}$ = maximum normal stress

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Selection of bearing

A bearing is a machine element that constrains relative motion to only the desired motion and reduces friction between moving parts. The design of the bearing may, for example, provide for free linear movement of the moving part or free rotation around a fixed axis; or, it may prevent a motion by controlling the vectors of normal forces that bear on the moving parts (Khurmi and Gupta 2005)

RESULTS AND DISCUSSION

Determination of weight of seed

Weight of seed W_s is

$W_s = M_s * g$

$W_s = 4\ kg * \frac{9.81m}{m^2} = 39.24\ N = 4\ kgf$

Design analysis of seed hopper

Volume of seed is:- $V_s = M_s/\gamma_s\ V_s = 5201.56\ cm^3$

Volume of seed box is:- $V_b' = 1.1\ V_s\ \ V_b' = 5721.72\ cm^3$

Volume of the trapezoidal section is calculated: $V_{b1} = (a + h \cot \theta) * h * l_b$

Assume: a = 7.5 cm

$\theta = 75^o$ (Angle of repose of wheat is from $23^o - 28^o$. The design of seedbox should be such that θ is more than for easy flowing of seeds. Therefore $\theta = 75^o$)

$5721.72\ cm^3 = (7.5\ cm + h \cot 75) * h * 45\ cm$

$12.058\ h^2 + 337.5\ h - 5721.72 = 0$

h = 11.9 cm

Assume h = 11 cm

To calculate $l = h \cot \theta = 11\ cm \cot 75° = 2.95\ cm$

To calculate top width $b = a + 2l = 7.5\ cm + 2 * 2.95\ cm = 13.4\ cm \approx 13.5\ cm$

Therefore the adjusted volume of the trapezoidal shape is:-

$V_{b1} = \frac{(b + a)}{2} * h * l_b = 5197.5\ cm^3$

Volume of rectangular section is calculated: $V_{b2} = b * h' * l_b$ Assume $h' = 2cm\ V_{b2} = 1215\ cm^3$

The total volume of the seed hopper is:- $V_b = V_{b1} + V_{b2} = 6412.5\ cm^3$

Since the volume of the hopper is based on the hopper structure ($V_b = 6412.5\ cm^3$) is greater than the volume of the hopper based on the seed volume ($V'_b = 5721.72\ cm^3$) the dimensions are sufficient.

Design analysis of fertilizer hopper

For easy construction, balanced operation of the animal-drawn planter and symmetry in size, the same size and shape of the fertilizer box is selected as that of the seed box.

Dimensions for trapezoidal section: a = 7.5 cm, h = 11 cm, l = 3 cm, b = 13.5 cm, $l_b = 45$ cm, $\theta = 75°$, Dimensions for rectangular section: h′ = 2 cm, b = 13.5 cm, $l_b = 45$ cm

Weight of fertilizer in the hopper: - $M_f = V_f * \gamma_f$

Density of fertilizer: - $\gamma_f = 1200\ kg/m^3 = 1.2\ g/cm^3$

M_f = 7695 gram, Say M_f = 7 kg, so fertilizer box can accommodate 4-7 kg

Design analysis of the planter size

Number of furrow openers in the planter is:- $Z = \frac{D}{d}$

$$D = \frac{horse\ power\ of\ bullocks(w)}{speed\ of\ the\ bullocks\left(\frac{m}{s}\right) * g}$$

horse power of bullocks = 0.58 hp

speed of the bullocks = 0.6 m/s

D = 73.5 kgf

d = draft of each row is from 10k gf-20 kgf for shallow depth (Sharma and Mukesh, 2010)

$Z = \frac{73.5kgf}{20kgf} = 3.6 \approx 3$

Then, working width of machine is:- W = Z * n = 45 cm

Design of seed chute

The seed chute is made up of ready-made rubber hose pipe having 1.5 cm diameter and 60 cm long.

Design of furrow opener

The shank-type opener is designed to provide a narrow opening which is ideal for smaller seeds. It is designed to have a block to facilitate seed placement from the hose. This opener is made from 0.476 cm mild steel plate.

Selection of standard (tyne) of furrow opener

The size of the furrow opener is:- tickness of the furrrow opener (t) = 7.06 mm = 0.706 cm

Design of furrow closer

Zero pressure press wheel type of furrow closer has a generally concave profile resulting from a hard molded rubber type, soft center rubber type (*i.e.* molded with hard edges and a soft flexible center). It is of 25 cm diameter wheel.

Design of the front wheel

The 30 cm diameter two front wheels have 8 spikes each to assure rotation of the wheels. The front wheel consisted of the wheel, the hub, the spokes, and the spikes. The wheels are designed from 3.8 cm x 0.476 cm flat bar that was rolled into a wheel. The hub was turned in the CNC lathe machine from a 3.8 cm diameter CRS. The spokes were from a 0.95 cm diameter CRS bar that was cut to size and then welded to the wheel and hub. The spikes, likewise, were cut to a length of 6.4cm from a 0.95 cm CRS bar and welded to the wheel.

Design of residue manager

Three notched disc coulters having 35 cm diameter are placed at the front of the planter for each row designed to remove residue. The 12 teeth notched coulter is made from 0.476 cm mild steel plate.

Design of the handle

The handle of the planter is designed to be an average for the different heights of person male/female which is 85cm from the ground so that it can reduce drudgery. This handle is made up of two mild steel thickness 0.476 cm welded with the main frame 5 cm from each side at the lower end and a rounded tube 12 cm length 0.5 cm internal diameter is welded at the handling edge.

Design of sprocket and chain

Speed of the driving in rpm is:- $N_w = \frac{V_w * 100}{60 * \pi} = 19.1$ rpm

Speed of the driven in rpm is:- $N_s = \frac{V_s * 100}{60 * \pi} = 8.75$ rpm

Speed ratio = $\frac{N_w}{N_s} = 2.18$

Assume Number of teeth of the diving sprocket (ground Wheel) (T_w) as 15

Therefore number of teeth on driven sprocket (seed metering Shaft) is:-

$$\frac{N_w}{N_s} = \frac{T_s}{T_w}$$

$T_s = 2.18 * 15 = 32.7 \approx 33$

Assume roller chain with chain pitch p = 15.875 mm

So, chain speed is: - Chain speed = $T_w * p * N_w = \frac{4.6m}{min}$ = 0.076 m/s

Therefore, from Chain table of Design Hand Book, simplex roller chain N^O 10B1 roll-on with the following specification is selected.

P = 15.875 mm, Minimum breaking load (Q) = 2263 kgf, Width between inner plates(w) = 11.7 mm Service factor = Load factor * lubrication factor * rating factor = 1.875

Factor of safety (F.O.S) = 3-5 for mild steel

Now horse Power transmitted by the chain

$$HP = \frac{Q * v}{4500 * \text{service factor} * F.O.S} = 0.25 \text{ hp}$$

Hence 0.25hp is greater than 0.012hp the selected chain is safe.

Then Chain length is: - Since the chain drive of the machine is at an angle 45° with the horizontal, take vertical and horizontal distance 45 cm.

Therefore, Center to center distance is: -

$$C = \sqrt{{h_c}^2 + {v_c}^2} = \sqrt{45\ cm^2 + 45\ cm^2} = 63.64 \text{ cm}$$

In order to accommodate the initial sag in the chain, the value of center-to-center distance is reduced by 2 to 5 mm $C_{corrected}$ = C – 5 mm = 632.4 mm

Therefore, approximate center to center distance in number of pitches is: - $C_p = \frac{C_{corrected}}{p} = 39.8$

Therefor length of chain $L_p = K_p * p = 1651$ mm = 1.651 m

Pitch diameter of sprocket: - For smaller sprocket $d_{(a1)} = p \csc (^{180}/_{T_1})$

$d_{(a1)}$ = 76.4 mm = 7.64 cm

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For larger sprocket $d_{(a2)}$ = p csc $(^{180}/_{T_2})$ = 167 mm = 16.7 cm

Design of the sprocket and chain at the seed metering Shaft and fertilizer metering shaft

Speed of the driving in rpm is: - N_s = 8.75 rpm

Speed ratio is: - Speed ratio = $\frac{N_s}{N_f} = 1$

Assume Number of teeth of the diving sprocket (seed metering shaft) (T_s) as 15

Therefore, number of teeth on driven sprocket (seed metering Shaft) is: - $\frac{N_s}{N_f} = \frac{T_f}{T_s}$, T_f = 15

Assume roller chain with chain pitch p = 15.875 mm

So, chain speed is: - Chain speed = T_s * p * N_s = 2.08m/ min = 0.035 m/s, P = 15.875 mm

Minimum breaking load (Q) = 2263 kgf and Width between inner plates(w) = 9.7 mm

Service factor = Load factor * lubrication factor * rating factor = 1.875

Factor of safety (F.O.S) = 3–5 for mild steel

Horse Power transmitted by the chain HP = $\frac{Q * v}{4500 * \text{service factor} * F.O.S}$

HP = 0.11hp

0.11 hp is greater than 0.012 hp, hence the selected chain is safe!

Design of the Shaft

Considerations: Wheel speed (V_w) = 0.6 m/s = 36 m/ min (based on the animal speed)

Main seed metering shaft = 0.275 m/s = 16.5 m/min [is assumed for minimum seed breakage

Diameter of ground wheel = 30 cm, Coefficient of rolling resistance of wheel = 0.3

Assume weight of machine = 50 kg, Weight on each wheel = 50 kg/5 = 10 kg

Rolling resistance force = coefficient of rolling resistance * weight on each wheel

Rolling resistance force = 3 kg

Torque produced by the ground wheel. (T_w = rolling resistance force * radius of wheel

T_w = 3kg * 0.15 m = 0.45 kg–m

Speed of the wheel in rpm (N_w) is $N_w = \frac{V_w * 100}{60 * \pi}$ = 19.1 rpm

Based on the wheel speed Horse Power at machine wheel is: -

HP = $\frac{2\pi * N_w * T_w}{4500}$ = 0.012 hp

Now, based on the Horse Power chain pulls for chain drive from ground wheel to the seed metering shaft is: -

$P_t = \frac{HP * 75}{V}$

Where: - V = $\frac{\pi d N_w}{60}$ = 0.0764 m/s

chain pulls in tension side is: - $P_t = \frac{HP * 75}{V}$ = 11.78 kgf

Also, the chain load (Q) is: - Q = K_1 * P_t

K_1 = 1.15 for mild steel Q = 13.547 kgf

Since the chain drive is acting at an angle θ = 45° with the horizontal the vertical chain load is:-

Q_v = Q sin θ = 9.58 kgf

Therefore, maximum bending moment in the axle is given by:

M_b = (weight on each wheel * overhung) + (Q_v * overhung)

Assume: - overhung of wheel = 15 cm,

Overhung of Sprocket = 5 cm

T_{eq} = 2.03 kgm

Factor of safety = 3

T_{eq} corrected = T_{eq} * F.o.s = 6.09 kgm

The diameter of the shaft based on the twisting moment is:-

T_{eq} corrected = $\frac{\pi}{16}$ d^3 τ_s

d = 19.35 mm

Therefore the safe diameter is:- d = 19.35 mm ≈ 20 mm

Design of fertilizer metering shaft:

Assume weight of sprocket as 0.3 kg, Length of the shaft is 55 cm

Design of beam for the main frame

Length of the beam = 100 cm

Total load applied on the beam = 15 kgf + 9.58 kgf = 24.58 kgf

Weight on each beam = 24.58 kgf/5 = 4.916 kgf

$$\sigma_{allowable} = \frac{\sigma_y}{F.O.S} = 4.59 \text{ kg/mm}^2$$

$$\sigma_{allowable} = M/Z$$

Bending moment on the beam is:-

$$M = \frac{W * L^2}{12}$$

M = 409.67 kgmm

Dimension of the beam is:- $Z = M/\sigma_{allowable}$

$Z = 89.25 \text{ mm}^3$

$$Z = \frac{bh^2}{6}$$

Assume h = 4.76 mm

$$89.25 \text{ mm}^3 = \frac{b * (4.76)^2}{6}$$

b = 4.3 ≈ 5 cm

Weight of the main frame

The volume of the main frame is calculated as: - $V_f = (w * h * t) N$

$V_f = (0.476 \text{ cm} * 5 \text{ cm} * 100 \text{ cm}) * 5 + (0.476 \text{ cm} * 5\text{cm} * 44 \text{ cm}) * 2 = 1399.44 \text{ cm}^3$

To find mass of the frame: - $M_f = V_f * \rho_s = 11.26$ kg

To determine weight of the frame: - $W_f = M_f * g = 110.46 N = 110.46 N$

Selection of bearings

Based on the shaft diameter dimension of the bearing is selected from the engineering toolbar (Khurmi and Gupta 2005).

For seed metering shaft

Bore diameter = 30 mm, Bearing number = 206, Outside diameter = 62 mm, Width = 16 mm

For fertilizer metering shaft

Bore diameter = 25 mm, Bearing number = 205, Outside diameter = 52 mm, Width = 15 mm

The parameters and calculations leading to the machine design are obtained, the total draft force required to pull the planter is 73.5 kgf, so two bulls each weighing ranging from 205 to 300 kg, having a power of 0.58 hp and also moving with a speed of 0.6 m/s can sufficiently pull the planters.

Then, we design the chain and sprocket parameters. The power transmitted by the chain to the main shaft is 0.25 hp which is greater than the 0.012 hp developed in the ground wheel shaft considering a service factor of 1.875; therefore, the selected chain is safe. Also, the power transmitted by the chain from the seed to the fertilizer metering shaft is calculated as 0.11 hp and is greater than the 0.012 hp developed by the wheel shaft considering a service factor of 1.875.

After that, we calculate the chain parameters thus the length of the chain between the ground wheel and the main shaft using equation (3.33) at an angle of 450 with the horizontal is 1.651 m. Then we assume the number of teeth of the driving sprocket is 15, where the number of teeth of the driven sprocket is calculated as 33. Applying the same chain equation, the length of the chain from the seed and fertilizer metering shaft with an angle of 00 with the horizontal is 0.56 m.

The ground wheel shaft experiences an equivalent twisting and bending moment of 6.09 and 6.03 kgm, respectively. The equivalent bending and twisting applied on the seed metering shaft are 15.39 and 9.84 kgm, respectively. Based on these stress values involved in the planter operation, a standard diameter of the ground wheel, seed metering, and fertilizer metering shaft of 20, 27, and 23 mm, respectively are obtained.

CONCLUSION

A economical animal drawn cereal grain no. till planter using local material was designed and fabricated. The equipment attaing better accuracy was recommended for further trials.

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Research Article

Effect of straw moisture on the performance of mulcher in paddy stubble field

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ABSTRACT

Globally, the most popularised issue is the burning of crop residues which has an adverse effect on the environment and human health. According to the estimates of Sardar Patel Renewable Energy Research Institute (2004), about 72 Mt crop residues are burnt on-farm. Apart from burning, crop residues have several uses *viz.*, soil mulching, manure, power generation, animal feed *etc.* Hence, straw mulching in paddy stubble fields was studied based on the effect of straw moisture on mulching parameters *viz.*, length of chopped material, cutting efficiency, and uniformity of spreading. The moisture content of paddy was taken at intervals after harvesting of the crop *i.e.*, 20, 30, and 40 days after harvest. The straw moisture content at these intervals is found to be 15, 19, and 24 per cent, respectively. The length of chopped material increased linearly with straw moisture content and varied from 15 to 24 cm. There is a decreasing trend of cutting efficiency with an increase in straw moisture content which varied from 65 to 86 per cent. The uniformity of spreading varied from 79.53 to 94.56 per cent and decreased with an increase in straw moisture content.

***Keywords*:** Straw moisture content, length of chopped material, cutting efficiency, uniformity of spreading.

India, the second largest agro-based economy with year-round crop cultivation, generates a large amount of agricultural waste, including crop residues. According to the Ministry of New and Renewable Energy (MNRE), approximately 500-550 million tonnes of crop residue is generated on-farm and off-farm annually from the production of 110 Mt of wheat, 122 Mt of rice, 71 Mt of maize, 26 Mt of millets, 141 Mt of sugarcane, 8 Mt of fibre crops (jute, cotton) and 28 Mt of pulses. Crop residue is used as fodder, fuel for other domestic and industrial purposes. However, there is still a surplus of 140 Mt out of which 92 Mt is burnt in the field each year.

Crop residue burning (CRB) has become a major environmental problem causing health issues as well as contributing to global warming. The government of India has attempted to curtail this problem through numerous measures and campaigns designed to promote sustainable management methods such as converting crop residue into energy. Crop residue burning is a common practice done by farmers. The reason behind these unhealthy practices is the stubble of greater height left over by the use of combine harvester, the short time span between crop harvesting and crop sowing, a shortage of labour, and the high cost of proper crop residue management. There is one more reason for CRB is nitrogen immobilization in soil due to residue incorporation in the soil, which results in the yellowing of the succeeding crop.

It is realized that if waste is treated in such a way that reduces its storage space, then its use for manurial purposes as well as industrial utilization becomes more viable. The shredded waste can be useful for the production of hard boards, particleboards, corrugated boards and boxes, paper pulp, and industrial applications. It is also a useful source of lignin in animal feeds. The use of organic manure improves the physical, chemical, and biological properties of soil by improvement of soil structure, water holding capacity, soil aeration, buffering of the soil surface temperature, reduction of the soil losses due to soil erosion *etc.* In order to utilize agricultural waste as an organic manure material, it requires to reduce the volume *i.e.*, to break into small pieces and used as mulch on the field.

Conservation agriculture is a resource-saving agricultural production system that aims to achieve production intensification and high yields while enhancing the natural resource base through compliance with three interrelated principles *viz.*, minimum mechanical soil disturbance, permanent soil organic cover, and species diversification. One of the principles of conservation agriculture is permanent soil organic cover which helps in

improving soil quality in terms of soil organic carbon, soil moisture retention, and essential nutrients. In consideration of this parameter, the evaluation of the mulcher was carried out to find the optimum efficiency of the mulcher in the paddy stubble field.

MATERIALS AND METHODS

The work was carried out at farmers' fields, where the evaluation was conducted at three different durations after the paddy harvest. The three intervals are according to the days after the harvest *i.e.*, 20, 30, and 40 days after the harvest. The following are the parameters selected for the study.

The moisture content of stubble was determined by the oven-dry method. The 20 samples were collected from the field, three samples were randomly selected and their initial weights were recorded using electronic balance. The samples were dried in a hot air oven set at a temperature of 105 °C and kept for a period of 24 h and samples were reweighed (Mohsenin, 1986).

$$\text{Stubble moisture content, \% (wb)} = \frac{W_w - W_d}{W_w} \times 100$$

Where,

W_w = initial weight of stubble sample, g

W_d = bone dry weight of stubble sample, g

The length of chopped material (Plate 1) was measured in the field at five different locations and the mean values were recorded (Senthilkumar *et al.*, 2010; Sridhar and Surendrakumar, 2016).

Cutting efficiency was calculated based on the height of stubbles in 20 m length measured prior to the shredding operation and the stubbles left behind in the same 20 m length were measured after the operation (Darekar Jyoti Anant and Veerangouda, 2020).

$$CE = \frac{W_1 - W_2}{W_2} \text{ X } 100$$

Where,

CE = Cutting Efficiency, %

W1 = Number of stubbles before cutting

W2 = Number of stubbles left after cutting

For measuring the uniformity of spreading (Plate 1), the chopped straw behind the machine after cutting was divided into three equal samples of size 1 x 1 m^2, and the weight of chopped straw in each plot was recorded using a weighing balance having the least count of 10 g. The results were represented in terms of the percentage of uniformity in each sample. Three replications of this procedure were done in each plot (Dalmis *et al.*, 2013).

RESULTS AND DISCUSSION

As discussed earlier the three parameters are studied at three different intervals of time *i.e.*, 20, 30 and 40 days after harvest.

The stubble moisture content varied between 15 and 24 per cent and the three different moistures selected are 15, 19 and 24 per cent. For the mentioned straw moisture levels, the following results were observed.

Length of chopped material

The effect of straw moisture on the length of chopped material is shown in Fig. 1. It shows the increasing trend with respect to straw moisture. The minimum length of chopped material of 15 cm was recorded at straw moisture of 15 percent, whereas the maximum length of chopped material of 22 cm was recorded at straw moisture of 24 per cent. The length of chopped material increased with increased straw moisture content since the high moisture does not encourage faster chopping action.

Cutting efficiency

The effect of straw moisture on cutting efficiency is shown in Fig. 2. It shows a decreasing trend with respect to straw moisture. The minimum cutting efficiency of 68 per cent was found at straw moisture of 24 per cent whereas the

Plate 1. Measuring of length of chopped material and weighing the collected samples.

maximum cutting efficiency of 86 per cent was found at 15 per cent straw moisture content. The higher the moisture content it effects the chopping action of the blades hence the cutting efficiency was reduced.

Uniformity of spreading

The effect of straw moisture on the uniformity of spreading is shown in Fig. 3. It shows a decreasing trend with respect to straw moisture. The minimum cutting efficiency of 85.6 per cent was found at straw moisture of 24 per cent whereas the maximum cutting efficiency of 90.2 per cent was found at 15 per cent straw moisture content. The higher the moisture content the minimum distance of the throw is observed hence the uniformity was decreased.

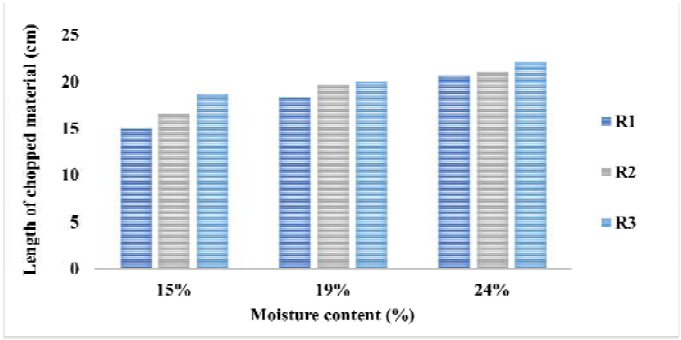

Fig. 1. Effect of straw moisture on length of chopped material.

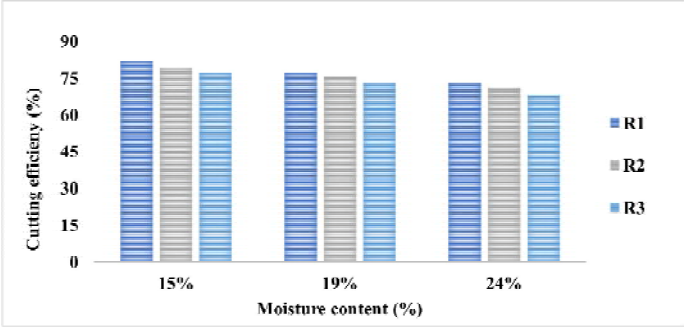

Fig. 2. Effect of straw moisture on cutting efficiency.

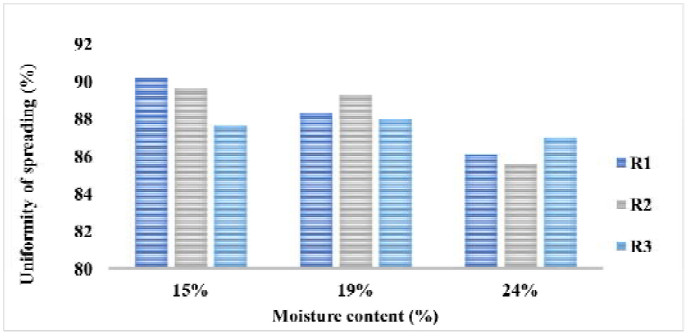

Fig. 3. Effect of straw moisture on uniformity of spreading.

CONCLUSION

The effect of straw moisture was greater in terms of the length of the cut, cutting efficiency, and uniformity of spreading. As the moisture increases the efficiency of the mulcher decreases hence, mulching action should be carried out at optimum stubble moisture.

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Research Article

Evaluation of chemical and sensory parameters of tomato-broccoli blended instant vegetable soup mix

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ABSTRACT

The aim of the study was to standardize appropriate blends of tomato and broccoli for the development of an instant vegetable soup mix. Soup mix was formulated with varied proportions of tomato and broccoli powder as T_1 (100:0 :: Tomato powder: Broccoli powder), T_2 (95:05 :: Tomato powder: Broccoli powder), T_3 (90:10 :: Tomato powder: Broccoli powder), T_4 (85:15 :: Tomato powder: Broccoli powder), T_5 (80:20 :: Tomato powder: Broccoli powder), T_6 (75:25 :: Tomato powder: Broccoli powder) and T_7 (70:30 :: Tomato powder: Broccoli powder) along with other ingredients *viz*., corn flour (thickening agent), salt *etc.* and were evaluated for their chemical and sensory characteristics at 30 days interval under ambient conditions. Results showed that treatment T_7 recorded the highest ascorbic acid (30.52 mg/100 g) and antioxidant activity (26.38 %). Sensory evaluation revealed that instant soup mix blended with 85:15 :: Tomato powder: Broccoli powder was most acceptable.

Key words: Antioxidant, ascorbic acid, instant soup mix, sensory evaluation, tomato, broccoli.

Soup is a liquid which is prepared from vegetables, pulses, fish or meat using water, juice or stock and some thickening agents and falls under the heterogenous category of food. Instant soup is a convenient food and takes less time to cook (Sarkar *et al*., 2017). It plays an important role in maintaining the nutritional levels of the people and also helps in making the crops available throughout the season. There is a big demand for instant dry soup mixes in the global market. Instant soup powders are similar kinds of instant foods which have extended popularity in recent years, by way of providing convenience, hygienic, and extensible shelf-life (Premavalli *et al*., 2005). Balanced nutrition is obtained by including whole cereals, vegetables and pulses in soup formulations.

Tomato (*Solanum lycopersicum* L.) belongs to the Solanaceae family and is the second most important vegetable crop. It is cultivated for fresh fruit and processed products. Tomatoes contain many health-promoting compounds including vitamins, carotenoids, and phenolic compounds. In addition to its economic and nutritional importance, tomatoes have become the model for the study of fleshy fruit development. It is a climacteric fruit and dramatic metabolic changes occur during its fruit development. They provide detectable flavour to any dish to which they are added. However, tomatoes are highly perishable in the fresh state leading to losses and wastage during peak season. Huge quantities of tomatoes are lost due to a lack of proper processing, storage and transportation facilities. Therefore, prevention of these losses and wastage is of major interest especially when there is a subsequent imbalance in demand and supply in the off-season (Akanbi *et al*., 2006). Hence, proper processing and storage into some preserved form during seasons of a glut will ensure its availability and utilization during the deficiency period.

Broccoli (*Brassica oleracea* var. italica) is an edible green plant in the Brassica family whose large flowering head and stalk are consumed as a vegetable (Miraj, 2016). It has been described as a vegetable with high nutritional value due to its important content of vitamins, antioxidants, anti-carcinogenic compounds, and health-promoting phytochemicals. This horticultural crop contains a high level of phytochemicals including glucosinolates and flavonoids which have chemoprotective properties of their major hydrolysis products, isothiocyanates (Campbell *et al*., 2007). Broccoli is also recommended as an excellent dietary component for diabetics due to its high protein and low sugar content. Wadmare *et al*. (2019) reported that moisture content in broccoli was found to be 86.36 %, carbohydrate 5.59 %, protein 4.89 %, and Fat 0.37%. Further, the mineral composition of broccoli showed 0.7 mg iron, 0.4 mg zinc, 47 mg calcium and 66 mg/100 g phosphorous.

Food dehydration refers to the complete removal of water from foods under controlled conditions. The advantages of dehydrated foods, particularly, dry soup mixes

include protection from enzymatic and oxidative spoilage along with flavour stability at room temperature over long periods of time (Rekha *et al*., 2010). Soup is a very fast form of cookery. It is probably one of man's oldest foods since it must have developed about the time that boiling was found to be a way of cooking food. The popularity of soups today is due to increased nutrition consciousness and the desire for simpler and lighter meals. Instant soups are very practical in preparation and take a short time to serve (Sunyoto *et al*., 2012). Therefore, the present investigation was undertaken with the objective to develop an instant soup mix from tomato and broccoli blends and analyze its chemical and sensory parameters during storage.

MATERIALS AND METHODS

Tomatoes were washed, blanched and cut into small slices (around 1 cm). Then, the slices were dipped in 2 per cent sodium benzoate solution at room temperature for 5 minutes and were drained thoroughly after the dip treatment (Aderibigbe *et al*., 2018). The slices were then dried in a tray drier for 16 hours at 50±2 ^{o}C. After obtaining constant moisture content, cooling was done at room temperature, the dried tomato slices were ground by using a grinder and sieved through a 325-micrometre sieve to obtain fine tomato powder. Broccoli was cleaned, washed under running tap water and surface water was dried. The florets were then separated from the bottom and blanching was done at 80 ^{o}C for 3 minutes in 0.1 per cent solution of sodium bicarbonate (Kaur *et al*., 2018). The broccoli florets were then crushed so as to reduce the time for drying and increase the drying rate. The crushed broccoli was spread evenly in a single layer on trays and drying was carried out at 50±2 ^{o}C for 8 hours with constant air flow rate. After obtaining constant moisture content dried broccoli was ground and sieved to obtain fine broccoli powder. Peeled onion and garlic samples were washed, sliced and dried in a tray drier at 60 ^{o}C for 5-6 hours and ground. The ground onion and garlic samples were sieved to obtain fine powder (Niththiya *et al*., 2014).

The dried tomato and broccoli powder were blended in different ratios T_1 (100:00:: Tomato powder: Broccoli powder), T_2 (95:05:: Tomato powder: Broccoli powder), T_3 (90:10:: Tomato powder: Broccoli powder), T_4 (85:15:: Tomato powder: Broccoli powder), T_5 (80:20:: Tomato powder: Broccoli powder), T_6 (75:25:: Tomato powder: Broccoli powder) and T_7 (70:30:: Tomato powder: Broccoli powder) to form nutritious and convenient soup mix. The formulated soup mix was packed in laminate pouches and its storability was studied for 90 days under ambient conditions. For the preparation of instant vegetable soup mix the tomato and broccoli powders were seasoned with dried onion powder (8%), garlic powder (8%), salt (2%), black pepper (2%) and commercially available corn flour (20%) to formulate seven different soup mixtures (three replications for each formula).

Chemical Analysis

Ascorbic acid was determined by a titrimetric method using 2,6-dichlorophenol indophenol dye (AOAC, 2012). Free radical scavenging activity was determined by DPPH (diphenyl picryl hydroxyl) method (Luo *et al*., 2009). Browning in the sample was estimated by the method of Ranganna (2014). Five grams of sample was macerated with 25 ml of 60 per cent ethyl alcohol and it was kept overnight in a beaker. The next day, it was thoroughly mixed and filtered. The filtrate was taken for measuring colour at 440 nm using 60 per cent aqueous ethyl alcohol as a blank. The increase in absorbance of sample extract at 440 nm was taken as a measure of non-enzymatic browning.

Sensory Evaluation

The sensory evaluation of tomato-broccoli blended instant vegetable soup mix was done by a panel of 10 semi-trained judges following the 9-point hedonic scale assigning (scores assigned as 9- "like extremely" to 1- "dislike extremely") as described by Amerine *et al*. (1965). The products were evaluated for colour, taste, consistency and aroma. The overall acceptability of the products was based on mean scores obtained from sensory characters. A mean score of 5.5 and above out of 9 was considered acceptable.

Economics

The cost of production of tomato-broccoli blended instant vegetable soup mix was determined by taking into consideration the cost of raw materials, chemicals and packaging materials *etc*. used in the preparation of the product. The data obtained were analyzed statistically using a Factorial completely randomized design for interpretation of the results through analysis of variance.

RESULTS AND DISCUSSION

Effect on ascorbic acid

The mean ascorbic acid content of the tomato-broccoli blended instant vegetable soup mix, showed an inclination of 63.03 per cent in the blend T_7 (70:30 :: Tomato powder: Broccoli powder). There was a significant increase in ascorbic acid content among the blends which might be due to the presence of higher ascorbic acid in broccoli powder (Table 1). Similar results were recorded by Farzana *et al*. (2017) in the soup powder supplemented with soy flour, mushroom, and moringa leaf. During storage of 90 days, 7.09 per cent decrease was observed in ascorbic acid content which might

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Table 1. Ascorbic acid content (mg/100 g) of instant vegetable soup mix.

Blends	Ascorbic acid (mg/100g) Storage period (days)				Mean (Blends)
	0	30	60	90	
T_1 (100:00 :: Tomato powder: Broccoli powder)	19.61	19.03	18.42	17.83	18.72
T_2 (95:05 :: Tomato powder: Broccoli powder)	21.55	21.04	20.39	19.73	20.68
T_3 (90:10 :: Tomato powder: Broccoli powder)	23.49	23.02	22.28	21.75	22.63
T_4 (85:15 :: Tomato powder: Broccoli powder)	25.47	25.00	24.27	23.71	24.61
T_5 (80:20 :: Tomato powder: Broccoli powder)	27.70	26.98	26.30	25.64	26.65
T_6 (75:25 :: Tomato powder: Broccoli powder)	29.36	28.96	28.23	27.60	28.53
T_7 (70:30 :: Tomato powder: Broccoli powder)	31.32	30.94	30.32	29.57	30.52
Mean (Storage)	25.50	24.96	24.31	23.69	-
Effects	**C.D (p≤0.05)**				
Blends	0.06				
Storage	0.05				
Blends × Storage	0.11				

be due to the oxidation of ascorbic acid into dehydro-ascorbic acid followed by further degradation to 2,3-diketogluconic acid and finally to furfural compounds which enter browning reaction (Sharma *et al*., 2013).

Effect on antioxidant activity

Antioxidant activity is an important component that scavenges free radicals which cause degenerative diseases. The mean antioxidant activity among control and blends showed a significant increase of 69.21 per cent in blend T_7 (70:30 :: Tomato powder: Broccoli powder) (Fig. 1). There were significant differences in blends with respect to an antioxidant activity which might be due to variation in the composition of vegetable powders. Singh and Kaur (2019) also observed similar findings in the baby corn soup mix. A decrease of 8.22 per cent was recorded during the storage of 90 days. Phenolic compounds and ascorbic acid content have been responsible for the antioxidant activity. A similar decrease in antioxidant activity was reported by Singh and Sagar (2010) in dehydrated drumstick leaves during a storage period of 3 months.

Effect on browning

Browning is a result of chemical processes and is a function of temperature, the structure of the material and residence time during processing as well as storage period and it may directly affect the sensory and nutritional quality of dehydrated products. Browning showed 34.51 per cent decline among control and broccoli blends (Fig. 2). The mean value of browning during 90 days of storage, showed a significant increase of 77.11 per cent. The increase in browning during storage might be due to the action of acidity, which enhanced hydrolytic reaction causing browning and acid also enhanced Maillard reaction and caramelization, which caused more browning in the products (Rahul, 2017).

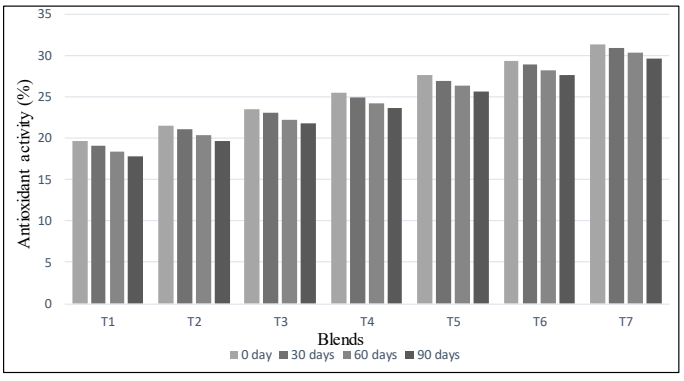

Fig. 1. Effect of blending tomato: broccoli on Antioxidant activity (%) of instant vegetable soup mix.

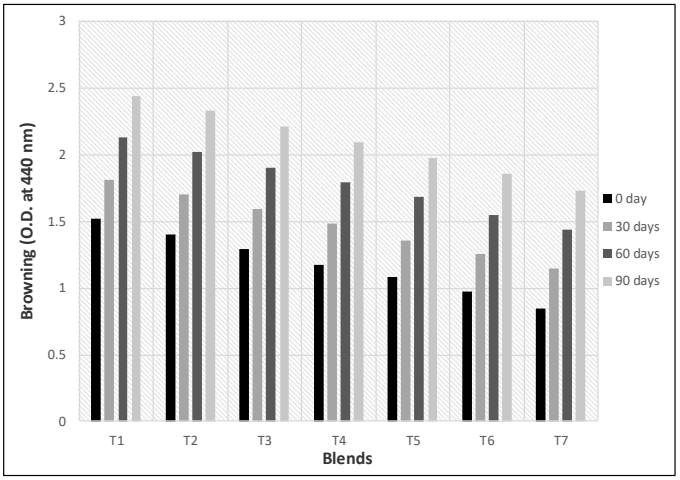

Fig. 2. Effect of blending on Browning (O.D. at 440 nm) of instant vegetable soup mix.

Effect on Sensory Parameters

Colour is one of the most significant quality parameters which attracts consumers to buy a product. The maximum mean colour was recorded in blend T_1 (100:00 :: Tomato powder: Broccoli powder) which was 12.30 per cent higher than blend T_7 (70:30 :: Tomato powder: Broccoli powder) (Table 2). The colour value decreased with blending as the product became darker due to an increase in broccoli powder in blends. As the storage period advanced the colour score decreased for the instant vegetable soup mix by 8.22 per cent which could be due to degradation of coloured pigments and browning caused by co-polymerization of organic acids of the product (Rahman, 2021).

Blend T_4 (85:15 :: Tomato powder: Broccoli powder) showed a maximum taste score which is 9.50 per cent higher (Table 2) than blend T_7 (70:30:: Tomato powder: Broccoli powder) which might be because of mix proportions of tomato and broccoli powder which gave good results. It was observed that when the proportion of broccoli powder increased beyond 15 per cent, it affected the taste score of the soup negatively. As the storage period advanced by 90 days, the mean taste score decreased by 6.23 per cent due to fluctuations in acids, pH and sugar-acid ratio (Safdar *et al*., 2014).

The blend T_4 (85:15 :: Tomato powder: Broccoli powder) showed a maximum mean consistency score of 7.96 whereas, a minimum mean consistency score of 7.11 were recorded in blend T_7 (70:30 :: Tomato powder: Broccoli powder) which might be due to an appropriate combination of tomato and broccoli powder for soup making (Table 3). During the

Table 2. Colour and Taste scores (Hedonic rating) of instant vegetable soup mix.

Blends	Colour				Mean (blends)	Taste				Mean (blends)
	Storage periods (days)					Storage periods (days)				
	0	30	60	90		0	30	60	90	
T_1 (100:00 :: TP:BP)	8.10	7.89	7.68	7.51	7.80	8.02	7.89	7.64	7.50	7.76
T_2 (95:05 :: TP:BP)	7.99	7.77	7.54	7.33	7.65	8.16	8.00	7.83	7.62	7.90
T_3 (90:10 :: TP:BP)	7.85	7.61	7.40	7.22	7.52	8.27	8.12	7.95	7.73	8.02
T_4 (85:15 :: TP:BP)	7.71	7.52	7.25	7.11	7.39	8.39	8.27	8.13	7.92	8.21
T_5 (80:20 :: TP:BP)	7.50	7.28	7.06	6.87	7.17	7.93	7.78	7.53	7.40	7.66
T_6 (75:25 :: TP:BP)	7.32	7.11	6.90	6.69	7.00	7.76	7.69	7.44	7.32	7.55
T_7 (70:30 :: TP:BP)	7.16	6.94	6.75	6.53	6.84	7.65	7.57	7.28	7.19	7.43
Mean (Storage)	7.66	7.44	7.22	7.03		8.02	7.90	7.68	7.52	
Effects	**C.D (p≤0.05)**					**Effects**		**C.(p≤0.05)**		
Blends	0.04					Blends		0.02		
Storage	0.03					Storage		0.02		
Blends × Storage	0.08					Blends × Storage		0.04		

BP Broccoli Powder

Table 3. Consistency and Aroma scores (Hedonic rating) of instant vegetable soup mix

Blends	Consistency				Mean (blends)	Aroma				Mean (blends)
	Storage periods (days)					Storage periods (days)				
	0	**30**	**60**	**90**		**0**	**30**	**60**	**90**	
T_1 (100:00 ::TP:BP)	7.96	7.67	7.31	7.02	7.49	7.98	7.77	7.61	7.49	7.71
T_2 (95:05 :: TP:BP)	8.11	7.81	7.42	7.15	7.62	8.12	7.87	7.76	7.62	7.84
T_3 (90:10 :: TP:BP)	8.30	7.99	7.60	7.34	7.80	8.29	8.15	7.95	7.83	8.05
T_4 (85:15 :: TP:BP)	8.43	8.15	7.76	7.51	7.96	8.42	8.27	8.10	7.91	8.17
T_5 (80:20 :: TP:BP)	7.82	7.58	7.20	6.91	7.37	7.89	7.71	7.53	7.42	7.63
T_6 (75:25 :: TP:BP)	7.75	7.45	7.06	6.76	7.25	7.75	7.58	7.45	7.30	7.52
T_7 (70:30 :: TP:BP)	7.64	7.30	6.91	6.62	7.11	7.51	7.43	7.32	7.15	7.35
Mean (Storage)	8.00	7.70	7.32	7.04	-	7.99	7.82	7.67	7.53	-
Effects	C.D (p≤0.05)					Effects		C.D (p≤0.05)		
Blends	0.03					Blends		0.05		
Storage	0.02					Storage		0.04		
Blends × Storage	0.07					Blends × Storage		0.08		

TP: Tomato Powder; BP: Broccoli Powder

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Table 4. Cost of production of instant vegetable soup mix.

Ingredients	Rate (₹)	Instant vegetable soup mix T_4 (85:15:: Tomato powder: Broccoli powder)	
		Quantity (g)	Amount (₹)
(A) Variable cost			
(a) Cost of inputs			
Tomato	30/kg	500 g	10
Broccoli	60/kg	50 g	2.5
Garlic	150/kg	8 g	1.2
Onion	25/ kg	8 g	0.2
Corn flour	200/kg	20 g	2.4
Black pepper powder	70/100g	2 g	0.3
Salt	18/kg	2 g	0.03
Laminate pouches	1/pouch	7	7
Total cost			23.63
(B) Cost of labour and fuel		@ 15%	3.54
Total variable cost			27.17
(C) Fixed cost			
Machinery depreciation @ 10% on the total machinery cost of 50,000 for 300 working days in a year			5000
Machinery depreciation for two days			33.33
(D) Profit @ 15 of total variable cost and fixed cost		@ 15%	9.07
(E) GST @ 18 of total variable cost, fixed cost and profit		@ 18%	12.52
Grand total for 100 g product			82.09
Cost per pouch for 15 g			12.31

storage period of 90 days, the consistency scores of the soup mix showed a decline of 12 per cent. Similar results regarding the decline in consistency score during storage were reported in mushroom whey soup powder by Singh *et al*. (2003).

The score for aroma was maximum in the blend T_4 (85:15 :: Tomato powder: Broccoli powder) and it was higher by 10.03 per cent than the blend T_7 (70:30 :: Tomato powder: Broccoli powder) which has minimum mean aroma value (Table 3). Kumar (2015) recorded similar results in soup powder prepared by incorporating a white button mushroom. With the advancement of storage, the aroma of the instant vegetable soup mix decreased by 5.75 per cent. The decrease in aroma score might be due to the degradation of volatile aromatic compounds which occur during storage (Rahul *et al*., 2019).

Cost of Production

The cost of production of instant vegetable soup mix (Table 4) T_4 (85:15 :: Tomato powder: Broccoli powder) which was adjudged as best on the basis of sensory evaluation was based upon the fixed and variable cost of all ingredients used and other factors such as processing charges, packaging materials, *etc*. The cost was calculated on the basis of the current market price of ingredients. The cost of production per 15 g of instant vegetable soup mix was worked to be [1] 12.31.

CONCLUSION

In conclusion, the soup mix formulations enriched with 30 per cent broccoli powder recorded the maximum ascorbic acid and antioxidant activity *i.e.* 30.52 mg per 100 g and 26.38 per cent, respectively. On the basis of sensory scores, blend T_4 (85:15:: Tomato powder: Broccoli powder) was adjudged superior among all the treatments. The cost of production was found to be ₹ 12.31. The instant vegetable soup mix formulated from the blends of tomato and broccoli can be a healthy substitute for people of all age groups with high nutritional value.

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Research Article

Development of a scale to measure the attitude of farmers towards the use of digital platforms in agriculture

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ABSTRACT

The aim of this study was to develop an attitude scale to measure the attitude of farmers towards the use of digital platforms in agriculture. It has been a long-time need for a proper scale to measure the attitude of farmers towards the use of digital tools for Agriculture and allied sectors, it was thought necessary to construct a scale for the purpose. Keeping this in view, an attempt has been made to develop a scale for measuring the attitude of farmers towards the use of digital platforms in agriculture. The method of summated rating scale, by Likert (1932) was used. Fifteen statements were selected from 50 statements for which 't' values were worked out, 't' value shows the level of differentiation between the high and low group for a statement. More than 25-30 per cent were selected which were with the highest 't' value. Finally, 15 statements were retained in the final scale for the study.

***Keywords*:** Digital tools, digital platform, farmers, attitude scale, Likert technique, reliability.

Agriculture is a gigantic sector of the Indian economy as it contributes about 17 per cent to the total Gross Domestic Product (GDP) and provides employment to over 60 per cent of the population. In spite of a large Indian economy, agriculture is lagging behind in many aspects and is characterized by a low degree of market integration and connectivity, low accessibility of reliable and timely information to the farmers, small land holdings, non-adoption or less adoption of improved technology.

India is primarily an agriculture-based country as agriculture contributes nearly one-seventh of the GDP in India. Agriculture is the most pivotal sector of the Indian economy in the current phase of development. Therefore, the transformation of traditional agriculture to modern agriculture is a challenge to fulfil the requirements of over increasing population

21st-century agriculture is highly knowledge-based. Information now has become one of the most critical inputs for profitable and sustainable agricultural development. Not only in all aspects of human life, making an efficient decision and securing the best opportunity information is a must. Information-based, decision-making agricultural system (Precision Agriculture) is designed to maximize agricultural production and is often described as the next great evolution in agriculture.

In today's situation, all the data are on the tip of your finger, you can collect them in a fraction of a moment. Information and Communication Technologies (ICTs) can play a significant role. It can enhance profitability at the farm level, production of market-oriented quality food products, and environment sustainability by reducing the impact of climate change. The ICTs farm-specific support services can be grouped into a) On-farm support services, b) Management and decision-making support services and c) On-site support services. All are directed to the delivery of appropriate knowledge and information to farmers. The use of ICT will boost the progress of the digital India programme, by harnessing the power and advantages of the virtual and online world.

Competency is a combination of a body of knowledge, skills and attitude that an individual possesses to perform a given task effectively and efficiently. Digital media use competency has major three components *viz*., knowledge, skill and attitude. Knowledge means the collection and retention of information in one mind. Skill is the ability to demonstrate a system and sequence of behaviour which results in something observable, something that one can see. Skill and knowledge are both required to perform a task. Attitudes mean a settled way of thinking or feeling about something. Thus, in order to perform any task effectively and successfully including establishing and running a farm unit and getting high profit by using new innovative ideas, a farmer needs to possess a set of knowledge, skill and attitude

which could be together labelled as 'competencies'. Competency is an underlying characteristic of a person which leads to his/her effective or superior performance in using digital platforms.

MATERIALS AND METHODS

Allport (1935) defined attitude as "a mental and neural state of readiness, organized through experience, exerting a directive or dynamic influence upon an individual's response to all objects and situations with which it is related". In Thurstone's study, attitude was defined as the "sum total of a man's inclinations and feelings, prejudice or bias, preconceived notions, ideas, fears, threats, and convictions about any specified topic" (Thurstone, 1928).

The Attitude towards Digital platforms in agriculture was operationalized as the degree of positive or negative feeling about agriculture technology. The method of summated rating technique suggested by Likert (1932) was followed in the development of the scale. The Attitude towards Digital platforms in agriculture was calculated.

The following steps were involved to develop the attitude scale.

Collection and editing of statements

In the first stage, many attitude scales measuring Attitudes towards Digital platforms in agriculture were examined in order to determine the dimensions of the concept, statements of attitude scale and process of developing an attitude scale. Semi-structured interviews and focus group discussions were also carried out with University faculties and experts to identify different dimensions of the concerned concept and develop related items. After reviewing secondary literature and interacting with experts in this field, an item pool consisting of 50 about the Attitude of farmers towards the use of Digital platforms in agriculture was developed.

Ordering and analysis of statements

Ordering of items is an important step in the development of the scale. The judges were asked to indicate their degree of agreement and disagreement in regard to the statement on the five-point (SA, A, U, D and SD) psychological continuum. Then these items were analyzed to see the degree of agreement and disagreement of the judges on a five-point continuum. A score of 4 was assigned to the SA (Strongly agree) response to the statement and 3, 2, 1 and 0 were assigned the A (Agree), U (Undecided), D (Disagree), SD (Strongly Disagree) response, respectively. The order of scoring was reversed for negative statements *i.e.,* the score of 0 was assigned to the SA (Strongly agree) response to the statement and 1, 2, 3 and 4 were assigned the A (Agree), U (Undecided), D (Disagree), SD (Strongly Disagree) response, respectively.

Relevancy test (Content validity test)

In this stage, a panel of experts was selected from different institutes. For the purpose of content validation, the initial draft of the attitude scale with 50 items on a five-point rating scale (1=Least relevant, 2=Less relevant, 3=Relevant, 4=Highly relevant, 5= Most relevant) was given to the panel for obtaining their opinion about whether the selected items were valid items to measure the Attitude towards Digital platforms in agriculture. These statements were sent to 150 different agricultural extension experts for their evaluation. Among 150 judges, only 45 judges sent their responses. Now out of 45 judges' responses, three responses were discarded due to vague evaluation. The relevancy score of each item was ascertained by adding the scores on the rating scale for all 42 judges' responses. From this data, relevancy weightage was worked out for all the statements by using the following formula.

Relevancy Weightage= MRi×5+HRi×4+Ri×3+LRi×2+LTRi×1/MPS

Where,

MRi = Number of judges who consider the i^{th} item as Most Relevant

HRi = Number of judges who consider the i^{th} item as Highly Relevant

Ri = Number of judges who consider the i^{th} item as Relevant

LRi = Number of judges who consider the i^{th} item as Less Relevant

LTRi = Number of judges who consider the i^{th} item as Least Relevant

MPS = Maximum possible score (50×5=225)

N = Number of judges (42)

Using this formula, the statements were screened for their relevancy. Accordingly, statements having relevancy weightage >3.75 were considered for the selection of statements.

Item analysis (Calculation of 't' value)

These 50 statements were subjected to item analysis to delineate the items based on the extent to which they can differentiate the respondent with high favourable attitude from the respondent with a less favourable attitude towards orange production technology. (Maximum possible score

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was 50×4=200 and minimum score is 50×0= 0). Based on the total scores obtained, the statements were arranged in descending order. Then 25 per cent of the statements with the highest total scores and 25 per cent of the statements with the lowest total scores were sorted out for the purposes of 't' value calculation. The 't' value of each statement was calculated by using the following formula as suggested by Edward (1969).

$$t = \frac{\bar{X}_H - \bar{X}_L}{\sqrt{\frac{\sum_{i=1}^{n}(X_H - \bar{X}_H)^2 + (X_L - \bar{X}_L)^2}{n(n-1)}}}$$

Where,

$$\sum(X_H - \bar{X}_H)^2 = \sum X_H{}^2 - \frac{(\sum X_H)^2}{n}$$

$$\sum(X_L - \bar{X}_L)^2 = \sum X_L{}^2 - \frac{(\sum X_L)^2}{n}$$

$\bar{X}_H$ = The mean score on a given statement for the high group

$\bar{X}_L$ = The mean score on a given statement for the low group

$\sum X_H{}^2$ = Some of the squares of the individual score on a given statement for a high group

$\sum X_L{}^2$ = Some of the squares of the individual score on a given statement for a low group

$\sum X_H$ = Summation of squares on the given statement for high group

$\sum X_L$ = Summation of squares on the given statement for low group

n = Number of subjects in the low and high group

t = Level of differentiation between the high and low group

$\sum$ = Summation

RESULTS AND DISCUSSION

Selection of statements

The 't' value shows the level of differentiation between the high and low groups for a statement. More than 25-30 per cent were selected which were with the highest 't' value. Finally, 15 statements were retained in the final scale for the study out of 50 statements (Table 1).

Reliability of scale

A scale is said to be reliable when it gives consistency to the same results on application to the same type of sample. The final set of 50 statements about the Attitude towards Digital platforms in agriculture was prepared (Table 1). There were 48 positive and 02 negative statements which represent the Attitude towards Digital platforms in agriculture on the five-point continuum. The new 20 farmers, who were not included in the sample size and the non-targeted farmers were selected from Jahanabad and Aurangabad. The scores were subjected to a product-moment correlation test in order to find out the reliability of the split-half test. The split half-test reliability coefficient r was 0.65, which was significant at 1 per cent level of probability. Reliability was calculated by using the below-mentioned formula (Spearman, 1910; Brown, 1910).

$r_{SB} = 2r_{hh}/1+r_{hh}$

Where,

r_{hh} = Pearson correlation between odd and even

The coefficient of correlation between odd and even scores was 0.65.

Validity of scale

The validity of a scale may have been defined as the truthfulness attribute of scale. The validity of any scale is the property ensuring that the obtained test scores measure the variables which is supposed to be measured (Kerlinger, 1973). The content of the scale is based on the relevant literature, expert opinions, extension personnel, and farmers as measured to check.

Similarly, several workers have developed the scales on various aspects. Bharamagoudar and Angadi (2015) developed a scale to measure the job perception of Panchayat development officers of north Karnataka which consisted of 4 components and 22 statements. Geeta *et al.* (2016) developed a scale to measure the impact of entrepreneurship development programmes which is useful for studying the impact of entrepreneurship development programmes. Supriya and Natikar (2018) developed a scale consisting of 4 components and 40 statements to measure the performance of Grama Sabha in the implementation of rural development programmes. Manjunath and Bheemappa (2021) stated that finally, 33 statements were retained on the scale which will

Table 1: List of Attitudes towards Digital platforms in agriculture with their RP- Relevancy Percentage, RW- Relevancy Weightage, MRS- Mean Relevancy Score and t values.

Statements	RP	RW	MRS	t-Value
I believe that digital platforms like Bihar Krishi App, IFFCO Kisan, Plantix, Kisan Suvidha, Kheti Badi and Krishi Mitra are smart to receive agricultural information.	357.14	3.571	0.75	0.2927
I feel that digital platforms in farming is a misuse of money.	185.71	1.857	0.39	4.06108
I think Kisan Choupal provides important information to the farming community.	357.14	3.571	0.75	0.4472
I think the digital platforms is a prompt system for agricultural activities.	314.29	3.143	0.66	5.0043*
I believe that application of digital platforms system means inviting unwanted interference in developmental efforts.	178.57	1.786	0.375	5.2318
I think that Farmer Portal, Internet Sathi and Digital Green are key systems to create awareness among farmers.	352.38	3.524	0.74	3.2863
I believe that Kisan Choupal helps in motivating people for productive work.	357.14	3.571	0.75	1.6329
I think bringing digital platforms in developing efforts is an impractical step.	228.57	2.286	0.48	8.5732*
I feel that digital platforms in the agricultural profession provide a chance to explore agricultural information from any location.	342.86	3.429	0.72	2.2777
I think digital platforms provide a chance to explore agricultural information round the clock.	359.52	3.595	0.755	3.2796
I think that applications of digital platforms are an effective way of managing agricultural labours.	297.62	2.976	0.625	6.6779*
I think that digital platforms help in knowing the availability of labours for agriculture efficiently.	269.05	2.690	0.565	4.1631*
I believe that Community Radio Station (CRS) helps in motivating people for productive work.	328.57	3.286	0.69	0.3611
I feel that applications of digital platforms help remarkably in connecting stakeholders with developmental efforts.	340.48	3.405	0.715	4.001*
I think digital platforms help farmers in trade agreements.	330.95	3.310	0.695	2.7529
I believe that digital platforms is an incredible resource for exchanging information.	347.62	3.476	0.73	1.1671
I believe that digital platforms help in motivating people for productive work.	323.81	3.238	0.68	5.6568*
Digital platforms provide possible solutions to the present agricultural situation.	333.33	3.333	0.7	5.0931*
Farmer Portal and IFFCO Kisan assist the farmer in planning and decision-making aspects of agriculture.	328.57	3.286	0.69	4.0931
I feel that Bihar Krishi App in the agricultural profession provides a chance to explore agricultural information from any location.	345.24	3.452	0.725	3.3796
All kinds of information exchange are possible only through digital platforms	269.05	2.690	0.565	4.8373*
Only resourceful farmers can get the benefit of the digital platforms	283.33	2.833	0.595	4.5392*
Digital platforms alone would solve the problems of farmers.	238.10	2.381	0.5	6.1263*
Bihar Krishi App alone would solve the problems of farmers.	216.67	2.167	0.455	2.9405
Plantix application Pest/disease outbreak warning system facilitates farmers to take preventive measures.	307.14	3.071	0.645	4.1602
Digital platforms avoid personal extension contact.	292.86	2.929	0.615	2.1287
Digital platforms provide a new opportunity to build skills and knowledge to the agriculture community.	328.57	3.286	0.69	4.3817*
Kisan Gyan Rath provides a new opportunity to build skills and knowledge about soil testing in the agriculture community.	323.81	3.238	0.68	5.4*
Digital platforms is a valuable tool, but it will never influence farmers' own decision-making.	261.90	2.619	0.55	4.0193
Weather forecasting through digital platforms assists farmers in timely decisions.	335.71	3.357	0.705	2.5455
Digital platforms are alternatives to the present extension system.	288.10	2.881	0.605	7.129
Digital platforms provide practical-oriented information.	302.38	3.024	0.635	4.0931
Bihar Krishi App can be accessed anywhere.	314.29	3.143	0.66	1.8973
Digital tools and services increase the confidence level of the farmers.	316.67	3.167	0.665	1.5491
Digital tools and services provide information very quickly.	364.29	3.643	0.765	1.8973
Facebook information helps in boosting up agriculture production.	292.86	2.929	0.615	1.1274
Whats App does not provide information related to regional agricultural problems.	216.67	2.167	0.455	0.6622
Information provided in Kiosks is difficult to understand.	211.90	2.119	0.445	2.6111
YouTube helps in better learning the skill of new agriculture technology.	359.52	3.595	0.755	1.8973
Agriculture information communicated through Krishi Darshan (DD Kisan) is understood even by an ordinary farmer.	345.24	3.452	0.725	2.5455
Whatever is communicated through Kisan Call Centre can be put into action by farmers easily.	314.29	3.143	0.66	1.5787
Digital platforms and services bring the people of remote areas into the national mainstream.	314.29	3.143	0.66	4.4387*
There is a lack of need-based information in different applications related to agriculture for different farming situations.	290.48	2.905	0.61	3.9336

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Statements	RP	RW	MRS	t-Value
The Internet provides the latest technical and scientific know-how about farming.	333.33	3.333	0.7	3.1305
e-NAM help in the decision-making process of farmers.	292.86	2.929	0.615	5.2318*
Television acts as a nerve centre, which transfers the information about plans and policies of Govt. to the farmers.	323.81	3.238	0.68	1.524
All kinds of information exchange are possible only through digital platforms (-)	211.90	2.119	0.445	5.3824*
Only resourceful farmers can get the benefit of digital platforms (-)	269.05	2.690	0.565	2.6111
Digital platforms in agriculture have improved the social status of the farmer.	292.86	2.929	0.615	3.638
Farmers' feedback is fast through mobile phone-based ICTs than through traditional methods	347.62	3.476	0.73	1.8973

*15 Statements which were selected for the study (Sl. No. 4. 8, 11, 12, 14, 17, 18, 21, 22, 23, 27, 28, 42, 45 & 47); RP- Relevancy Percentage, RW- Relevancy Weightage, MRS- Mean Relevancy Score, (-) Negative statements

be helpful for the policymakers and administrators to analyze and understand rural youth's behaviour and to develop coping strategies for attracting and retaining rural youth in agriculture.

CONCLUSION

This study aims at constructing a scale to measure the attitude of farmers towards the use of digital tools in agriculture. The attitude scale consists of 15 statements, with high reliability, and more predictive validity. This scale can be used in future studies on perceptions and feeling about the farmers towards the use of digital tools in agricultural production. It will be helpful to the policymakers and administrators to develop suitable strategies towards the use of digital tools by knowing the attitude of farmers towards Digital technologies.

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Research Article

Comparative economic analysis of tomato cultivation under sprinkler irrigation system in southern Haryana

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ABSTRACT

In the present paper, an attempt has been made to study the comparative economic analysis of tomato cultivation under a sprinkler irrigation system in southern Haryana using the primary data from 35 randomly selected farmers. A multistage random sampling technique was used for the selection of sample farmers. Two districts *viz*., Bhiwani and Rewari were selected for the study. From each district, two blocks namely Tosham and Loharu from Bhiwani district and Khol at Rewari and Nahar from Rewari districts were chosen further. The primary data were collected by personal interviews of the selected farmers with the help of a specially designed schedule. Simple statistical tools like averages, percentages, and Benefit-Cost ratio (BC ratio) were used to compare and interpret the results properly. The total variable cost incurred for the cultivation of tomato accounted for ₹ 140203.20 and ₹ 144476.20 in Rewari and Bhiwani districts, respectively. The results also showed that the total cost incurred in Rewari district (₹ 217714.10 ha^{-1}) was lower than the cost incurred by Bhiwani district farmers (₹ 238229.80 ha^{-1}). Net returns received were higher in Rewari district (₹ 325393.00) as compared to Bhiwani district (₹ 295486.90) because of the high returns per hectare in Rewari district. The BC ratio over total cost in Rewari, Bhiwani, and overall were 2.49, 2.24, and 2.36, respectively.

***Keywords*:** BC ratio, comparative economic analysis, sprinkler irrigation, tomato.

Sprinkler irrigation is a method of irrigation for efficient use of water in crops. A sprinkler irrigation system allows the application of water under high pressure with the help of a motor. It emits water similar to rainfall through a small diameter nozzle placed in the pipes. Water is distributed through a network of pipes, sprayed into the air, and irrigates most of the soil types due to the wide range of discharge capacity. Initially, it was used in plantation crops such as tea and coffee but could not become popular due to the availability of water in large quantities. Recently, the realization of the need for effective utilization of water and the declining groundwater table has made sprinkler irrigation more popular. Closely grown crops such as millets, pulses, wheat, sugarcane, groundnut, cotton, vegetables, fruits, flowers, spices, and condiments have been found to be suitable to cultivate under sprinkler irrigation.

Vegetables play an important role in the agricultural economy and nutritional security of the country due to their short lifespan, high output, nutritional diversity, economic viability, and potential to generate both on- and off-farm employment. India continues to be the second largest producer of vegetables in the world next to China with a total production of approximately 200 million metric tons in the fiscal year 2022 (Anonymous, 2022a). Among the states in India, during this period Uttar Pradesh produced the largest share of vegetables, accounting for 14.8 per cent, and West Bengal came in second with a share of 14 per cent (Anonymous, 2022c). India's diverse climate also ensures the availability of all varieties of fresh vegetables. As per National Horticulture Database (Second Advance Estimates) published by National Horticulture Board, India produced 200.45 million metric tonnes of vegetables during 2020-21 (Anonymous, 2022d). The area under cultivation of vegetables stood at 10.86 million hectares. Major vegetables grown in India are Potato, Onion, Tomato, Cauliflower, Cabbage, Beans, Egg Plants, Cucumber, Frozen Peas, Garlic and Okra.

Tomato (*Lycopersicon esculentum* Mill.) is a significant Solanaceae family crop. It is also known as "Love Apple". Tomato is a rich source of vitamins A and C, minerals, potassium, and fibres. Tomato is one of the most important protective food crops in India. It originated from locals of tropical America. In the sixteenth century, it began to spread to other parts of the world, and in the last nine decades, it has been widely accepted. It is the world's biggest vegetable crop, cultivated for its juicy fruit. It is considered as an important business nutritional vegetable crop.

The area, production, and productivity of tomato in India were about 831 thousand hectares, 20300 thousand

metric tonnes, and 24.42 metric tonnes per hectare, respectively, as per the first advance estimates of the year 2021-22 (Anonymous, 2021b). India is the second largest vegetable producer in the world (Anonymous, 2022d). The major tomato-producing states in the country are Madhya Pradesh, Andhra Pradesh, Karnataka, Tamil Nadu, and Orissa with production of 2970.00 (14.63%), 2217.00 (10.92%), 2077.00 (10.23%), 1489.03 (7.34%), and 1432.29 (7.06%) thousand metric tonnes, respectively during 2021-22 (Anonymous, 2022e). Haryana ranked fourteen in position with the production of 440.00 thousand metric tonnes accounting for 2.17 per cent share of the country (Anonymous, 2022e).

MATERIALS AND METHODS

The multistage sampling design was adopted in the selection of districts, blocks, villages, and tomato growers. In the first stage, Bhiwani and Rewari districts of Haryana state were selected purposively for the study, on the basis of the high rate of adoption of sprinklers due to scarcity of water. From each district, two blocks with the highest number of sprinklers were purposively chosen for study. From the selected blocks in each district, a list of all the villages in a block where the sprinkler irrigation system was used by the farmers was prepared separately and two villages from each block were selected randomly for further sampling. A total of 35 tomato growers were selected for the study. For the collection of information from farmers, a well-structured interview schedule was prepared after detailed discussion with progressive farmers, development officials, and scientists working in various departments of the University. The relevant information pertaining to cropping pattern, source of irrigation, inputs used, output attained, prices of inputs and output as well as factors that constrain the production of tomato for the year 2021-22 was extracted through interaction with selected farmers. Analytical techniques like average, percentage, costs, returns, Benefit-Cost ratio (BC ratio) *etc.* were employed to draw valid inferences from the study.

RESULTS AND DISCUSSION

Comparative economics of tomato cultivation under sprinkler irrigation system in Bhiwani and Rewari districts

Comparative economic analysis of tomato cultivation under sprinkler irrigation in selected districts was made on a per-hectare basis. The cost and returns of tomato cultivation under Rewari and Bhiwani districts of Haryana are presented in Table 1. As shown in Table 1 total cost in Rewari district was worked out to be ₹ 217714.10. The cost structure of the variable cost in Rewari district also showed that the highest proportion amounting to ₹ 25880.95 (11.88%) was spent on harvesting followed by plant protection, irrigation, loading and unloading charges, fertilizers and manure with

Table 1: Comparative economics of tomato crop grown under sprinkler irrigation system in southern Haryana (₹ /ha).

Particulars	Rewari district			Bhiwani district			Overall Average		
	No./Qty	Value	Per cent	No./Qty	Value	Per cent	No./Qty	Value	Per cent
Preparatory tillage	4.50	6428.57	2.95	4.60	6785.00	2.84	4.55	6606.78	2.89
Pre sowing irrigation	-	1154.28	0.53	-	1137.50	0.47	-	1145.89	0.50
Nursery raising & Transplanting	-	5714.28	2.62	-	5133.33	2.15	-	5423.80	2.37
Bed preparation	-	1815.47	0.83	-	1908.33	0.80	-	1861.90	0.81
Seed (gram)	114.00	5660.71	2.60	86.33	5465.00	2.29	100.16	5562.85	2.44
Total Fertilizer Investment	402.05	13913.70	6.39	423.68	14680.00	6.16	412.86	14296.85	6.27
Irrigation	15.80	18173.81	8.34	16.25	18636.67	7.82	16.02	18405.24	8.07
Hoeing/Weeding	3.15	11928.57	5.47	3.33	13116.67	5.50	3.24	12522.62	5.49
Plant Protection	8.55	22753.57	10.45	10.20	24704.50	10.37	9.37	23729.04	10.40
Harvesting	-	25880.95	11.88	-	26150.00	10.97	-	26015.48	11.41
Loading & Unloading Charges	-	17607.14	8.08	-	17307.50	7.26	-	17457.32	7.65
Interest on working capital @ 7%	-	9172.17	4.21	-	9451.71	3.96	-	9311.94	4.08
Total Variable Cost	-	140203.20	64.39	-	144476.20	60.64	-	142339.70	62.43
Management Charges	-	14020.32	6.43	-	14447.62	6.06	-	14233.97	6.24
Risk factors	-	14020.32	6.43	-	14447.62	6.06	-	14233.97	6.24
Transportation charges	-	21494.05	9.87	-	34775.00	14.59	-	28134.53	12.34
The rental value of land	-	27976.19	12.84	-	30083.33	12.62	-	29029.76	12.73
Total costs	-	217714.10	100	-	238229.80	100	-	227971.90	100
Production (q)	271.75	543107.10	-	261.50	533716.70	-	266.62	538411.90	-
Return over variable cost	-	402903.90	-	-	389240.50	-	-	396072.20	-
Net return	-	325393.00	-	-	295486.90	-	-	310440.00	-
Cost of production	-	801.15	-	-	911.01	-	-	856.08	-
BC ratio	-	2.49	-	-	2.24	-	-	2.36	-

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₹ 22753.57 (10.45%), ₹ 18173.81 (8.34%), ₹ 17607.14 (8.08%), ₹ 13913.70 (6.39%), respectively. Likewise, principal components of fixed cost in decreasing order are the rental value of land, transportation charges, management charges, and risk factors accounted for ₹ 27976.19 (12.84%), ₹ 21494.05 (9.87%), ₹ 14020.32 (6.43%) and ₹ 14020.32 (6.43%), respectively. In the case of Bhiwani district total cost was observed to be ₹ 238229.80. Principal components of variable cost in decreasing order are harvesting cost, plant protection, irrigation, loading and unloading charges accounting for ₹ 26150.00 (10.97%), ₹ 24704.50 (10.37%), ₹ 18636.67 (7.82%) and ₹ 17307.50 (7.26%), respectively. Similarly, principal components of fixed cost in decreasing order are transportation charges, rental value of land, management charges, and risk factors accounted for ₹ 34775.62 (14.59%), ₹ 30083.33 (12.62%), ₹ 14447.62 (6.06%) and ₹ 14447.62 (6.06%), respectively. It is clear from the results that the total variable cost in Bhiwani district was higher as compared to Rewari district. One of the reasons for the high variable cost in Bhiwani district is more quantity (10.20) of plant protection applied in the area as compared to Rewari district (8.55). The results also showed that the total cost incurred in Rewari district (₹ 217714.10 ha^{-1}) was lower than the cost incurred by Bhiwani farmers (₹ 238229.80 ha^{-1}). The reason behind this is the high transportation charges (₹ 34775.00) in Bhiwani district as compared to Rewari district (₹ 21494.05). In the study area of Bhiwani, most of the farmers are selling their produce to Chandigarh market for which the transportation charge was ₹ 55-60 $crate^{-1}$ whereas Rewari farmers are selling their produce in Gurugram and Rewari market for which the transport charge was ₹ 35 and ₹ 15 $crate^{-1}$, respectively. The results showed that higher production was obtained in Rewari district (271.75 q ha^{-1}) as compared to Bhiwani district (261.50 q ha^{-1}). The overall average for both the districts for variable cost and total cost was observed to be ₹ 142339.70 and ₹ 227971.90. Net returns were received to be higher in Rewari district (₹ 325393.00) as compared to Bhiwani district (₹ 295486.90) because of the high returns per hectare in Rewari district. As a result, the benefit-cost ratio (BC ratio) was also to be obtained higher in the case of Rewari farmers compared to Bhiwani farmers *i.e.*, 2.49 and 2.24, respectively exhibiting that sprinkler irrigation systems were observed beneficial in tomato cultivation in the study area.

Similar observations were made by Sunita (2014) in her study on sprinkler and surface method of irrigation in Jhunjhunu district of Rajasthan. The economic analysis of tomato production were made by Kumar *et al*. (2016) under poly house and open field conditions, and Gaikwad *et al*. (2020) during *kharif* season in Nashik district of Maharashtra state.

CONCLUSION

The study concluded that in comparative economic analysis, in the case of variable cost, maximum difference was observed for plant protection and weeding charges, both of which are higher in Bhiwani district. The total cost of tomato cultivation in Bhiwani district was higher as compared to Rewari district. The major reason behind it was the high transportation charges in Bhiwani district. Farmers realized a higher yield of tomato in Rewari district as compared to Bhiwani district. The returns over variable cost, net return, and BC ratio were also higher (₹ 402903.90, ₹ 325393.00, and 2.49, respectively) in the case of Rewari district as compared to Bhiwani district (₹ 389240.50, ₹ 295486.90, and 2.24, respectively).

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