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Registered under Society Registration Act 21, 1860
Main office : A-601, Indira Nagar, Lucknow - 226 016, India
www.ecoagrijournal.com
E-mail : ecofriendlyagriculture@gmail.com
ecofriendlyapproaches@gmail.com

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REVIEW

Insect growth regulators: practical use, limitations and future

Satnam Singh, Suneet Pandher, Rakesh Kumar Sharma[1] and Rajinder Kumar[2]

Punjab Agricultural University, Regional Station, Faridkot - 151 203, India
[1] PAU Regional Station, Ballowalsaunkri, Punjab, [2]Punjab Agricultural University, Ludhiana
E-mail: satnam@pau.edu

ABSTRACT

Compounds interfering with normal growth and development of insects are categorized under the group IGRs. The group includes moulting hormone analogues, anti moulting hormone analogues, juvenile hormone analogues, anti juvenile hormone analogues, insect neuropeptides, chitin synthesis inhibitors, chitin degradation inhibitors and sclerotization inhibitors. Exogenous applications of MHAs (Mimic®, Interprid®, Mach®, Confirm®) lead to increased titre of ecdysone which can not be metabolized or excreted rapidly to prevent hormonal imbalance resulting in moulting promotion and consequent death of insects but due to their hydrophilic nature they cannot penetrate insect cuticle, thus effective only upon digestion. JH and its analogues (Altosid®, Enstar®, Insegar®, GenTrol®, Mator®, Kabat®, Admiral®, Logic® and Aware®) result in deranged development and several deformities such as supernumerary larvae, larval-pupal, larval-adult, pupal-adult intermediates and adultoides however, low persistence and stage specificity are limiting their field use. CSIs (Applaud®, Consult®, Match®, Nomolt®, and Baycidal®) act by inhibiting chitin synthetase and cause moulting aberrations associated with several morphological abnormalities. Neuropeptides are safe and selective compounds for control of insect pests, but their heat labile nature, costlier synthesis, inability to penetrate the insect cuticle are the major constraints in their use. Alternatively, the neuropeptides genes along with chitinase gene could be the potential candidates for designing the effective biopesticides (entomopathogenic bacteria, viruses, nematodes, etc.) through genetic engineering.

Key words: Moulting hormone analogues, anti moulting hormone analogues, juvenile hormone analogues, anti juvenile hormone analogues, chitin synthesis inhibitors, insect neuropeptides, insect-pest management

It was in 1962 that the Rachel Carson's famous book "Silent Springs" acquainted the world with the ill effects of synthetic insecticides such as DDT and soon after, an intensive search for the alternate methods or more precisely eco-friendly methods of insect-pest control followed. Insects differ from vertebrates w.r.t. three respects i.e. endocrine system, structure of integument and communication system, which can be manipulated for pest management. Based on endocrine system and integument, a new class of comparatively safe insecticides came into existence known as insect growth regulators (IGR's) (a name familiar to plant growth regulators (PGRs) that influence plant growth and phenology) or bio-rational insecticides or 3rd generation insecticides and defined as "compounds interfering with normal growth and development of insects". Based on the endocrine system and insect cuticle, the IGRs have been classified as follows:

Endocrine System

Juvenile Hormone Analogues (JHAs), Anti Juvenile Hormone Analogues (AJHAs), Moulting Hormone Analogues (MHAs), Antimoulting Hormone Analogues (AMHAs) and insect neuropeptides

Insect cuticle

Chitin Synthesis Inhibitors (CSIs), chitin degradation inhibitors, and sclerotization inhibitors. JHAs and CSIs are the most exploited commercially followed by MHAs, but the work on the other IGRs is still in infancy.

IGRS based on endocrine system

The insect endocrine system chiefly consists of a group of neurosecretory cells in the brain which are linked to the corpus cardiacum by a network of nerves called the nervi corpori cardiacum I (NCC I) and NCC II. The neurosecretory cells of the brain secrete the brain hormone or PTTH (prothoracico trophic hormone) or activating hormone or neurohormone which in turn activates the PTG (prothoracic gland) to produce MH (moulting hormone). This hormone initiates the process of moulting by increasing the level of 20 E (20 hydroxyecdysone) in haemolymph with the result, insect stops feeding and apolysis (separation of epidermis from the old cuticle) occurs. The layer between the old cuticle and the epidermis is filled with moulting fluid, which contains enzymes in inactive form responsible for the digestion of old cuticle. With the decrease in the 20 E titer the enzymes in the moulting fluid get activated and the digestion of procuticle takes place. This is followed by release of

eclosion hormone, triggering ecdysis (shedding of old cuticle) and completion of molting cycle. The type of moult is determined by another hormone secreted by the activated corporus allatum known as juvenile hormone (JH) or neotenin or statusquo hormone. The earlier concept of quantitative effect i.e. if the JH titer is high, the larva will moult into larva; if low, then larva into pupa and virtual absence of JH molts pupa into adult, was disproved and replaced with qualitative developmental programming and stressed that the genome of each cell receives individually the message of either being activated by JH or not (Sláma, 1999). Thus, the exogenous application of these hormones can upset the insect homeostasis resulting in physiological disruption and consequently death of insect. Additionally, their highly specific nature holds promise for utilization for insect control (Bowers, 1971).

Ecdysteroids or Moulting Hormone Analogues (MHAS)

The non steroidal synthetic analogues of moulting hormone (MH) or ecdysone are known as ecdysoids while steroidal ones termed as ecdysteroids (Sláma, 1995). The first ecdysteroid was isolated as a molting hormone (ecdysone) in 1954 by Butenandt and Karlson. Chemically, ecdysoids are bisacylhydrazines. The first commercial MHA was discovered by Rohm and Hass Chemical company in 1983 (Hsu, 1991). Soon after, a simpler analogue RH-5849 was made and used in most of the symptomological studies. It had been effectively tested and used under the field conditions for control of larval Lepidopterans, Coleopterans and Dipterans (Aller and Ramsay, 1988). This was superseded by another cost effective analogue tebufenozide available under the name RH 5992, Mimic®, Confirm® and Romdan® and effective against various Lepidopterous insects such as C*ydia pomonella, Cnaphalocrocis medinalis, Spodoptera exigua, Helcoverpa spp* (Heller *et al.*,1992). Two products, methoxyfenozide (RH 2485, Interprid®) effective against Codling moth, oriental fruit moth and European corn borer and halofenozide were developed by the joint venture of Rohm and Hass and American Cynamide Company. These were similar to tebufenozide but differed in respect that both were plant systemic, thus also effective against the soil dwelling insects. The product halofenozide, coleopteran specific (RH 0345, Mach-2®) has been found effective against white grubs, armyworms, cutworms and commercially recommended for use on turf grass against *Popila japonica* in Australia (RhoMid, 1996). Myers and Hull (2003) reported lepidopteran specific tebufenzoide and methoxyfenozide (MHAs) to be effective in causing the significant reduction in fertility and fecundity of tufted apple moth *Platynota idaeusalis* on apple in USA, while tebufenozide appeared the most potent ecdysteroid agonist against *E.* kuehniella in Algeria (Hami *et al.*, 2005). In spite of great potential in pest control much of commercial exploitation has not been achieved. Besides, some plants carrying phytoecdysteroids have also been known. About 111 families of plants are known to contain 69 phytoecdysteroids (Bergamasco and Horn, 1983). The first ecdysteroids, inkosterone and ecdysterone were isolated from the plant *Achyranthes fauriei* (Nakanshi *et al.,* 1966*)*. Sumitomo company and Mark Research Labs in 1996 isolated two products viz, 3, 5 di-tret butyl 4 hydroxy N isobutyl benzamide (DTBHIB) and 8-0-acetyl harpidge respectively from *Ajuga reptans* which showed moulting hormone activity against *Drosophila*. Some other plant species carrying MHA factors are *Podocarpus nakaii* carrying Ponasterone A, B, C and D (Nakanshi *et al.,* 1966), ecdysone from *Pteridium equilium* (Takemoto *et al.,* 1967) and ecdysterone from *Polypodium vulgarae* (Krick, 1977). Although these analogues showed promise against large number of insect species, yet these are less likely to be effective candidates in pest control due costly and laborious synthesis owing to their complex steroidal structure, inability to penetrate the insect cuticle because of large number of hydroxyl (OH) groups present in their structure, environmental instability and moreover, their close proximity to steroid hormone in higher animals puts a cautious approach on their use.

Lafont and Dinan (2009) have summarized that though ecdysteroids have limited success as pest control agents but their analogues are highly useful for determining the structural and spatial requirements for binding to nuclear and taste receptors of insect pests and also, the elevation of phytoecdysteroid levels in selected plant species could protect crop species against phytophagous pests (insects and nematodes).

Anti Moulting Hormones Analogues (AMHAS)

The AMHAs and AJHAs (antijuvenile hormone analogues) are not the hormones but name given to the compounds antagonizing the action of moulting hormone and juvenile hormones, respectively. Chemically AMHAs are azosterols and non-steroidal compounds; they act by antagonizing the conversion of phytosterols to cholesterol which is required as a base for the synthesis of ecdysone in insects thus the moulting hormone titer is affected and consequently moulting in delayed (Walker and Svoboda, 1973). Commercially these have not been much exploited but some plants carrying these compounds such as ajugalactone from *Ajuga decumbens* (Robbins *et al.,* 1970); ajugarins from *Ajuga remota* (Kubo *et al.,* 1976) have been identified. Azadirachtin from *Azadirachtin indica* acted on the corporus cardiacum of *Locusta migratoria* causing disturbance to ecdysteroid titer (Joshi *et al.,* 1989), while plumbagin from African medicinal shrub *Plumbago capensis*

acted directly on prothoracic gland (PTG) causing inhibition of ecdysone production (Garcia *et al.,* 1990). Arbones *et al.,* (1990) prepared some fluorinated analogues of the imidazole IGR KK-42 which showed ecdysone antagonistic effects against some insects. Such antagonistic effects have been explored in the mealworm, *Tenebrio molitor* where reduced reproduction was observed in the pest (Berghiche *et al.*, 2008). The use of such chemicals for insect control at this stage seems skeptical because of high cost of azosterols and their probable interference with steroid hormone regulation in higher animals (Saxena, 1983). However the discovery of compounds such as plumbagin and azadirachtin is expected to provide impetus for the futuristic development of some effective moulting hormone antagonists.

Juvenile Hormone Analogues (JHAS)

In 1956, Prof. C. M Williams for the first time isolated "golden oil" from giant silk moth *Hyalophora cecropia* (Linn) and called it juvenile hormone, when this was injected in the pupae of silk worm it induced the formation of second pupae and inhibited metamorphosis. In 1965 Prof. C M Williams and Prof. Karl Sláma working in a laboratory at Harvard observed that the paper from the balsam fir tree *Abes balsemia* used for the rearing of hemipteran bug *Pyrrhocoris apterus* prevented its metamorphosis. This was known by the name "paper factor" world wide and the compound was later identified as juvabione. Foreseeing the great potential of these compounds in pest control, Prof. C M William designated them as "Third generation pesticides". Six naturally occurring JHs are reported to be present: the JHO, JH I, JH II and 4 methyl JH II occur only in Lepidoptera whereas the JH III occurs in non- Lepidopterous insects except higher Diptera where it is replaced by JH III-bisepoxide (Dhadialla *et al.,*1998). The first commercial analogue of JH, methoprene was registered in USA in 1973 for mosquito control and in UK in 1980 for pharaoh ant control. The synthetic analogues of JH are known as juvenoids which cause morphogenetic, gonadotrophic and diapause disrupting effects in insects. Morphogenetic control is ideally suited for controlling insects that are pests as adults. Morphogenetic effects include deranged development, supernumerary larvae, larval-pupal, larval-adult and pupal adult intermediates, adultoides. Small amount of JH is required for the ovarian maturation or vitellogenesis but the exogeneous application of higher JH titer on adult females renders their eggs unviable and thus eggs fail to hatch, if somehow they hatch the effect is manifested in the last larval instar which is unable to metamorphose into adult. Exogenous application of JH on the diapausing pupae activates prothoracic gland and prevents it from entering into diapause thus disrupting the insect hemostasis (Retnakaran *et al.,* 1985). JH suppresses the expression of some of the antibacterial peptides that are involved in the suppression of bacterial infection, lowers the sensitivity of plasmatocytes to an insect cytokine, plasmatocyte-spreading peptide in addition to regulation of other transformative processes such as migratory behavior, wing length, color polymorphism and caste determination (Palli, 2009). Above all, the timing of application of JHAs is crucial for insect pest management.

Some authors classify JHAs broadly into two groups: the terpenoid JHAs such as methoprene and kinoprene and the phenoxy JHAs (fenoxycarb and pyriproxyfen). Methoprene is available in various trade names *viz*, Altosid® which is a food additive and passes through the animal digestive system with such strong integrity that it suppresses the development of mosquito maggots in the cattle dung; Kabat® is used against stored tobacco insects; Apex® against sciarid flies of mushroom; Precor® against pharaoh ants and Minex® against fruit and vegetable pests. Hydroprene (Gentrol®, Mator®) used against cockroaches acts by causing sterility and pyriproxyfen (Admiral®, Knack®, Esteem®, Arc®) is effective only under green house conditions due to lack of environmental stability . Fenoxycarb is available as Logic® for fire ants, Varikill® for mosquitoes, Insegar® commercially recommended for fruits pests in Switzerland and Italy, Torus® for fleas and Pictly® for cockroaches. The compound kinoprene (Enstar II®) is effective against aphids, scales, whitefly and mealy bugs and diofenolan (Context®, Aware®) against scale insects, eggs of Lepidoptera and ornamental insect pests (Dhadialla *et al.,* 1998; Retnakaran *et al.,* 1985).

JHAs against vegetable and fruit insect pests

Foliar spray of R-20458 @ 10ml/insect and 15ml/insect on cauliflower leaves resulted in 60 and 100 per cent mortality of *Spodoptera litura* (Singh and Singh, 1988). Singh and Sidhu (1990) reported toxic effects and abnormal morphogenesis with foliar spray hydroprene and R-24058 on *Lipaphis erysimi* on cabbage. Pyriproxyfen @ 40 ppm (@ 150 l ha^{-1}) on cabbage resulted in 90 per cent mortality of *Myzus persicae* and significant increase in yield over control (Hatakoshi *et al.,*1991). Pyriproxyfen (0.05%) at 45 days interval gave effective control of citrus whitefly *Dialeurodes citri* (Golkes *et al.,* 1990). Fenoxycarb (Insegar® 25WP) @ 0.8kg a.i ha^{-1} resulted in reduction of damage by tufted applemoth *Platynota idaeusalis* to 8.1 per cent as compared to control. Pyriproxyfen (Tiger®) kept the *A. aurantii* population below the ETL level on citrus (Peleg, 1994) and could cause direct mortality, reduce longevity and inhibit progeny production of onion thrips, *Thrips tabaci* under laboratory conditions (Liu, 2003). In addition, treating mass-reared male fruit flies with methoprene along with protein diet improved the

efficacy of SIT for a number of pest fruit flies (Pereira *et al.*, 2009).

JHAs against stored grain and household insect pests

The first JHA, methoprene, was used primarily against household pests because of its low activity against agricultural pests and low residual on plants under field conditions. Fenoxycarb @ 10 and 5mg kg^{-1} provided 48 weeks protection against lesser grain borer *Rhizopertha dominica* on maize and paddy, respectively (Semson *et al.*, 1990). ZR-777 @ 5mg/female resulted in 11 fold decrease in fecundity of red rust flour beetle *Tribolium castaneum* from 45.62 to 4.12 eggs/female along with the egg hatching inhibition (Singh, 1994). Pyriproxyfen @ 91.9 mg kg^{-1} resulted in suppression of adult emergence of *Callosobruchus maculatus* and 100 per cent control on stored cowpea seeds for 8 months. Hydroprene (Pointsource®) resulted in arrested larval growth, morphological deformities, incomplete emergence and twisted wings of *Tribolium castaneum* and *T. confusum* (Arthur, 2003). Fenoxycarb was able to cause morphogenetic aberrations in *Cluex pipiens* (Grenier and Grenier, 1993). Pyriproxyfen (0.1%) resulted in 85 per cent deformed males and females along with significant reduction in the progeny of mole cricket *Scaptericus abbreviatues* (Perkman and Frank, 1998). Bait composed of peanut oil, lolco blue, corn grit and pyriproxyfen (0.5%) used against pharaoh ants *Monomorium pharonis* led to 73 per cent reduction in worker population and 31-49 per cent reduction in queen population 8 weeks after treatment. The IGR did not kill the worker rapidly helping in the distribution of bait to other colonies. Carbamate juvenoid W28 @ 10 and 20mg/female against ovoviviparous cockroach *Blaberus craniifer* resulted in reduction of mating success and complete sterilization, respectively. Miller and Neck (2004) evaluated two methods for the control of German Cockroach *Blatella germanica,* first (TBBC) traditional base board cark and cervix with the insecticidal formulation and the second was IGR based, comprising of first vacuuming the units and then application of hydroprene. The population remained less than 5/unit for 8 months declining from 24.7/unit to 3.9/unit with IGR which was bit costly (4.06$/unit) as compared to the TBCC (1.50$/unit) where it remained steady through out the period.

In addition to the discussed synthetic JHAs, a large number has been identified in a number of plant spp. Juvocimene I and II isolated from sweet basil *Ocimum basilicum* and dihydroisanidiane from *Piper reticulatum* showed JH activity against milkweed bug *Oncopeltus* fasciatus (Bowers and Nishida 1980). Roots of the American coneflower, *Echinacea angustifolia* carry echinolone which exhibit JH activity against *O. fasciatus* and *Tgenebrio molitor* (Jacobson *et al.,* 1975). Crude extract of *Nama rothrockii* and *N. sandwicense* possess JH activity against *O. fasciatus* resulting in formation of supernumerary instar (Binder *et al.,* 1991). The compounds like, karanjin from *Pongamia glabra* possesses JH activity against *Lipaphis erysimi*, rhodojaponin III from *Rhododendron* spp against *Pieris rapae* (Zhong *et al.,* 2001) and sujiol from *Juniperus communis* against *Spodoptera litura* (Vardhini *et al.,* 2001).

Although the JHAs cannot provide solution to every pest problem but they do have some advantages over the conventional insecticides such as, simple structure which makes their synthesis easy and less costly, highly specific thus low mammalian toxicity and no threat to the non-target, non-persistent and lesser chances of development of cross resistance due to their novel mode of action. Balanced against these potential advantages some disadvantages are slow mode of action, stage specificity thus damage due to the immature stages cannot be prevented, lack of environmental stability and some reports of development of resistance to these compounds (Ma *et al.*, 2010). The discovery of new UV-stable JHAs such a pyriproxyfen and fenoxycarb has opened the way for the futuristic development of juvenoids as main stream crop protection agents.

Anti Juvenile Hormone Compounds (AJHA)

These are known as JH antagonists and are classified on the basis of mode of action as compounds which inhibit the early steps of JH biosynthesis *viz,* compactin, fluoromevalonate (Fmev), allylic compounds, ETB (ethyl 4-[2-(tret-butylcarboxyloxy) butoxy]-benzoate) and imidazoles. The second category include the compound which inhibit the later steps of JH biosynthesis *viz,* piperonyl butaoxide and the third category included the allotocidal compounds *viz*, precocenes and EMD [ethyl (E)-3-methyl-2-dodecenoate]. From practical point of view, chromene derivatives, precocenes are the most important as these compounds are oxidized within insect body into highly reactive metabolities which interfere at cellular level resulting in destruction of cells of corpus allatum gland, responsible for secretion of JH (Unnithan *et al.*, 1980). Bowers *et al.,* (1976) were the first to isolate two chromenes namely, 7 methoxy 2, 2–dimethylchromene and 6, 7 dimethoxy –2, 2 dimethylchromene from the extracts of common bedding plant, *Ageratum houstonianum* and were later designated at precocene I and precocene II, respectively. These were described as "fourth generation insecticides" due to their vast potential in insect-pest control. Later on Fagoonee and Umrit (1981) isolated Precocene III from the plant of same genera *A. conyzoides*. The first plant to possess compounds having both JH and AJH activity was *Nama sandwicense* causing nymphs of *O. fasciatus* to mature precociously to diminutive sterile adultoides and formation of

supernumerary instar. Precocenes induced variety of physiological and behavioural changes including precocious metamorphosis of the immature stages, sterilization of adult females, diapause induction and inhibition of sex pheromone production (Bowers, 1983). Apart from their AJH effects on insects, precocenes have also been observed to induce JH type effects in *Locusta migratoria migratorioides* (Miall and Mordue, 1980), green peach aphid *Myzus persicae* (Mitler and Hales, 1984) and brown plant hopper, *Nilaparvata lugens* (Pardeep and Nair, 1989). Some advantages of AJHAs which foresees their wide scope in insect–pest management are shortening life cycle of immature stages which results in diminished feeding, precocious males unable to mate with normal females, precocious sterile females and the most important is that they are effective against different developmental stages under field conditions. But some major limitations in their commercialization are the limited specificity of precocenes for hemimetabolus insects, identification of the plant species carrying these compounds and their synthesis.

Insect neuropeptides

The endocrine activity in nerve cells was demonstrated by Kopeck in 1922 and this for the first time proved in the animal kingdom that the nerve tissues produce hormone. Insect neuropeptides have diverse physiological roles in insects, such as diuresis, ecdysis, pheromone biosynthesis and control of muscle activity. The first neuropeptide proctolin was isolated by Staratt and Brown in 1975, 180g of neuropeptide was isolated from 12 kg of *Periplaneta americana*. At present 40 neuropeptides are known but none has been commercialized as insect control agents due to poor bioavailability, pharmacokinetics and short half-life (Scherkenbeck and Zdobinsky 2009). Some of the major neuropeptides characterized and their role has been described by Gade and Gharam (2003) (Table 1). The peptide code mentioned is on the basis of the insect from which it has been isolated e.g. Aedae TMOF (trypsin modulating oosatic factor), the first three letters (Aed) are taken from generic name *Aedes* and last two letters (ae) from the first two letters of species name *aegypti*. TMOF from grey flesh fly *Neobelleria bullae* and *Aedes aegypti* is responsible for inhibition of trypsin biosynthesis thus arresting the egg development.

If the genes encoding these neuropeptides could be expressed in entomopathogenic virus, bacteria, fungi and nematodes, this would affect the titer of neuropeptides in infected insects resulting in physiological disruption, thus increasing the efficiency of the entomopathogens. These neuropeptides have not been commercially exploited but several major advances in research on different aspects have

Table 1. Some major insect neuropeptides characterized and their role

Peptide name	Peptide code	Role
TMOF	Aedae TMOF Neobu TMOF	Inhibition of trypsin biosynthesis
AKH	Locmi-AKH- I, II, III	Mobilization of energy substrates
DH	Manse-DH	Water balance
PBAN	Helze-PBAN	Stimulation of pheromone synthesis
Allatosin A,B,C type	Dippu-AST1, Grybi-B1, Manse-AST	Control of JH synthesis
Diapause hormone	Bommo-DH	Diapause regulation

(Gade and Gharam, 2003)

been carried out e.g. a diuretic hormone gene from *Manduca sexta* when expressed in *Bombyx mori* NPV and tested on the same insect, it targeted its malphigian tubules and resulted in 20 per cent reduction in the ST_{50} (the time for which the 50 per cent population survives when infected with virus) as compared to the infection with wild type virus (Maeda, 1989). An another gene PBAN (pheromone biosynthesis anti neuropeptide) from *Helicoverpa zea* when incorporated into *Autographa californica* MNPV and tested on host *Tricopulsia ni* resulted in 19-26 per cent reduction in the ST_{50} value as compared to the infection by the wild type virus. Similary modified JHE, Juvenile hormone esterase gene from *Manduca sexta,* modified by site directed mutagenesis (JHE is a stable protein but pharmokinetic studies reveal that half life of JHE in haemolymph of *M. sexta* is only one day and is readily removed by pericardial cells, so the lysine from position 29 and 522 has been replaced by arginine residues and the two types of JHE obtained are JHE 29 and JHE 522 respectively. The JHE with both the position replaced with arginine is known as JHE KK or modified JHE), when incorporated in AcMNPV and tested against *M. sexta* resulted in 66 per cent reduction in feeding damage as compared to the infection by the wild type virus (Bonning and Hammock, 1997).The major limitations of these neuropeptides which hinders in their commercialization are photo instability, inability to penetrate insect cuticle and heat liable nature due to their proteinacious nature. But they have a vast future, by developing peptidomimetics which means processing enzyme antagonists and agonists that can penetrate insect cuticle and block or over stimulate the peptide synthesis at cell level, incorporation of neuropeptides and antineuropeptide genes in entomopathogenic microorganisms or host plants and development of field stable formulations. Exploiting insect neuropeptides for pest management needs thorough

knowledge of the cellular and molecular basis of their actions besides well defined methods for obtaining antagonists on the basis of a known neuropeptide agonist (Altstein and Nässel, 2010). Recently, studies on penetration ability and bioactivity of unmodified linear neuropeptides of four insect neuropeptides of the pyrokinin (PK)/pheromone biosynthesis activating neuropeptide (PBAN) family yielded significant results (Hariton *et al.*, 2009). Another approach based on designing pseudopeptides by substituting some amino acids to render the molecule more stable to peptidase attack and more bioavailable has been introduced (Nachman *et al.*, 2008 & 2009).

Chitin Synthesis Inhibitors (CSIs)

Chitin synthesis inhibitors are the compounds which inhibit the synthesis of chitin by acting on the enzyme chitinase. Chitin formation is carried out by the action of various enzymes whereby monosaccharide glucose is converted into a polysaccharide, â-1-4 acetyl glucosamine units. The target site for the benzoylphenylurea analogs is chitin synthetase blocking the terminal polymerization step catalyzed by the enzyme chitin synthetase during the process of biosynthesis of chitin, thus resulting in no chitin formation and insect dies of desiccation. They also inhibit a number of other enzymes and DNA biosynthesis in larval cells (Mayer *et al.*,1990).The insecticidal activity of benzolphenylurea analogs was discovered by Philip Duphar Company in 1970 and the 1st effective product against insects with code DU 19.111 was made from combination of herbicide dichlobenil and urea herbicide diuron. The first commercial product of such kind was Dimilin® (diflubenzuron) (Retnakaran *et al.*, 1985).

Various commercial formulations of CSIs available under different trade names are being used against wide spectrum of the insect-pests (Table 2). Buprofenzin (Applaud ® and Accolade®) has insecticidal as well as acaricidal action with both stomach and contact poison mode of action and the affected adults lay sterile eggs. Diflubenzuron (Dimilin®, Adept and Micro®) with high environmental stability has been registered along with Flufenoxyuron in India (CIBRC, 2006). Hexflumuron (Sentricon®) has been used to develop the sentricon system for control of termites. Under this system, monitoring stations are placed at different places near the house foundation. Once the termite activity is detected, it is replaced by IGR bait, the termite returns to colony before dying thus recruiting the other termites towards the bait and eliminating the whole colony. Lufenuron (Match®, Program®) is recommend for the control of dog fleas @ one tablet for month at meal time; when flea bites the dog, lufenuron enters the flea system and gets deposited on eggs thus preventing them from hatching.

CSIs for management of vegetable and fruit insect-pests

Chlorfluazuron @ 1.5 l ha^{-1} against potato colardo beetle *Leptinotarsa decemlineata* was as good as OP's and SP's in term of yield (Bykhoverts and Kilienko, 1991). Clift and Ferray (1992) in Australia tried triflumuron and diflubenzuron on mushroom pests *Lycoriella mali* and *Heteropeza pygemaea* and found that both compounds controlled the pests @ 35mg kg^{-1} in casing and 10mg kg^{-1} in compost; in addition triflumuron gave best results when combined with malathion (0.01%). Use of diflubenzuron @ 3mg/kg of casing and cryomizine (chitin growth promoter) @ l0 mg kg^{-1} of casing against mushroom sciarid by *Lycoriella auripila* resulted in 13 per cent increase in yield (White, 1999). Diflubenzuron @ 250 ppm caused 83.3 per cent mortality of cucurbit fly *Dacus culurbitae* (Reddy *et al.*, 1993). Buprofezin was found better than commonly used dimethoate to control *Trialeurodes vaporarium* (Zhong and Ghao, 1994). Buprofezin and Novaluron are being widely used for the control of mealybug and *Spodoptera* in cotton, respectively (Anonymous, 2011). Novaluron gave good results against brinjal fruit and shoot borer by lowering shoot infestation (Anil and Sharma, 2010).

Diflubenzuron against pear spills *Cacopsylla pyricola* resulted in significant reduction in psylla population and cost of application, which was half that of conventional treatment (Booth and Reidl, 1996). Management of citrus psylla *Cacopsylla pyri* for four years with lufenuron and diflubenzuron resulted in significant reduction in psylla population and leaf fall (Polensy, 2001). Lufeneuron proved a better option for the control of Mediterranean fruit fly *Ceratitis capitata* in Spain as compared to malathion and mass trapping, as the Lufenuron acts by causing the sterilization of the flies (Llopis *et al.*, 2002).The lowest average infestation of codling moth *Cydia pomonellla* (2.60%) and increase in yield (59.71kg $tree^{-1}$) of apples was reported by use of cascade 10 DC (flufenoxyuron) as compared to Match 50 EC. Buprofezin gave high level control of citrus mealy bug *Pseudococus affinis* (Malagon *et al.*, 1991). CSIs, novaluron, diflubenzuron, and teflubenzuron have been proved very effective against chilli fruit borer (*S. litura*) and mushroom sciarid fly *Lycoriella ingenua* (Amalendu *et al*, 2008 & Erler *et al.*, 2011). Studies by Zhu *et al.* (2012) suggested that sublethal concentrations of hexaflumuron reduced *S. litura* larval survival and interfered with hemolymph physiological balances.

CSIs for the management of stored grain and veterinary insect-pests

Diflubenzuron (Baysir 8514®) @ 5 ppm against saw toothed grain beetle *Oryzaephillus surinamenses* resulted in 99 per cent egg and 97.5 per cent larval mortality (Parween,

Table 2. Commercially available CSI formulations against different insect pests

Common name	Trade name	Target
Buprofezin	Applaud, Accolade, Tribune	Rice homopterans, vegetable and fruits pests Cotton mealybug
Chlorfluazuron	Atabron, Helix	Lepidoptera and Hooper
Diflubenzuron	Dimilin, Adept, Micro,	Gypsy moth, boll weevil, midges, mosquitoes, citrus and vegetable pests,
Flufenoxuron	Cascade	Phytophagous mites
Fluazuron	Acute	Cattle ticks
Flucycloxuron	Andalin	Spider mites and Diptera
Hexaflumuron	Consult, Sentricon, Truneo, Recruit	Lepidopterans, Coleopterans and termites
Lufenuron	Match, Program	Dog fleas, cotton pests
Novaluron	Rimon	Lepidoptera and Diptera
Teflubenzuron	Nomolt	Lepidoptera and Hemiptera
Triflumuron	Baycidal	Dictyoptera and Coleoptera

(Retnakaran *et al.,* 1985; Pedigo, 2002; Anonymous, 2011)

2001). Teflubenzuron @ 400 and 600 ppm against rice grain moth *Corcyra cephalonica* resulted in ovicidal action, interference with all the developmental stages and mortality of eggs at black head stage (Chakraborti and Chaterjee, 2001). Teflubenzuron and hexaflumuron @ 200mg kg^{-1} of cowpea resulted in reduction in adult emergence of pulse beetle *Callasobruchus maculatus* in addition to control range of 96.2 and 94.3 per cent up to 1 and 8 months with teflubenzuron, respectively whereas 93.8 and 82.25 per cent up to 1 and 8 months with hexaflumuron, respectively (Su *et al.,* 2003). Hexaflumuron resulted in reduced fecundity, longevity and egg viability of *C. maculates* (Kellouche and Soltani, 2006). Triflumuron (Baycidal®) @ 2g/200 ml/m^2 was sufficient for reducing the larval and adult stocks of meal worm *Alphitobius diapernius* in broiler and turkey houses (Salvin *et al.,* 2003). Diflubenzuron against hornfly *Haemotobia irritans* on cow resulted in significant reduction in number of flies per cow (Steelman *et al.,* 2003). Silva and Mendes (2002) reported diflubenzuron @ 300, 100 and 500 ppb lethal to *Haematobia irritans* as pupal malformation occurred in breeding media containing different diflubenzuron concentrations and same product @ 3g kg^{-1} of chicken feed gave the effective control of synathropic flies of poultry. Lufenuron an effective control agent of dog flea *Catenocephalides felis* on cats and dogs has been commercially recommended for use (Fahmy and Dien, 2002). Even at sub-lethal concentrations, it has a very good larvicidal and ovicidal activity in *T. castaneum* (Arora *et al.*, 2012).

Plant based CSIs

Plants also carry many compounds which act as chitin synthesis inhibitors such as plumbagin a naturally occurring CSI present in the roots of tropical medicinal shrub *Plumbago capensis* which inhibits ecdysis in several Lepidopteron pests including *Helicoverpa* spp (Kubo and Klocke, 1983). These plumbagins possess high ovicidal activity against eggs of different stages of *Dysdercus koenigii* (Gujar *et al.,* 1999).Azadirachtin, potent neem based CSI, limits reproductive potential of stored product insect pests (Khattar, 2011). Polyoxins and Nikkomycins were identified in *Sacchromyces cacaoi* var *asoensis* and *S. tendae*, respectively, former acts to kill fungal pathogen and latter as both fungicide and miticide (Cohen, 2001).

IGRs for the management of crop pests

Benzolphenyl urea DBI-3024 (Bistrifluron® 10SC) from the Korean company, Dongbu Hannong Chemical Co. against *Bemisia tabaci* resulted in significant reduction in crop injuries due to the pest and no cross resistance to OPs (Kim *et al.,* 2000). Buprofenzin (Knack®) gave about 12 weeks control of *B. tabaci* with significantly less cost than conventional programme in Arizona U.S.A (Ellsworth *et al.,* 1997). Pyriproxyfen (RH2485) @ 300g a.i ha^{-1} against bollworms resulted in reduction in locule and square damage and increase in seed yield as compared to control (Banerjee *et al.,* 2001). The rain fast compound novaluron (Rimon®) against *Spodoptera exigua, S. littoralis* and *B. tabaci* was quite effective under field conditions with residual activity for 10-30 days, safe to non-target and no cross resistance to other groups. The percent boll damage was only 7.90 with IGR as compared to crypermethrin (19.90) and control (65.00), also the maximum yield (8.20q/ha) was obtained with CSI followed by cypermethrin (6.20q/ha) and control. (0.90 q/ha) (Ishaaya *et al.,* 2003). Lufenzuron (Match 5EC®) against

Helicoverpa armigera on cotton resulted in significant reduction in weight of different instars, in addition to development of cavities in fore wings, reduction in adult emergence, significant decline in fecundity (Butter *et al.,* 2003). Flucycloxuron, CSI, treatment gave delayed metamorphosis, characterized by partial moulting and abnormal wing development of red cotton stainer, *Dysdercus koenigii* (Fabricius) (Khan and Qamar, 2011). Methoxyfenozide @ 200g a.iha was effective against *H. armigera* on cotton (Mohan and Uthamaswamy, 2001) while, the foliar application of diflubenzuron has shown excellent residual activity against eggs of *H. armigera* and *S. litura* (Arora and Sidhu, 1994). JH mimic NC196 @ 300-600g a.i ha^{-1} suppressed the *Nilaparvata lugens* population for 1 month on paddy. Diflubenzuron @ 750g a.i ha^{-1} against *H. armigera* on chickpea resulted in significant reduction in pod damage (15.70%) as compared to control (37.10%) and also the yield in treated (22.60q ha^{-1}) was more as compared to control (18.20q/ha). Diflubenzuron @ 150-170 g a.i ha^{-1} against the same pest on pigeon pea resulted in significant reduction in larval population with a yield of 15.6 q ha^{-1} (Kumar and Dahiya, 1997). Fenoxycarb, diflubenzuron, priyproxyfen, kinoprene against western flower thrips *Frankiniella occidentalis* were effective in reducing the pest emergence under green house (Ludwig and Oetting, 2001). Methoprene caused a sinificant reduction in adult progeny in all rice psocid species *Liposcelis bostrychophila* Badonnel, *L. decolor* (Pearman), *L. entomophila* (Enderlein), *L..paeta* Pearman (Psocoptera: Liposcelididae), and *Lepinotus reticulatus* Enderlein (Trogiidae) at the application rates of 5 and 10 ppm, however at 1 ppm, numbers of adults were reduced for all species on wheat and maize.

Use of IGRs with insecticides

IGRs have been efficiently used for controlling various insect pests along with insecticides. Diflubenzuron alone and in combination with two carbamate insecticides, carbryl (0.15%) and BPMC (Fenucarb 0.025 %) against spotted leaf beetle *Epilachna vigintioctopunctata* on brinjal resulted in 93.7 per cent reduction in population with 19.79 ton/ha yield, while the yield in control and diflubenzuron alone was 7.25 ha^{-1} and 12.55 tons ha^{-1}, respectively (Srinivas *et al.,* 1986). Hexaflumron + chlorpyriphos, cypermethrin gave effective control of *Leptinotarsa decemlineata* on potato as there was improvement in the IGR activity with low doses of insecticide (Dobernic, 1996). Combination of tebufenozide and ë-cyhalothrin against Mexican rice borer, a serious pest of sugarcane in South Texas resulted in significant reduction in bored canes, increase in yield and juice quality in addition to safety to braconid parasitoid *Alloroghus pyralophagus* (Legaspi *et al.,* 1999). Use of chitin promoter cryomazine in combination with imdaclorprid against *Leptinotarsa decimlineata* prolonged the duration of II instar along with inhibition of feeding (Furlong and Gorden, 2001). Adulticidal compound (Pyretheroid - Cyflutherrin) and a larvicidal compound (triflumuron) when used in combination greatly reduced the adult and larval stores of mealworm from broiler and turkey houses (Salvin *et al.,* 2003). Combination of novaluron 10 EC, cypermethrin and acephate gave effective control of cotton bollworms (Rao *et al.,* 2001).

Efficiency of IGRS with biocontrol agents

Bt products along with IGR (Chlrofluazuron- Atabron ®) have been recommended to achieve effective use as well as resistance management in New Guinea (Saucke *et al.,* 2000). Buprofenzin in combination with *Eretmocerus eremicus*, a hymenopterous parasitoid of white fly *Bemisia argentifolii* @ one female at weekly intervals $plant^{-1}$ on poinsettia crop resulted in nymphal population below the ETL under glass house while, buprofenzin in combination with two predators *Clistostethus arcuatus* and *Orius albidipennis* against *Bemisia tabaci* resulted in 100 per cent control of whitefly whereas the predator and IGR alone resulted 98 and 66 per cent control, respectively (Driesche *et al.,* 2001). Combination of *Nosema locustae* and flufenoxuron (5.0%) against rice grasshopper *Oxya chinensis* resulted in 73.9 per cent control of pest in 7 days with effectiveness up to 45 days compared to protozoan alone @ 1010 spores ha^{-1} giving 65.4 per cent control up to 30 days. Triflumuron @ 10mg sqm^{-1} (fortnightly) along with hymenopterous parasitoid *Dirhinus himalayanus* 6 number sqm^{-1} (3 months) against *Musca domestica* resulted in significant reduction in puparium and fly density (Srinivasan and Amalraj, 2003). Chlorfluazuron and thuricide (*Bt)* in combination gave effective control of *Plutella xylostella* with no deleterious effects on parasitoid *Diadegma semiclausum* and *Cotesia plutellae* (Saucke, 2000).

Development of resistance to IGRS

William proposed the use of compounds with insect hormone activity as "third generation insecticides", as it was believed that insects would be unable to develop resistance to molecules that mimic their own hormone. This presumption has not proved true as there are several documented instances of IGR resistance. Gupta and Gupta (1994) reported that diflubenzuron @ 0.0016ml/adult of rice grain moth *Corcyra cephalonica* resulted in 84.21 per cent sterility in the F_1 whereas this was reduced to 21.43 per cent at F_4 with the same dose of chemical, thus giving an indication of development of resistance by the insect to this compound. Resistance to pyriproxyfen in *B. tabaci* started developing after one year of use in green house and after 10 years compound use in cotton (one application) exhibited

high level of resistance (Horowitz *et al.,* 2002). Gorman *et al.,* (2002) reported resistance to buprofezin in green house whitefly *Trialeurodes vaporariorum* in U.S.A. @ 1000mg l^{-1} in glass house and also cross resistance to unrelated IGR leflubenzuron and nicotine. Ishaaya *et al.,* (2003) reported 4 fold resistance in *S. littoralis* to teflubenzuron on cucumber and 1200-2000 folds in *B. tabaci* to pyriproxyfen on cotton. Significant levels of resistance have been exhibited to both diflubenzuron and deltamethrin by *Cydia pomonella* (Sauphanor *et al.,* 2000). The most notorious pest housefly exhibited low levels (RF<10-fold) of resistance to the IGRs, diflubenzuron, methoprene, novaluron, pyripoxyfen, and triflumuron from Antalya province (Cetin *et al.,* 2009). Studies suggest oxidative metabolism as the primary reason for development of resistance to tebufenozide and methoxyfenozide, however, at the cellular level, there is evidence that there could be alterations in the target site(s). Decrease in oviposition along with the lower toxicity indicated a possible fitness cost related to development of resistance (Smagghe *et al.*, 1998).

Effect of IGRs on non-target

Effect on beneficial insects and non target arthropods

Application of methoprene to last instar larvae resulted in 30 per cent increase in silk production due to the elongation of life cycle (Akai *et al.,* 1971). Diflubenzuron (0.1%) resulted in 24.8 to 38.4 per cent mortality of *Chrysopera carnea* eggs (Arora and Varma, 1993). Hatching inhibition of eggs of *Rodolia cardinalis*, predator of cottony cushion scale was observed on 3, 7 and 19 days old plants treated with buprofenzin, triflumuron, pyriproxyfen. Tebufenozide @ 0.67kg a.i ha^{-1} resulted in significant reduction in per cent parasitism by *Cactolaccus grandis* egg parasitoid of boll weevil *Anthomos grandis* (Elzen *et al.,* 2000). Willrich and Boethael (2001) reported diflubenzuron @ 0.035kg a.i ha^{-1} safe to parasitoid of velvet bean caterpillar *Capidosoma floridum.* Three years studies to evaluate the effect of buprofenzin and pyriproxyfen on 20 predator taxa (*Encarsia spp, Chrysoperla spp, Eretomocerus* spp etc) in cotton ecosystem revealed that 8 predator taxa density were reduced significantly with IGR compared to conventional insecticides which resulted in reduction of population of all 20 predator taxa (Naranjo *et al.,* 2004). *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) a common natural predator of defoliating caterpillar was indirectly affected through consumption of prey contaminated with CSI diflubenzuron (Castro *et al.,* 2011). CSIs, teflubenzuron and hexaflumuron, showed high toxicity to non target soil arthropod *Folsomia candida (*Collembolan) among six IGRs tested (Campiche *et al.*, 2006). On the contrary, Triflumuron had shown no effect on the rate of infection and recycling capacity of the parasitoid *Dirhinus himalayanus* in nature facilitating the combined use of both parasitoid and IGR in fly control programme (Srinivasan and Amalraj, 2003). Studies, so far, suggest that IGRs at sub lethal doses, may be integrated in IPM while considering the timing and method of application so as to avoid harmful effects on beneficials.

Effect on vertebrates

MHA's are non mutagenic with low acute toxicity to fish, birds and mammals but methoprene exhibits teratogenic effects on rats at 1000 mg/kg. JHA's are also non mutagenic, non oncogenic without any developmental and reproductive toxicity. In case of CSI's the reproductive, teratogenic and mutagenic effects are negative while the oncogenetic effects with compound chlorobenzol chlorophenyl Urea (CCU) are in criticism (Gernier and Gernier, 1993; Retnakaran *et al.*, 1985). One of the studies suggest that exposure to Lufenuron in the food chain may lead to undesirable consequences in vertebrates (Pinakin *et al.*, 2011).

Future and conclusions

IGRs have great potential for insect control but costlier synthesis, environmental instability and narrow host range limits their commercialization. JHAs and CSIs among IGRs can become a viable component of IPM programme if used judiciously as many commercial formulations of these are available. The novel modes of action or IGRs make them less prone to cross-resistance while, low mammalian toxicity, biodegrability and specific nature of these compounds make them eco-friendly. Stage specificity of JHAs may limit their use under field conditions since the most damaging stage of some insect pests is in the entire larval stage but on the contrary, could be useful in mosquito control programs (Tunaz and Uygun 2004). Ecdysone agonists are most efficacious when ingested but new formulations or chemistries with contact or systemic action could be fruitful area of research. Among the IGRs, nonsteroidal ecdysone agonist bisacylhydrazine insecticides are the best understood at the molecular level in terms of the mode of action, molecular targets and selective toxicity etc unlike JHAs and CSIs. Better understanding of JHAs and CSIs at molecular level and their biosynthetic pathways could pave way for development of novel IGRs. IGRs cannot be the panacea for all the pest problems but can be efficiently and effectively integrated with other tools of pest management. There is an urgent need to have better field stable formulations of IGRs while the insect neuropeptide and chitinase genes can be potential candidates to design effective biopesticides.

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Nutrient supply package and varietal suitability for organic farming in rice

A. Upendra Rao*, K.M. D. Murthy, S. Krishnam Raju, D. Adi Lakshmi and T. V. Sridhar

Andhra Pradesh Rice Research Institute- Maruteru - 534 122, ANGR Agricultural University, Hyderabad, AP, India

ABSTRACT

Field experiments were conducted for two consecutive *kharif* seasons of 2007 and 2008 on Godavari alluvial soils of Maruteru, A.P. with three organic nutrient supply packages and five varieties with an objective to find an appropriate 100 per cent organic nutrient supply system and suitable variety. The pooled data of two years revealed that supply of cent per cent recommended N thorough 1/3rd each of green leaf manure + poultry manure + groundnut cake recorded significantly higher yield attributes, grain yield, higher root volume and root biomass, N and K uptake at harvest. Among varieties MTU 7029 recorded higher yield attributes, grain yield, higher root volume, root biomass, weed biomass and nitrogen and phosphorus uptake at harvest. Physical quality parameters, like hulling per cent and head rice recovery was higher in MTU 1061. Grain quality parameters were superior in BPT 5204. Pest and disease incidence was significantly higher in BPT 5204 and MTU 7029 and were lowest in MTU 1001.

Key words : Organic farming, rice, nutrient supply packages

Organic agriculture is gaining momentum throughout the world due to increased awareness about the ill effects of chemicals used in agriculture on human and animal health, soil quality and environment. Use of chemical inputs has increased the crop yield but caused many environmental problems including soil, air and water pollution and finally human health hazards and making the crop productivity unsustainable (Eid *et al*., 2006). Repeated crop failures, decreasing marginal returns due to stagnating productivity and rising input cost, emergence of niche markets and patrons of alternate production strategies ushered the rediscovery of organic production system in India. Today organic agriculture is one of the fastest growing production systems in the Agriculture sector (Ramesh *et al*., 2005). Rice is one of the major contributor of total food grain production of India (43%), is now witnessed the yield stagnation and declining productivity due to continuous use of high level of chemical fertilizers which had also led to soil degradation problems. Organic rice pose better nutritional quality (Saha *et al*., 2007) and fetches higher market price. Studies suggest that yield could be sustained without increasing the chemical nutrient inputs but by tightening the nutrient cycles (Stockdale *et al.,* 2001) and diversifying the soil biota (Ramesh and Rao, 2009). There were sporadic evidences for influence of different combination of organic sources of nutrition and varietal response to organic rice culture. Hence, experiment was done to generate scientific data on combination of organic sources and varieties for organic rice in coastal humid climate.

MATERIALS AND METHODS

Field experiments were conducted for two consecutive *kharif* season of 2007 and 2008 on Godavari alluvials (Vertic chromusters) at Andhra Pradesh Rice Research Institute, Maruteru, A.P. India (26.38^{0}N, 84. 44^{0}E and 5 m above mean sea level). The soil is clay loam having pH 7.0, CEC of 41.3 meq 100g^{-1} of soil, organic carbon 0.86 per cent, available nitrogen 295 kg ha^{-1}, available P_2O_5 36 kg ha^{-1} and K_2O 312 kg ha^{-1}. The top 30 cm soil had a bulk density of 1.57 g cc^{-1}. The trial was conducted in split plot design with three replications. Main plots consists three organic nutrient supply packages N1 - 1/3rd of 100 per cent recommended N thorough FYM + Vermicompost + Neem cake. N2 - 1/3rd of 100 per cent recommended N through green leaf manure + poultry manure + groundnut cake. N3 – 50 per cent recommended N as FYM + rock phosphate to substitute the P requirement of crops + PSB + *Azospirillum* + *Sesbania* intercropping. Sub plots consists five varieties viz. BPT 5204, MTU 7029, MTU 1061, MTU 1001, MTU 1064. Thirty days old seedlings were planted at a spacing of 20 X 15 cm with 2-3 seedlings hill^{-1}. Water was maintained at a depth of 2 cm up to panicle initiation and 5 cm thereafter up to one week before harvest. The field was drained before application of fertilizers and one week before harvest. Weeds were

*Author for Correspondence: Dr. A. Upendra Rao, Associate Professor of Agronomy, Agricultural College, Naira, Srikakulam (Dist), A.P. Pin: 532 185, Mobile : 09440149488 E- mail : *draurao@gmail.com*

controlled by hand weeding twice at 20 and 40 days after transplanting. Organic manures were applied based on their nutrient content and incorporated two weeks before planting. The experiment received uniform plant protection and cultural management practices throughout the period of crop growth. Neem oil and *Pseudomonas* was used for pest and disease management. Data on yield attributes and yield, pest and diseases were collected following standard procedures from 10 randomly marked hills. Root volume at flowering was calculated using water displacement method. Root biomass and weed biomass was estimated at flowering duly following standard procedure. NPK uptake was calculated by multiplying nutrient content with dry matter production at harvest. The quality parameters were assessed as per the procedure given by Ghosh (1971). Data were analyzed using ANOVA and the significance was tested by Fisher's least significance difference (p= 0.05) by pooling two years data.

Nutrient content of different organic manures

Organic manure	N (%)	P (%)	K (%)
FYM	0.86	0.39	0.51
Vermi compost	1.68	2.21	0.67
Poultry manure	2.27	0.72	1.41
Green leaf manure	2.05	0.49	1.62
Neem cake	3.70	0.94	1.19
Ground nut cake	7.13	1.38	1.26

RESULTS AND DISCUSSION

Nutrient supply packages, and varieties significantly influenced the tiller production, yield attributes and yield, however the interaction between nutrient supply packages, and varieties was not significant. Among the three organic nutrient supply packages, supply of 100 per cent recommended N thorough 1/3rd each of green leaf manure + poultry manure + groundnut cake recorded significantly higher number of tillers, panicles, total grains as well as filled grains panicle^{-1} (Table 1) and these were ultimately resulted in higher grain yield. Quick release of N from poultry manure compared to other organic manure can be attributed for this superior performance. The other two nutrient supply packages were at par in production of tillers, yield attributes and yield. The thousand grain weight was unaffected by different nutrient supply packages. Among the varieties, MTU 7029 recorded the highest number of tillers, panicles, total grains as well as filled grains panicle^{-1} and thousand grain weight (Table 1), which were ultimately resulted in higher grain yield. As MTU 7029 has the capacity to produce higher grain yield under lesser N availability compared to other varieties might be the reason for this superior performance of MTU 7029. The other varieties were at par in production of tillers, yield attributes and yield. Superior performance of MTU 7029 over other varieties under organic farming was also reported by Raju *et al.*(2004).

Both nutrient supply packages, and varieties influenced significantly the root parameters, weed biomass and nutrient uptake (Table 2). Among the organic nutrient supply packages, supply of 100 per cent recommended N thorough 1/3rd each of green leaf manure + poultry manure + groundnut cake recorded higher root volume and root biomass, weed biomass and NPK uptake at harvest. Better

Table 1. Effect of organic nutrient supply packages and varieties on yield attributes and grain yield of rice

(Pooled data of 2 years)

Treatment	Grain yield (kg ha^{-1})	Tillers sqm^{-1}	Panicles sqm^{-1}	Total grains panicle^{-1}	Filled grains panicle^{-1}	1000 grain weight (g)
Organic nutrient supply package						
N_1	3432	314	194	109	92	20.70
N_2	4042	346	223	121	106	21.45
N_3	3366	329	202	110	88	20.20
SEm ±	107	8.7	6.8	3.2	3.9	0.63
CD (P=0.05)	297	24	19	9	11	NS
Variety						
BPT 5204	3154	310	188	95	77	17.24
MTU 7029	4219	342	228	120	99	21.45
MTU 1061	2909	298	174	96	74	21.03
MTU 1001	3016	317	191	93	75	23.04
MTU 1064	3164	324	193	108	84	20.84
SEm ±	131	12.6	10.2	5.8	4.9	0.71
CD (P=0.05)	270	26	21	12	10	1.46

Table 2. Effect of organic nutrient supply packages and varieties on root parameters, weed biomass and NPK uptake by rice (Pooled data of 2 years)

Treatment	Root volume ml plant^{-1}	Root biomass g hill^{-1}	Weed biomass (g)	Uptake at harvest (kg ha^{-1}) N	P	K
Organic Nutrient supply package						
N_1	21.3	9.16	15.73	98.3	23.8	127
N_2	23.5	10.08	17.26	107.1	25.7	133
N_3	22.9	9.73	14.97	95.6	28.9	121
SEm ±	0.65	0.41	0.71	1.75	1.46	2.01
CD (P=0.05)	1.8	1.13	1.97	4.8	4.05	5.6
Variety						
BPT 5204	21.5	9.69	18.77	111.5	23.8	121
MTU 7029	29.7	12.15	15.21	126.1	28.5	132
MTU 1061	21.2	9.73	17.35	112.7	23.6	138
MTU 1001	20.4	8.58	19.06	104.3	21.3	127
MTU 1064	23.1	10.26	16.49	120.5	24.9	141
SEm ±	1.36	1.12	1.08	2.25	1.88	3.1
CD (P=0.05)	2.8	2.3	2.2	4.6	3.9	6.4

and early nutrient availability might have helped in improved root volume and weight in this treatment, which in turn increased the uptake of N and K. Significant increase in uptake of P with application of 50 per cent recommended N as FYM + rock phosphate to substitute the P requirement of crops + PSB + *Azospirillum* + *Sesbania* intercropping might be due to the fact of increased availability of P compared to other treatments. Among the varieties, MTU 7029 recorded conspicuously higher root volume and root biomass, weed biomass and nitrogen and phosphorus uptake at harvest. The performance of MTU 1064 was also impressive particularly with potassium uptake. While these parameters were lower in MTU 1001. Higher root volume, root biomass and lesser weed competition in MTU 7029 and MTU 1064 might have enhanced the nutrient uptake by these varieties compared to other varieties.

The quality parameters were not altered conspicuously by different nutrient supply packages as well as their interaction with varieties. Similar findings of non significant differences in milling quality and cooking quality of rice among different combinations of organic sources of nutrition were reported by Davari and Sharma (2010). However, varieties showed measurable differences in quality parameters (Table 3). Among the varieties the hulling per cent was higher in MTU 1061 followed by MTU 7029, milling per cent was higher in MTU 1001 followed by MTU 7029, per cent head rice recovery was higher in MTU 1061 followed by MTU 1064 and the L : B ratio was noticeably higher in BPT 5204. Grain quality parameters like volume expansion ratio, elongation ratio, amylase content and protein content were higher in BPT 5204, while these parameters were lowest in MTU 1001. Better grain quality in BPT 5204, conspicuous differences in quality aspects of rice varieties under organic farming was also reported by Raju *et al*. (2004).

Influence of organic nutrient supply packages and varieties, pest and disease incidence was conspicuous but the interaction effect of nutrient supply packages and varieties was absent (Table 4). Supply of 100 per cent recommended dose of N thorough 1/3rd each of green leaf manure + poultry manure + groundnut cake noticeably increased the number of white ears sqm^{-1}, BPH hill^{-1} and per cent sheath blight incidence. Whereas the per cent blast incidence was higher in 50 per cent recommended N as FYM + rock phosphate to substitute the P requirement of crops + PSB + *Azospirillum* + *Sesbania* intercropping. Soils with a high functional diversity of microorganisms, which occur very often under organic agriculture practice, develop disease and insect suppressive properties and can help to induce resistance in plants (FileBbach *et al.,* 2007). This supports the findings of Kajimura *et al*. (1995) who noted low densities of BPH and WBPH in organically cultivated fields and those with low N content. Among the varieties infestation of stem borer, BPH, Leaf folder, per cent incidence of sheath blight and blast were noticeably higher in BPT 5204 and MTU 7029. The pest and disease incidence was lowest in MTU 1001. Differences in pest and disease resistance among different varieties might be the reason for differences in incidence of pest and diseases in BPT 5204 MTU 7029 and MTU 1001. Similar findings were reported by Ratnasudhakar *et al*. (2004).

Table 3. Effect of organic nutrient supply packages and varieties on quality parameters of rice

(Pooled data of 2 years)

Treatment	Hulling (%)	Milling (%)	HRR (%)	L/B Ratio	VER	ER	Amylase Content (%)	Protein Content (%)
Organic nutrient supply package								
N_1	78.1	73.5	65.2	2.69	4.11	1.64	23.7	7.71
N_2	78.3	74.8	67.3	2.87	4.06	1.62	24.1	7.78
N_3	79.4	74.1	66.0	2.71	3.95	1.68	22.2	7.69
SEm ±	1.21	1.15	1.07	0.21	0.28	0.11	0.18	0.14
CD (P=0.05)	NS	NS	NS	NS	NS	NS	NS	NS
Variety								
BPT 5204	76.5	70.4	62.6	3.02	5.25	1.79	25.4	7.90
MTU 7029	79.1	75.7	62.5	2.61	4.47	1.55	24.0	7.85
MTU 1061	79.5	75.1	68.7	2.81	4.62	1.69	23.7	7.79
MTU 1001	78.9	77.0	64.5	2.73	4.21	1.53	22.7	7.69
MTU 1064	78.3	71.3	65.4	2.62	4.45	1.67	23.4	7.73
SEm ±	0.08	1.12	1.05	0.08	0.27	0.11	0.43	0.09
CD (P=0.05)	0.16	2.31	2.2	0.16	0.56	0.22	0.89	0.19

HRR - Head Rice Recovery; VER- Volume Expansion Ratio; ER- Elongation Ratio

Table 4. Effect of organic nutrient supply packages and varieties on pest and disease incidence in rice

(Pooled data of 2 years)

Treatment	Dead hearts sqm^{-1}	White ears sqm^{-1}	BPH $hill^{-1}$	Leaf folder (%)	Sheath blight incidence (%)	Blast incidence (%)
Organic nutrient supply package						
N_1	15.8	24.1	21.7	5.3	16.5	8.7
N_2	17.3	26.0	35.0	5.8	19.1	9.2
N_3	14.5	20.5	26.3	4.7	15.8	11.0
SEm ±	1.07	1.24	1.73	0.67	1.12	0.85
CD (P=0.05)	NS	3.4	4.8	NS	3.1	2.3
Variety						
BPT 5204	18.7	26.2	32.0	6.5	19.5	14.8
MTU 7029	19.3	25.7	30.6	5.9	22.1	11.3
MTU 1061	15.6	18.5	22.1	3.8	14.7	8.3
MTU 1001	14.4	16.3	18.1	4.6	11.4	6.5
MTU 1064	16.1	21.8	26.7	5.1	16.4	7.2
SEm ±	1.52	2.18	2.45	1.11	1.64	1.35
CD (P=0.05)	3.1	4.5	5.0	2.3	3.4	2.8

It can be concluded that, there was conspicuous difference in 100 per cent organic nutrient supply packages. Supply of 100 per cent recommended N thorough $1/3^{rd}$ each of green leaf manure + poultry manure + groundnut cake was found superior in terms of yield attributes, yield, root parameters and nutrient uptake. Noticeable varietal response was observed under organic farming. MTU 7029 can be chosen for yield, BPT 5204 can be chosen for better grain quality, MTU 1001 can be better varietal option in pest and disease endemic areas.

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Agronomic evaluation of biodynamic practices for organic cultivation of basmati rice based cropping system

D.K. Singh, Jyoti Pandey, Geeta Kaur and Anoop Kumar Dubey

Department of Agronomy, College of Agriculture, GBPUA&T, Pantnagar – 263 145, Uttarakhand, India
E-mail : dhananjayrahul@rediffmail.com

ABSTRACT

An experiment was conducted in the year 2009 - 10 and 2010 - 11 at Breeder Seed Production Center of Pantnagar to evaluate the performance of different cropping systems under biodynamic practices. The treatments were, cropping system rice – chickpea - *Sesbania* & rice - vegetable pea - maize + moong and six sub plots in split plot design. Among two cropping system rice – chickpea - *Sesbania* cropping system recorded significantly higher values for grain yield (3629 kg ha^{-1}), straw yield (5177 kg ha^{-1}), nutrient uptake and almost all macro nutrients in soil. Treatment T_1 (FYM + VC + NC + EC) and T_5 (T_1 + BD + Panchgavya) recorded significantly higher grain yield, i.e. 3683 kg ha^{-1} and 3144 kg ha^{-1}, respectively. T_5 recorded significantly higher nitrogen uptake (80.7 kg ha^{-1}), while T_1 recorded higher values for uptake of phosphorus, potassium and sulphur (i.e. 23.6, 95.2 and 18.0 kg ha^{-1}, respectively). Highest organic carbon was observed in T_5 treatment (1.01%), available nitrogen in T_4 (379 kg ha^{-1}), available phosphorus and sulphur was found to be maximum in T_5. Available potassium in soil was found in T_1 treatment of nutrient management.

Key words: Organic, biodynamic, cropping system, green manuring, nutrient uptake

Green revolution was achieved due to greater use of synthetic agro-chemicals such as fertilizers and pesticides with adoption of nutrient responsive high yielding varieties of crops, which boosted the production. However, now this increase in production is showing downward trend and in many cases there are indications of declining productivity and production. Besides this, environmental and health problems associated with the use of agro-chemicals have been documented which has brought a major shift of people towards organic farming (Proctor, 2002). Organic farming is a form of agriculture which avoids or largely excluded the use of synthetic fertilizers and pesticides, plant growth regulators and livestock feed additives. As far as possible organic farmers rely on crop rotation, crop residues, animal manures and mechanical cultivation to maintain soil productivity and tilth to supply plant nutrient and control weeds, insects and other pests. So we need biodynamic agriculture. Biodynamic agriculture is an advanced form of organic farming. It includes the normal organic farming practices, such as the use of compost, green manure and crop rotation.

MATERIALS AND METHODS

The experiment on biodynamic practices was initiated in *kharif* 2009-10, and it continued up to 2010-11 at Seed Production Centre, Pantnagar. The experiment was carried out in spilt plot design having two cropping system basmati rice - chickpea and basmati rice - vegetable pea – maize + moong in main plot and 6 biodynamic practices T_1 = FYM + VC + NC + EC (1/4 + 1/4 + 1/4 + 1/4), T_2 = BD, T_3 = T_1 + Panchgavya, T_4 = T_1 + BD, T_5 = T_1 + BD + Panchgavya and T_6 = Control in sub plot. Under basmati rice - chickpea cropping system, green manuring with *Sesbania* was done in the plot prior to *kharif* crop. Data of yield, yield attributes and nutrient uptake by plants were recorded after each season of crop harvest.

The soil of the experimental site was sandy loam in texture. Soil sample were collected from each plot and analyzed for organic carbon, available nitrogen, phosphorus, potassium, sulphur and micro-nutrients by adopting standard procedure and data analyzed statistically.

RESULT AND DISCUSSION

Effect on yield and nutrient uptake

Results revealed that the significantly higher rice grain (8.46% and 25.80% in 2009 and 2010, respectively) and straw yield (11.38% and 17.29% in 2009 and 2010, respectively) was recorded in basmati rice - chick pea - *sesbania* cropping system over rice - vegetable pea – maize + moong. Increased in the grain yield significantly owing to the residual effect of *sesbania* incorporation to rice crop over no green manuring. These finding are the support of Tiwari *et al*., 2004 in maize - wheat cropping system. Significant variations were observed for different nutrient management under different treatment combinations during investigation. Treatment T_1

recorded significantly superior values for grain yield (3683 kg ha^{-1}) but at par with T_3, T_4 and T_5 in the year 2009. Increased in the yield was to the tune of 17.7 per cent with the treatment T_1 and that of 15.0 per cent with T_5 over control. Similar finding was obtained by Wani *et al.*, 2008 in grain yield of wheat and chickpea. However, T_5 registered higher value of straw yield of rice for 2009, grain yield for the year 2010, and at par with T_3, T_4 & T_1 respectively (Table 1). Significantly maximum straw yield during 2010 was found with the treatment T_4 which was statistically at par with T_3, T_1 and T_5.

The yield attributes of rice was not influenced significantly by different cropping system except effective tillers sqm^{-1} which was significantly highest in rice - chickpea over rice-vegetable pea – maize + moong cropping system. In both years among the biodynamic treatments significantly higher number of effective tillers sqm^{-1} was recorded under T_5 in both the years which was at par with T_1 in 2009 and T_4, T_3 and T_1 treatments in 2010. Likewise significantly higher weight of grain per panicle was recorded T_1 in 2009 and in 2010 with T_5 than other biodynamic and nutrient management treatments. In 2009, higher thousand grains weight was recorded with T_1 and was at par with T_5.

No significant variation was observed in nutrient uptake by basmati rice due to cropping system in 2009, while in the year 2010 the uptake was significantly higher under rice - chickpea cropping system for the entire nutrient studied. Among the biodynamic treatment, significant difference was

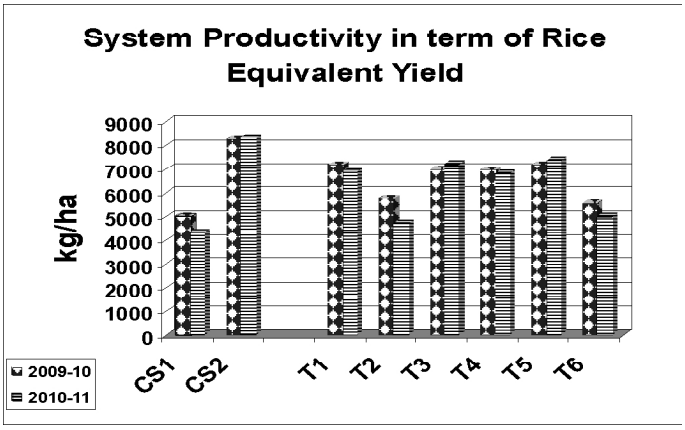

Fig. 1. System Productivity in terms of rice equivalent yield

recorded in nutrient uptake by rice for both the years except for phosphorus and sulphur uptake in 2009. Higher nitrogen uptake was recorded under T_5 in 2009 and it was at par with T_3, T_4 and T_1 respectively. In the year 2010 it was significantly higher with T_5. However, phosphorus, potassium and sulphur registered higher value with T_1 for the year 2009. The entire uptake by basmati rice was significantly higher with T_5 (Table 2).

System productivity

Results revealed that the total system productivity was higher under Rice-vegetable pea-maize+ moong cropping system for both years. Among biodynamic treatments, T_1, T_3

Table 1. Yield and yield attributes of basmati rice

Treatment	Grain yield (kg ha^{-1})		Straw yield (kg ha^{-1})		Effective tillers sqm^{-1}		Wt. of grain panicle^{-1} (g)		1000 grain wt. (g)	
	Main plots (Cropping systems)									
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Rice-C. pea-*Sesbania*	3629	3028	4882	5177	268	288	1.44	1.32	27.02	23.5
Rice-V. pea-maize + moong	3346	2407	4383	4414	240	228	1.42	1.21	27.13	23.0
SEm±	87.7	74	74.9	61	5.6	4.4	0.02	0.01	0.13	0.3
CD (p=0.05)	342	289	290.2	240	21.7	17.3	NS	0.05	NS	NS
	Sub-plots (Biodynamic practices)									
T_1=FYM+VC+NC+EC (1/4+1/4+1/4+1/4)	3683	2911	5359	4917	273	266	1.50	1.26	28.31	23.4
T_2=BD	3153	2178	4059	4528	240	234	1.40	1.25	26.42	22.8
T_3=T_1+Panchgavya	3569	2932	5305	4923	250	270	1.43	1.27	26.87	22.3
T_4=T_1+BD	3590	2939	5322	5094	245	275	1.41	1.27	27.28	23.2
T_5=T_1+BD + Panchgavya	3598	3144	5405	4900	275	277	1.45	1.34	27.63	23.4
T_6=Control	3129	2202	4045	4411	240	228	1.36	1.20	25.91	22.9
SEm±	95.0	103	64.1	110	4.4	3.8	0.01	0.02	0.25	0.5
CD (p=0.05)	280.3	303	189.1	325	15.9	11.2	0.04	0.06	0.74	NS

Table 2. Total nutrient uptake by basmati rice

Treatment	N uptake (kg ha^{-1})		P uptake (kg ha^{-1})		K uptake (kg ha^{-1})		S uptake (kg ha^{-1})	
	Main plot (Cropping system)							
	2009	2010	2009	2010	2009	2010	2009	2010
Rice-C. pea - *Sesbania*	77.4	61.9	21.8	14.4	86.6	74.1	15.5	6.7
Rice-V. pea-maize + moong	72.3	52.0	21.4	12.2	86.2	62.4	15.6	5.6
SEm ±	2.01	1.3	0.64	0.2	1.7	0.8	0.37	0.2
CD (p=0.05)	NS	5.1	NS	0.8	NS	2.9	NS	0.6
	Sub-plot (Biodynamic practice)							
T_1=FYM+VC+NC+EC (1/4+1/4+1/4+1/4)	75.1	57.6	23.6	13.1	95.2	70.2	18.0	6.4
T_2 = BD	64.0	46.2	20.0	10.3	77.8	59.3	13.8	5.1
T_3 = T_1 + Panchgavya	78.8	58.9	22.2	14.7	89.8	78.4	15.6	7.2
T_4 = T_1 + BD	76.6	62.5	21.3	14.3	87.4	72.8	14.6	6.3
T_5 = T_1 + BD + Panchgavya	80.7	70.4	23.3	17.1	92.7	81.2	17.4	7.9
T_6 = Control	59.8	46.0	18.1	10.3	75.8	47.6	14.0	3.8
SEm ±	3.55	1.6	0.15	0.4	4.7	1.5	0.12	0.2
CD (p = 0.05)	10.5	4.9	NS	1.2	13.9	4.3	NS	0.6

and T_5 showed almost similar system productivity however; T_5 biodynamic treatments resulted the highest system productivity as it contains FYM + VC + NC + EC + BD + Panchgavya (Fig. 1).

Soil nutritional status

Result revealed that soil nutritional status after completion of two crop cycle was significantly influenced by different cropping systems except available sulphur. Soil organic carbon, available nitrogen and potassium were found to significantly higher under rice -chickpea cropping system, while available phosphorus was significantly higher under rice-vegetable pea - maize + moong cropping system. Among biodynamic treatments significantly higher organic carbon was found with T_5 which was at par with T_3, T_1, and T_4. Gupta *et al.,* (1988) noticed an increase in organic carbon content in soil due to continuous addition of FYM. Similar results were obtained by Bharadwaj and Omanwar (1994). Available nitrogen was significantly higher with T_1. Available phosphorus and sulphur recorded significantly maximum values in treatment T_5 after the two years crop cycle. Significantly highest available potassium was recorded in T_1 for both the years studied by Bhoi, 2007-08 showed same results.

Micronutrient status of soil was significantly influenced by different cropping systems. Significantly superior available Iron and Zinc were recorded in rice - vegetable pea – maize + moong cropping system (Table 3).

Significant variation was observed for available micro-nutrient by different biodynamic practices. Significantly superior available Iron was recorded with T_4 and it was at par with T_5, T_3 and T_1. Significantly highest Zinc was recorded in T_1.

There was improvement in yield and soil status of plots during experimentation as rice – chickpea - *Sesbania* cropping system proved to be superior in enhancing grain yield and T_5 and T_4 nutrient management and biodynamic practice was proved to be more productive in terms of nutrient uptake. Nutrient supply through organic nutrient sources along with BD preparation and Panchgavya under T_5 alone and in combination with rice – chickpea - *Sesbania* cropping system have a potential of improving the yield, yield attributes & physico - chemical soil properties. The increment was higher in case of combined application of organics and fermented liquid manures as compared to organic manures alone under T_5 which may be ascribed to enhanced mineralization of organic matter due to enhanced microbial activity. This facilitated continuous supply of readily available nutrients to plants from organic manures and thus restoring / enriching available pool of soil nitrogen. This provides a long term impact of these practices on ecosystem and environment.

Table 3. Nutritional status of soil after completion of two crop cycle

Treatment	Organic C (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})	Available K (kg ha^{-1})	Available S (kg ha^{-1})	Available Fe (ppm)	Available Zn (ppm)
Main plot (Cropping system)							
Rice - C. pea - *Sesbania*	0.98	347	26.8	256	34.0	31.4	0.72
Rice - V. Pea – maize + moong	0.92	334	30.3	198	33.0	33.1	0.87
SEm ±	0.01	2.1	0.2	2.6	0.5	0.6	0.01
CD (p = 0.05)	0.04	8.0	0.7	10.1	NS	2.4	0.02
Sub-plot (Biodynamic practice)							
T_1= FYM + VC + NC + EC (1/4 + 1/4 + 1/4 + 1/4)	0.99	374	32.3	251	35.5	32.2	1.06
T_2 = BD	0.85	297	23.3	197	29.1	31.0	0.63
T_3 = T_1 + Panchgavya	1.00	371	30.6	237	34.9	32.6	0.75
T_4 = T_1 + BD	0.99	345	29.6	238	34.3	33.8	0.89
T_5 = T_1 + BD + Panchgavya	1.01	367	33.1	223	37.3	33.4	0.74
T_6 = Control	0.87	290	22.5	217	29.8	30.7	0.71
SEm ±	0.02	4.2	0.8	4.3	0.8	0.6	0.01
CD (p = 0.05)	0.06	12.5	2.4	12.7	2.3	1.8	0.04

Initial organic carbon= 0.93%, Available N=227 kg ha^{-1}, Available P= 34.6 kg ha^{-1}, Available K= 133 kg ha^{-1}, Available S= 19.0 kg ha^{-1}, Available Fe = 22.1 ppm & Available Zn = 0.70 ppm.

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Studies on performance of organic farming and chemical farming in rice – rice system

*A. Upendra Rao, K.M.D. Murthy, T.V. Sridhar and D. Srinivas

Andhra Pradesh Rice Research Institute - Maruteru - 534 122; ANGR Agricultural University, Hyderabad, AP, India

ABSTRACT

Field experiments conducted consecutively for three years during *kharif* and *rabi* seasons on Godavari alluvial soils revealed that, application of 100 per cent recommended NPK along with $ZnSO_4$ @50 kg ha^{-1} resulted highest grain yield, gross return, net return and rupee per rupee invested over exclusive organic farming practices. Integrated nutrient management during *kharif* and for system application of 100 per cent recommended NPK along with $ZnSO_4$ @50 kg ha^{-1} during *rabi* showed more sustainability in yields among all the treatments. The organic carbon content was conspicuously increased with all the exclusive organic nutrient management practices as well as integrated use of nutrients. The status of available soil potassium was remarkably decreased in all the exclusive organic nutrient management practices and integrated nutrient management treatments.

Key words: Rice, organic farming, yield, sustainability, economics

The traditional organic agriculture in India was transformed to inorganic in mid sixties of the previous century as a result of Green Revolution and attained self sufficiency in food production by use of high external inputs. Rice is one of the major contributors to the success by contributing approximately 43 per cent of total food grain production of India. In recent years, yield stagnation and declining productivity is observed due to continuous use of high level of chemical fertilizers, which has led to soil degradation problem. At the same time organic farming is gaining momentum throughout the world, including India, due to the farmer's movement, growing awareness for environment and health among the consumers and promotion from the policy makers. Keeping the above aspects in view, the present study was undertaken to compare the performance of organic farming, chemical farming and integrated nutrient management in rice - rice system.

MATERIALS AND METHODS

Field experiments were conducted on rice (*Oryza sativa* L.) consecutively for three years during *kharif* and *rabi* seasons of 2005-06, 2006-07 and 2007-08 on Godavari alluvials (Vertic chromusters) at Andhra Pradesh Rice Research Institute - Maruteru, A.P. India (26.38^0N, 84.44^0E and 5 m above mean sea level). The soil is clay loam having pH 7.1, organic carbon 0.9 per cent, available P_2O_5 38 kg ha^{-1} and K_2O 344 kg ha^{-1}. The trial consists of eight treatments. T_1: 50 per cent recommended NPK + 50 per cent N as FYM; T_2 : 100 per cent recommended N (1/3 each through FYM + vermicompost + neem cake); T_3: T_2 + intercropping with sesbania. T_4: T_2 + organic practices for weed and pest control; T_5: 50 per cent N as FYM + rock phosphate to substitute the P requirement of the crops + PSB + Azospirillum; T_6: T_2 + biofertiliser containing N & P carriers (Azo + PSB); T_7: 100 per cent NPK + 50 kg $ZnSO_4$ ha^{-1}; T_8 : T_2 + organic pest control. MTU 3626 (135 days duration) during *kharif* and MTU 1010 (120 days duration) during *rabi* were the test varieties, planted 24 to 26 days old seedlings at a spacing of 20 X 15 cm during *kharif* 15 X 15 cm during *rabi* with 2-3 seedlings $hill^{-1}$. Weeds were controlled by application of pre emergence herbicide Pretilachlore @ 0.75 kg a.i ha^{-1} followed by one hand weeding at 40 days after transplanting except in T_4, where weeds were controlled manually. Water was maintained at a depth of 2 cm up to panicle initiation and 5 cm thereafter up to one week before harvest. The field was drained before application of fertilizers and one week before harvest. Manures and fertilizers were applied as per the treatments requirement through Urea, SSP, MOP. Entire P and K and 1/3 recommended N was applied as basal dose and remaining N was applied in two splits at active tillering and panicle initiation stage. Organic manures were applied based on their nutrient content and incorporated two weeks before planting. The experiments were received uniform plant protection and cultural management practices throughout

***Author for correspondence:** Dr. A. Upendra Rao, Associate Professor of Agronomy, Agricultural College, Naira, Srikakulam (Dist), A.P. Pin : 532 185, Mobile : 09440149488 E- mail: *draurao@gmail.com*

the period of crop growth except in T_4, where neem oil and *Pseudomonas* was utilized for pest and disease management. The data was analysed by pooled analysis using three years data. Sustainability Index was calculated using formula given by Gangwar *et al*. (2004)

RESULTS & DISCUSSION

Three years pooled data revealed that, application of 100 per cent recommended NPK along with $ZnSO_4$ @50 kg ha^{-1} resulted the highest grain yield, which was significantly superior over all the organic farming practices irrespective of the combinations. However, it was at par to integrated use of 50 per cent NPK + 50 per cent N as FYM during both the seasons as well as system as a whole (Table 1, 2 & 3). Higher grain yield with application of 100 per cent recommended NPK along with $ZnSO_4$ @50 kg ha^{-1} might be due to sufficient nutrient supply as per the crop needs resulted in favourable growth and yield structure compared to exclusive organic nutrient management practices. Raju *et al.* (1992) reported adequate availability and translocation of nitrogen and other nutrients to sink manifests marked yield improvement in rice. Similar results of higher yields of rice with 100 per cent RDF over organic farming was reported by Jadhav *et al.* (2007). However, the yield reduction of 16.1 per cent during

Table 1. Grain yield, sustainability and economics of rice - rice system as influenced by different nutrient management practices during *kharif* (Pooled data of three years)

Treatment	Grain yield (kg ha^{-1})	Sustainability Index	Gross returns (Rs ha^{-1})	Net returns (Rs ha^{-1})	Rupee per rupee invested (Rs/Rs)
T1	4437	0.84	30015	12851	0.75
T2	3889	0.68	26143	2689	0.11
T3	4005	0.82	27000	2613	0.11
T4	3780	0.80	25413	463	0.02
T5	3774	0.71	25371	7722	0.44
T6	3970	0.72	26744	2996	0.13
T7	4772	0.80	32203	16856	1.10
T8	3833	0.67	25900	2020	0.08
SEm ±	114.0	-	907	482.0	0.029
CD (P=0.05)	345	-	2751	1463	0.089

T_1: 50% rec. NPK + 50% N as FYM; T_2 : 100% Rec. N (1/3 each through FYM + vermicompost + neem cake); T_3: T_2 + intercropping with *sesbania*. T_4: T_2 + organic practices for weed and pest control; T_5: 50% N as FYM + rock phosphate to substitute the P requirement of crops + PSB + Azospirillum; T_6: T_2 + biofertiliser containing N & P carriers (Azo + PSB); T_7: 100% NPK + 50 kg Zn SO_4 ha^{-1}; T_8: T_2 + organic pest control

Table 2. Grain yield, sustainability and economics of rice - rice system as influenced by different nutrient management practices during *rabi* (Pooled data of three years)

Treatment	Grain yield (kg ha^{-1})	Sustainability Index	Gross returns (Rs ha^{-1})	Net returns (Rs ha^{-1})	Rupee per rupee invested (Rs/Rs)
T1	5581	0.77	39031	19250	0.97
T2	4382	0.72	30659	5310	0.21
T3	4593	0.76	32059	5546	0.21
T4	3971	0.75	27945	1433	0.05
T5	4112	0.73	28860	10817	0.60
T6	4386	0.62	30879	5236	0.20
T7	5713	0.80	39597	20110	1.03
T8	4318	0.72	30208	4797	0.19
SEm ±	178.0	-	1027	599.0	0.031
CD (P=0.05)	541	-	3116	1817	0.097

Table 3. Grain yield, sustainability and economics of rice - rice system as influenced by different nutrient management practices (Pooled data of three years)

Treatment	Grain yield (kg ha^{-1})	Sustainability Index	Gross returns (Rs ha^{-1})	Net returns (Rs ha^{-1})	Rupee per rupee invested (Rs/Rs)
T1	10018	0.86	69046	32101	1.72
T2	8271	0.81	56802	7999	0.32
T3	8598	0.84	59059	8159	0.32
T4	7751	0.82	53358	1896	0.07
T5	7886	0.79	54231	18539	1.04
T6	8356	0.76	57623	8232	0.33
T7	10485	0.84	71800	36966	2.13
T8	8151	0.80	56108	6817	0.27
SEm ±	160	-	1237	812	0.05
CD (P=0.05)	486	-	3751	2462	0.16

kharif, 19.6 per cent during *rabi* and 18 per cent for the system was recorded with the best exclusive organic treatment over application of 100 per cent recommended NPK along with $ZnSO_4$ @50 kg ha^{-1}. After four years of experimentation 25.4 per cent yield reduction was recorded with organic farming treatments over 100 per cent RDF in *kharif* rice (Yadav *et al.*, 2002). Yield obtained under organic farming was 14-51 per cent lower than inorganic and integrated nutrient management in rice after seven years across the country (Prasad *et al*., 2008). Sustainability Index revealed that Integrated nutrient management during *kharif* and for System, application of 100 per cent recommended NPK along with $ZnSO_4$ @50 kg ha^{-1} during *rabi* showed more sustainability among all the treatments. Higher sustainability with integrated nutrient use compared to chemical fertilization in rice - rice system was reported by Mohanty *et al*. (2007). Among different organic practices, application of 100 per cent recommended N (1/3 each through FYM + vermicompost + neem cake) + intercropping with *Sesbania* and 100 per cent recommended N (1/3 each through FYM + vermicompost + neem cake) + organic practices for weed and pest control showed more stability compared to other practices.

Economic analysis of the three years showed that the highest gross returns were recorded with application of 100 per cent recommended NPK along with $ZnSO_4$ @50 kg ha^{-1} followed by Integrated use of 50 per cent NPK + 50 per cent N as FYM and were significantly superior over exclusive organic nutrient management practices during both the seasons as well as system as a whole (Table 1,2 & 3). The gross returns were at par with different exclusive organic nutrient management practices. The net return and rupee per rupee invested were also significantly higher with application of 100 per cent recommended NPK along with $ZnSO_4$ @50 kg ha^{-1} compared to exclusive organic nutrient management practices during both the seasons as well as system as a whole. However, these parameters were at par to integrated use of 50 per cent NPK + 50 per cent N as FYM during *rabi*. Higher cost of production besides reduced yields lead to decreased returns from different exclusive organic farming treatments. Similar findings were also reported by Basavarajappa *et al*. (2007) and Singh *et al*. (1996). Among different exclusive organic nutrient management practices, application of 50 per cent N as FYM + rock phosphate to substitute the P requirement + PSB + Azospirillum recorded significantly higher net returns and rupee per rupee invested during both the seasons as well as system as a whole compared to other exclusive organic treatments. The treatments involved organic practices for weed and pest control during recorded significantly lower net returns and rupee per rupee invested during both the seasons as well as system as a whole.

The fertility status of the soil after four years of the study showed remarkable variations due to effect of different sources of nutrients (Table 4). There was significant decrease in soil pH over initial values with exclusive organic nutrient management practices and though there was decrease in pH with 100 per cent chemical fertilization and integrated use of nutrients, the change was not statistically measurable. There was significant increase in EC, which was noticed with100 per cent chemical fertilization and integrated use of nutrients. The Organic carbon content was conspicuously increased with all the exclusive organic nutrient management practices as well as integrated use of nutrients.

Table 4. Chemical parameters of the soil as influenced by different nutrient management practices in rice-rice system after *rabi* (Pooled data of three years)

Treatment	pH	EC (ds/m)	Organic carbon (%)	Available P_2O_5 (kg ha^{-1})	Available K_2O (kg ha^{-1})
Initial	7.1	0.40	0.90	38.00	344
T1	6.72	0.47	1.06	40.15	247
T2	5.92	0.34	1.29	45.49	264
T3	6.00	0.32	1.28	46.29	272
T4	5.80	0.42	1.24	44.43	257
T5	5.92	0.37	1.16	42.78	265
T6	6.19	0.44	1.28	43.11	270
T7	6.95	0.48	0.95	39.22	262
T8	5.90	0.38	1.18	43.18	261
SEm ±	0.25	0.02	0.03	1.74	7.30
CD (P=0.05)	0.74	0.06	0.09	5.3	23.0

Incorporation of organic amendments resulted in increased organic carbon status might be due to improvement of physical and biological properties of the soil (Nayyar, 2002). The available phosphorus status was improved with most of the organic farming treatments and the difference was measurable with 100 per cent recommended N (1/3 each through FYM + vermicompost + neem cake), 100 per cent recommended N (1/3 each through FYM + vermicompost + neem cake + intercropping with *Sesbania* and 100 per cent recommended N (1/3 each through FYM + vermicompost + neem cake) + organic practices for weed and pest control. Whereas, there was no improvement in available phosphorus status with 100 per cent chemical fertilization and integrated use of nutrients. The status of available soil potassium was decreased in all the treatments over initial values, however the decrease was significant in all exclusive organic nutrient management practices and integrated use of nutrients showed that the nutrient replenishment was not with the tune of the crop needs by different organic sources.

The results indicated that organic farming has an edge over inorganic farming to sustain the soil organic carbon, but this practice is highly expensive, less productive and not profitable as per the existing market. Integrated nutrient management found to be good option to stabilise the production and profitability besides maintaining soil health.

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Effect of different doses of vermicompost on gerbera cv. Red Gem.

Madhumita Choudhury Talukdar and Luna Barooah

Department of Horticulture, Assam Agricultural University, Jorhat-785 013, Assam, India

ABSTRACT

A field study was conducted in the experimental farm of Department of Horticulture, Assam Agricultural University, Jorhat with a view to find out the effect of different doses of vermicompost on growth, flowering and sucker production of gerbera cv. Red Gem. The treatments consist of different doses of vermicompost viz. 5, 10, 15, 20, 25 and 30 t ha^{-1} of T_1, … T_6 respectively, which were compared with control and NPK (recommended dose) treatment. The results of the experiment revealed that among the different treatments, application of vermicompost @15 t ha^{-1} (T_3) significantly increased the growth and flower characters and sucker production followed by vermicompost @10 t ha^{-1} (T_2) and NPK (T_8). Maximum plant height (48.66 cm), number of leaves per plant (42.66), maximum leaf length (38.00 cm), minimum days to full bloom (69.26 days), number of flowers plant^{-1} (33.00), maximum stalk length (48.00 cm), maximum size of flower (9.10 cm) and vase life (8.66 days) were observed under T_3 (vermi compost @15 t ha^{-1}).

Key words: Vermicompost, gerbera

Gerbera (*Gerbera gamesonii* Bolus) is one of the important commercial cut flower of the world belongs to the family Asteraceae (Compositae). It is native of South Africa and is known as African daisy, Transvaal daisy and Barbeton daisy. Gerbera is perennial in nature and can be grown in open as well as in protected conditions. There are two types of gerbera, one is general gerbera and other one is black centered gerbera. Black centered or black heart gerberas are mainly grown under polyhouse or protected condition but general gerberas can be grown both open and polyhouse condition. A general gerbera variety, Red Gem is found suitable for cultivation in open field of Assam in terms of highest number of flower and sucker production. Considering the recent concept of sustainable agriculture and increasing cost of inorganic fertilizers it is important to use cost effective organic fertilizers or vermicomposts which has a special significance in crop production. Keeping these points in mind the present experiment was undertaken with the aim to optimize doses of vermicompost for gerbera Cv. Red Gem.

MATERIALS AND METHODS

A field experiment was carried out during 2009-10 at the Experimental Farm, Department of Horticulture Assam Agricultural University, Jorhat. The soil was sandy loam with pH of 4.90; organic carbon content 0.64 per cent, available N 0.07 per cent and available P_2O_5 and K_2O was 47.52 and 74.25 kg ha^{-1}, respectively. The experiment was laid out in randomized block design with three replications. The treatments include different doses of vermicompost viz 5, 10, 15, 20, 25, 30 t ha^{-1}, Control and NPK (20:20:20). The suckers were planted in the main field at the spacing of 30 x 30cm. All doses of vermicomposts were applied as basal dose. The control was maintained without applying vermicompost and fertilizer, while the treatment of recommended dose was maintained by application of half dose of N and full dose of P and K as basal. The remaining dose of N was applied one month after planting. Observations were recorded on plant height, number of leaves, leaf area, days to first flower emergence, number of days to full bloom, number of flowers, size of flowers, shelf life and vase life of cut flowers. The data were analyzed statistically and presented in Table 1 and 2.

RESULTS AND DISCUSSION

A perusal of the data (Table 1, 2) revealed that maximum plant height (48.66 cm), number of leaves (42.66), leaf length (38.00 cm), leaf breadth (13.16 cm), minimum days to flower emergence (61.26 days) to full bloom (69.26), number of flowers plant^{-1} (33.00), stalk length (48.00 cm), size of flower (9.10 cm), shelf life (16.83 days), vase life (8.66 days) was recorded under T_3 followed by T_2 and T_8. It may be due to better nutrient availability due to increased activity of microbes in vermicompost. It is well established that some microbes like bacteria, fungi, actinomycetes, algae etc. are capable of producing plant growth substances like auxins, gibberellins, etc. in appreciable quantity during vermicomposting (Arancon *et al*., 2004), which might have influenced the plants growth positively. Atiyeh *et al*. (2002)

Table 1. Effect of vermicompost on growth of gerbera. cv. Red Gem.

Treatment	Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf breadth (cm)	Days to flower bud visibility
T_1	41.50	35.33	32.83	10.50	64.00
T_2	42.83	36.33	34.00	10.00	61.73
T_3	42.50	37.66	32.16	11.00	65.20
T_4	43.66	39.33	33.33	11.83	63.33
T_5	48.66	42.66	38.00	13.16	61.26
T_6	45.50	42.33	35.33	13.33	62.86
T_7	40.50	33.33	30.50	9.66	67.60
T_8	44.66	39.66	35.73	12.76	60.20
SE	0.62	0.44	0.44	0.44	0.98
CD at 5%	1.34	0.96	0.95	0.95	2.12

Table 2. Effect of vermicompost on flowering of gerbera. cv. Red Gem.

Treatment	Days to full bloom	No of flowers $Plant^{-1}$	Stalk length (cm)	Size of flower (cm)	Self life (days)	Vase life (days)
T_1	71.80	23.86	42.33	8.38	11.83	7.13
T_2	72.33	25.13	43.50	8.67	12.66	7.40
T_3	72.20	28.00	43.16	8.86	13.16	8.06
T_4	70.53	30.00	44.66	9.05	16.26	8.40
T_5	69.26	33.00	48.00	9.10	16.83	8.66
T_6	69.33	32.60	47.33	8.96	18.10	8.40
T_7	75.46	21.20	38.66	7.40	13.50	5.96
T_8	69.50	28.80	45.66	9.00	16.93	8.06
SE	0.95	1.98	0.59	0.16	0.48	0.26
CD at 5%	2.05	4.27	1.27	0.34	1.04	0.56

found that fresh weights of marigold plant shoots at 121 days after seeding were greatest in potting mixtures containing 30 per cent and 40 per cent pig manure and vermicompost. However, they were least in potting mixtures of 90 per cent and 100 per cent vermicompost. Arancon *et al.* (2008) examined the influence of vermicomposts produced from cattle manure, food wastes and paper wastes on Petunia and results showed that germination, growth and flowering of Petunia plants were enhanced due to the application of vermicompost.

Increasing of flower numbers by vermicompost amendments is also in agreement with a report by Gajalakshmi and Abbasi (2004), where use of vermicompost led to significant improvements in both growth and flowering of crossandra compared to untreated control plants. Vermicompost contains most nutrients in plant available form such as nitrates, phosphates and exchangeable calcium and soluble potassium (Edwards, 1998; Orozco *et al*., 1996). Vermicompost have large particulate surface areas that provide many micro sites for microbial activity and for the strong retention of nutrients (Shi-wei and Fu-Zhen, 1991). Vermicomposts are rich in microbial populations and diversity, particularly fungi, bacteria and actinomycetes (Edwards, 1998; Tomati *et al*., 1987), which consistently promote biological activity. This helps the plants to germinate, flower and yield better than in commercial container media, independent of nutrient availability (Atiyeh *et al*., 2000). Vermicompost contains plant growth regulators and other plant growth influencing materials produced by microorganisms (Grappelli *et al*. 1987, Tomati *et al*., 1987).

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Effect of climate change on the productivity of mango cv. Banganpalli under Andhra Pradesh agroclimatic conditions

A. Bhagwan, A. Kiran Kumar, K. Purushotam and A. K. Misra[1]

Fruit Research Station, Sangareddy, Medak, A.P., India
[1]Central Institute for Subtropical Horticulture, Rehmankhera, P.O. Kakori, Lucknow, U.P., India
E mail : aravabhagwan@rediffmail.com; misra_a_k@yahoo.co.in

ABSTRACT

Regular bearing mango cv. Banganpalli of Andhra Pradesh showed extremely poor and delayed flowering and progressive decrease in productivity during 2007, 2008, 2009 and 2010. The estimated period of peak flower bud differentiation under Andhra Pradesh conditions is likely to be from November to December. Maximum and minimum temperature during November to February greatly influences the productivity of mango cv. Banganpalli. The number of fruit per tree of mango cv. Banganpalli of above 20 years (age) at Fruit Research Station, Sangareddy from 2006 to 11 (6 years) were correlated with corresponding temperature during flower bud differentiation and flowering. Significantly highest number of fruit tree^{-1} (530) was recorded during the year 2006. From 2007, the number of fruit tree^{-1} significantly decreased till 2010. Night temperature of less than 15^{0}C for maximum no of days has been recorded during November-January during 2006 and 2007 which is conducive for flower bud differentiation. However, during 2008, 2009 and 2010 night temperature of less than 15^{0}C was recorded for minimum number of days during January-February resulting in to late, erratic and multiple flowering flushes and subsequently recorded lowest number of fruits tree^{-1} compared to the year 2006. Further, during the same years the flower panicles were exposed to the more than 35^{0}C during February resulting in poor fruit set and flower drop, which ultimately resulted in the drop of the number of fruit tree^{-1} during 2008-10. Analysis of the temperature data showed that the shift of low night temperature of less than 15^{0}C from November-December to January-February and subsequent sudden rise in temperature of above 35^{0}C during February has progressively delayed the flowering and subsequently exposed the emerging flower panicle to high day temperatures might have resulted in less no of perfect flowers, poor fruit set and decrease productivity of mango cv. Banganpallalli during the last 4 years.

Key words: *Mangifera indica*, flowering, fruit set, temperature

In mango the flower bud differentiation and emergence of panicle is preceded by flower induction. Besides nutritional and environmental factors, mango flowering is greatly influenced by the environmental factors like temperature and rainfall (Chacko, 1984). In Andhra Pradesh conditions, the entire process from induction to flowering is completed during the period of November to February. The peak flower bud differentiation occurs from November to December with flowering during January-February. In Andhra Pradesh, mango is grown in three regions viz. Telangana, Rayalaseema and Coastal areas. The mango requires chilling temperature for the flower bud differentiation for a certain period. For proper flower bud differentiation, shoot maturity, stress and chilling temperature, especially night temperatures, are important factors (Nunez and Davenport, 1993). A night temperature of less than 15^{0}C for two to three weeks is necessary for proper flower bud differentiation (Davenport, 2000, 2003; Nunez and Davenport, 1995). Generally in Telangana and Rayalaseem regions, such low night temperature, reaches during the month of November-December. By first fortnight of January, as the night temperature rises, the flower panicles emerge. The total flowering period for commercial cultivar of mango Banganpalli is typically 25 days. The fruit set occurs during the last week of January to first fortnight of February, when the temperature are conducive for the fruit set. Of late, for the past four years, the flowering in mango under Andhra Pradesh (India) condition is erratic and late. Further, the flowering is occurring in three of four flushes resulting into poor fruit set in early flushed flowers. The problem is further compounded by the incidence of pest and diseases, when the late or early emerged flowers are exposed to conducive temperature conditions for the development of hopper and powdery mildew, which are the important problem during flowering period. The overall decrease in fruit set in late emerged flower panicles and fruit retention is reducing progressively the productivity of mango over past four years. The data on the bearing of the mango cv. Banganpalli over the past four year at Fruit Research Station, Sangareddy corroborates the reduced productivity. In the farmers field the late flowering is compounded by poor water management apart from difficulty in the management of mango hopper and powdery mildew resulting into poor crop load and economic loss. To identify whether, the temperature

aberration during the past five years (2006-10) is contributing the low productivity, the temperature from November to March of 2006-10 is compared with yield data during corresponding period.

MATERIALS AND METHODS

The daily record of maximum and minimum temperature and rainfall for the period of November to March during the year 2006-10 was recorded by Automatic weather station at Fruit Research Station, Sangareddy (A.P). The daily and weekly averages along with deviation from normal (aberrations) for corresponding weeks of the years 2006 to 2010 of the above weather parameters were obtained and plotted to identify the aberrations and correlated with the yield (number of fruits tree^{-1}) of the mango cv. Banganpalli. Yield record of a total of the 17 mango trees (cv. Banganpalli) of above 20 years of age, where the yields have stabilized, were taken and subjected to statistical analysis and correlated with the temperatures. All the general production and plant protection measures were equally taken for all the 17 trees from 2006 to 2010.

Mango phenology

In Andhra Pradesh, the flower bud differentiation occurs in the month of November and December. For the proper flower bud differentiation night temperature of less than 15^{0}C for 2-3 week is essential (Davenport, 2000). In addition to the temperature requirement, proper shoot maturity in terms of C to N ratio and stress condition (withholding irrigation during November and December) are essential factors for the proper flower bud differentiation in mango. The peak vegetative phase, which starts from July, ends by November month with the arrival of winter season. During November and December the flower bud differentiation initiates and the mango trees enter dormancy. As the temperature rises during January, the differentiated flower panicles emerge and by the end of January, the flower opens completely, followed by the fruit set. The normal peak flowering of mango occurs during the month of January. The fruits set generally occur during February when the mean day temperatures are below 35^{0}C. The peak fruit development occurs during the month of Februray, March and April. The mango fruit cv. Baganpalli development stops by the end of April and the fruit matures by 2nd fortnight of May. The harvesting period of mango cv. Banganpalli starts from 2nd fortnight of May and extends till 1st fortnight of June.

Climate in Andhra Pradesh

The Andhra Pradesh is blessed with very good agro-climate throughout the year. The normal annual rainfall of Andhra Pradesh is 800-1000mm. The rainy season in Andhra Pradesh extends from June to September. However, rarely late showers are received during October-November. The maximum and minimum temperature during rainy season is 32^{0}C and 22^{0}C respectively. The normal winter season starts from the 2nd fortnight of November and continues till 2nd fortnight of January. The average maximum temperature and minimum temperature is 29^{0}C and 12^{0}C respectively during the month of December. From 2nd fortnight of January the temperature starts rising. The average maximum and minimum temperature is 34^{0}C and 17^{0}C during the month of February. Typical summer starts from March and continues till the end of May. The average peak summer temperature is 40^{0}C. The maximum temperature of 44-45^{0}C for 2-3 days on an average is reached during the month of May.

RESULTS AND DISCUSSION

The number of fruits tree^{-1} of mango cv. Banganpalli differed significantly during the year 2006 to 2010 (Table 1). Significantly maximum number of fruits tree^{-1} (530) was recorded during the year 2006. From the year 2007 to 2010, there was progressive decreas in number of fruits tree^{-1} with significantly minimum (273) recorded during the year 2010, which was at par with 2009 (280). Further, the number of fruit tree^{-1} during the year 2007 (395) and 2008 (386) were at par.

It was observed that during the year 2005, minimum temperature of less than 15^{0}C was recorded for maximum days during November and December (Table 2) resulting in to normal flowering. Minimum night temperature of less than 15^{0}C for 2-3 week during the inductive period is essential for mango flowering (Davenport, 2000). Low minimum temperatures in November are congenial for the mango flower bud differentiation with normal good flowering during the month of January in southern Indian conditions (Ravishanker *et al.*, 1979). Further normal mean day temperatures of less than 35^{0}C (Table 2) were recorded during the months of March, 2006 resulting in good fruit set. All the normal climate parameters during 2006 were responsible for the significantly higher fruit number tree^{-1} (530) as compared to subsequent years, 2007 to 2010.

During the year 2006 and 07, the minimum temperature of less than 15^{0}C shifted to December 2006 and January 2007 (Table 2 and 3), resulting in late flowering during the month of February. The late emergence of flower panicle during 2007 and 08 than the normal time (as during 2006) reduced significantly the number of fruits tree^{-1} (395 and 386).

During 2007-09, even though minimum temperature of less than 15^{0}C was achieved during November, lowest

mean night temperature of 9.3^{0}C during December, 2007 and 9.6^{0}C during January, 2008 (Table 2 and 3) has resulted in very late and multiple flowering flushes. Ravishanker et *al.,* (1979) have found that continued low temperature appears to exert a depressing effect on further development of the flower buds of mango. The emergence of the flower panicle was delayed greatly even up to end of March, 2008 due to low mean night temperatures of 19.0^{0}C during the period. This might have resulted in significantly reduced number of fruits tree^{-1}(386) which is at par with 2007 (395.8) where similar aberrations in climate were recorded.

During the 2008, minimum temperature of less than 15^{0}C was recorded for 18 days only during the month of December (Table 2). Further, this low temperature was discontinuous during January, 2009. For flower bud differentiation minimum temperature of less than 15^{0}C for a continuous period of 2-3 week is essential (Davenport 2000, 2003). Further, under South Indian conditions higher night temperature of above 17^{0}C was found to be detrimental for the flower bud differentiation (Murthy and Upreti, 2004). Due to this reason, the percentage flowering was less in Medak district of Andhra Pradesh. Further, the late emerged flower panicle was exposed to high mean day temperature of above 35^{0}C, which might have resulting in to poor fruit set due to poor pollen viability (Issrakaraisila and Considine, 1994). As a result of less flowering and poor fruit set the yield in terms of number of fruits tree^{-1} was significantly reduced (280) when compared to previous years (Table 1)

During the year, 2009, minimum temperature of less than 15^{0}C for sufficient period (2-3 weeks) was not recorded during November, December, 2009, January and February, 2010 (Table 2 and 3). Due to this reason, the flowering was

Table 1. Yield data (No. of fruits tree^{-1}) of mango cv. Banganpalli during 2006-11

Year	No. of fruits tree^{-1}
2006	530.3^a
2007	395.8^b
2008	386.0^b
2009	280.0^c
2010	273.0^c
S. Em	35.87
CD (at 5%)	100.8

Each value is a mean of 17 trees.
Figures with same alphabet did not differ significantly

Table 2. Number of days below 15^{0}C (minimum temperature) during the year 2005-09

Month and year	No. of days below 15^{0}C (Mean min. temp.)	Month and year	No. of days below 15^{0}C (Mean min. temp.)
November, 2005 (Fruiting season 2006)	19 (14.2)	December, 2005 (Fruiting season 2006)	20 (13.6)
November, 2006 (Fruiting season 2007)	1 (17.0)	December, 2006 (Fruiting season 2007)	22 (13.2)
November, 2007 (Fruiting season 2008)	12 (14.6)	December, 2007 (Fruiting season 2008)	30 (9.3)
November, 2008 (Fruiting season 2009)	6 (14.8)	December, 2008 (Fruiting season 2009)	18 (12.1)
November, 2009 (Fruiting season 2010)	3 (16.4)	December, 2009 (Fruiting season 2010)	10 (14.4)

Figures in parenthesis are monthly mean temperatures during the corresponding period.

Table 3. Number of days below minimum temperature 15^{0}C and above maximum temperature 35^{0}C during the year 2006-10

Month/Year	No. of days below 15^{0}C (Mean min. temp.)	Month/Year	No. of days below 15^{0}C (Mean min. temp.)	No. of days above 35^{0}C (Mean max. temp.)
January 2006	30 (12.9)	February 2006	23(13.5)	2(32.5)
January 2007	23 (13.6)	February 2007	3 (15.4)	0 (32)
January 2008	25 (9.6)	February 2008	12 (15.8)	0 (28.4)
January 2009	6 (15.4)	February 2009	0 (16.4)	18 (30.1)
January 2010	6 (15.4)	February 2010	0 (17.5)	18 (33.9)

Figures in parenthesis are monthly mean temperatures during the corresponding period.

only 30-40% in the Andhra Padesh resulting in low yield during the period. Further, the late emerged flower panicle were exposed to high day temperature of above 36^0C (Table 4) during March 2009 which might have reduced the fruit set. A mean day temperature of above 35^0C results in the poor pollen viability (Issrakaraisila and Considine, 1994) and fruit set in mango. All the above deviation in the climate when compared to 2006 resulted in drastic reduction in number of fruits tree^{-1} (273) of up to 50% when compared to year 2006-07 (530).

It is therefore inferred that in Andhra Pradesh that due to climate change especially the temperature changes, during the flowering and fruiting of mango adversely affect the flowering of mango in cv. Banganpalli. The erratic, and late flowering in different flushes of mango during the past 4 years can be correlated to the temperature (both day and night) fluctuation from November to May (the peak flowering and fruiting season of mango). It is also noted from the feedback of the farmers, that the effects of climate change are severe in those orchards which are deficient in soil moisture and poorly maintained orchards.

Table 4. Number of days below minimum temperature 15^0C and above maximum temperature 35^0C during the year 2006-10

Year	No of days below 15^0C (Mean min. temp.)	No of days above 35^0C (Mean max. temp.)
March 2006	2 (19.7)	13 (33.5)
March 007	0 (19.7)	0 (34.2)
March 2008	0 (19.0)	0 (31.1)
March 2009	0 (17.1)	30 (36.2)
March 2010	0 (20.6)	30 (38.4)

Figures in parenthesis are monthly mean temperatures during the corresponding period.

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Response of different combination of organic manures for production of turmeric (*Curcuma longa* L.)

S.P. Singh

AICRPS on Spices, Department of Horticulture, Tirhut College of Agriculture, Dholi, Muzaffarpur, RAU, Bihar, India

ABSTRACT

A field experiment was conducted during kharif season of 2007-2008 and 2009-2010 to study the effect of different combination of organic manures on growth, yield and cost : benefit ratio of turmeric (*Curcuma longa* L.). Soil application of different organic manurial combination significantly influenced the growth and yield attributes of turmeric during three year of consecutive experimentation. However, soil application FYM 30 t ha^{-1} + vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1} produced the maximum plant height (118.06 cm), number of tillers per plant (5.22), number of leaves per tiller (13.53) and fresh projected yield (48.82 t ha^{-1}), giving maximum profit of Rs. 3.64 per unit cost as compare to other organic manorial combination.

Key words: Turmeric (*Curcuma longa* L.), FYM, vermicompost, neem oil cake, yield attributes.

Turmeric (*Curcuma longa L.*) is an important spices as well as medicinal plant extensively used in Ayurveda, Unani and siddha medicines as home remedy for various diseases. Turmeric is used as a food additive (spices), preservative and colouring agent.

Organic farming provides better and balanced environment, better food and living condition to the human beings. Among the spices crops, turmeric is an important spice grown commercially for local demand and also for export. In turmeric, the information on the effect of organic farming using various organic manures and organic growth stimulants are very meager.

MATERIALS AND METHODS

The experiment was conducted three consecutive years from 2007-2010 at experimental plot at Tirhut College of Agriculture, Dholi, Muzaffarpur (Bihar) under All India Coordinated Research Project on Spices (ICAR). Experiment was laid out in Randomized Block Design with three replication and fourteen treatments. Treatment details are as follows.

T_1 : FYM 30 t ha^{-1} alone

T_2 : Vermicompost 20 q ha^{-1} alone

T_3 : FYM 30 t ha^{-1} + Neem oil cake 8 q ha^{-1}

T_4 : Vermicompost 20 q ha^{-1} + Neem oil cake 8 q ha^{-1}

T_5 : FYM 30 t ha^{-1} + Vermicompost 20 q ha^{-1} + Neem oil cake 8 q ha^{-1}

T_6 : FYM 20 t ha^{-1} + Vermicompost 20 q ha^{-1} + Neem oil cake 8 q ha^{-1}

T_7 : FYM 10 t ha^{-1} + Vermicompost 20 q ha^{-1} + Neem oil cake 8q ha^{-1}

T_8 : FYM 30 t ha^{-1} + Vermicompost 15 q ha^{-1} + Neem oil cake 8q ha^{-1}

T_9 : FYM 20 t ha^{-1} + Vermicompost 15 q ha^{-1} + Neem oil cake 8q ha^{-1}

T_{10} : FYM 10 t ha^{-1} + Vermicompost 15 q ha^{-1} + Neem oil cake 8q ha^{-1}

T_{11} : FYM 30 t ha^{-1} + Vermicompost 10 q ha^{-1} + Neem oil cake 8q ha^{-1}

T_{12} : FYM 20 t ha^{-1} + Vermicompost 10 q ha^{-1} + Neem oil cake 8q ha^{-1}

T_{13} : FYM 10 t ha^{-1} + Vermicompost 10 q ha^{-1} + Neem oil cake 8q ha^{-1}

T_{14} : Absolute Control

The crop was sown in last week of May every year. The plot size was 3.0 x 1.0 m. The plants were spaced at row to row distance of 30 cm and rhizome/plant to rhizome/plant 20 cm. All the treatments of organic fertilizer were incorporated in the soil during field preparation and there was no application of organic and inorganic in control plot (Absolute Control).

For recording the observation, the technique of random sampling was adopted and five plants per net plot were randomly selected in each treatment from all the replication for recording the observation on growth character like height of the plant (cm), number of tiller $plant^{-1}$, number of leaves $tiller^{-1}$, yield $plot^{-1}$ (kg/$3m^2$), projected yield (t ha^{-1}) and cost : benefit ratio.

RESULTS AND DISCUSSION

Soil application of organic manures significantly improved the plant height (cm), number of tillers plant^{-1}, number of leaves tiller^{-1}, yield plot^{-1} and ha^{-1} (Table 1). The plant height was significantly increased with all the treatments of organic manure as compared to absolute control and treatments FYM 30 t ha^{-1} (T_1), vermicompost 20 q ha^{-1} alone (T_2) and FYM 30 t ha^{-1} + neem oil cake 8 q ha^{-1} (T_3). Maximum plant height of 118 cm was recorded with the treatment FYM 30 t ha^{-1} + vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_5) followed by treatment FYM 30 t ha^{-1} + vermicompost 15 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_8) i.e. 112 cm. The lowest plant height of 93.48 cm was noticed with the treatment of vermicompost 20 q ha^{-1} alone. Since, the plant height is an important yield attribute in turmeric, any practice to alter the plant height would influence the rhizome yield as reported by Vincent (1980). Similar results are reported by Sanker *et al.* (2009) in onion, Singh *et.al.* (2009) in ginger and Balkrishnamurthi *et.al.* (2009) in turmeric.

In present experiment, the soil application of organic manures increased the plant height which may be due to increased uptake of nitrogen. Constituent of protein and protoplasm vigrously induce the vegetative development of the plants. The plant height in single dose of manure was comparatively low, which could be due to poor nutrient absorption from soil. Combined application of farmyard manure + vermicompost + neem oil cake gave the maximum plant height compared to absolute control. The increased water holding capacity, reduction of bulk density, improved particle density, pore space, texture, soil available nutrient status favorably influenced the root growth and development, there by indirectly influencing the increased plant height.

The number of tillers was significantly increased with soil application of organic manures like FYM 30 t ha^{-1} + vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_5), FYM 20 t ha^{-1} + vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_6), FYM 30 t ha^{-1} + vermicompost 15 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_8), FYM 30 t ha^{-1} + vermicompost 10 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_{12}) as compared to absolute control. Among the treatments, maximum number of tillers (5.22) was recorded with soil application of FYM 30 t ha^{-1} + vermicompost 20 t ha^{-1} + neem oil cake 8 q ha^{-1} (T_5). The lowest number of tillers (3.29) was recorded with the treatment of vermicompost 20 q ha^{-1} alone and absolute control (T_{14}). Since, the number of tiller is an important yield attribute in turmeric, it would influence the rhizome yield and mother rhizome as reported by Balkrishnamurthy *et.al.* (2009) in turmeric and Singh *et.al.* (2009) in ginger. FYM with narrow C : N ratio may produce more humic acid and the humic substances contained in its form chelates with phosphorus.

Table 1. Response of different treatments of organic components on growth of turmeric plant

Character	Height of the plant (cm)			Pooled	No of tillers plant^{-1}			Pooled	No of leaves plant^{-1}			Pooled
Treatment	2007-08	2008-09	2009-010	mean	2007-08	2008-09	2009-010	mean	2007-08	2008-09	2009-010	mean
1	2	3	4	5	6	7	8	9	10	11	12	13
T_1	95.33	103.07	83.87	94.09	2.60	3.67	4.00	3.42	12.80	13.80	9.53	12.04
T_2	93.00	104.13	83.33	93.48	3.07	3.60	3.20	3.29	13.07	13.40	9.13	11.86
T_3	103.33	107.27	85.13	98.57	3.47	3.27	3.87	3.53	13.00	13.40	9.40	11.93
T_4	109.00	105.20	86.93	100.37	3.53	3.67	3.40	3.53	12.93	13.46	9.00	11.79
T_5	126.67	126.00	101.53	118.06	5.40	5.47	4.80	5.22	14.60	14.67	11.33	13.53
T_6	116.33	117.47	99.07	110.95	4.40	4.57	4.13	4.36	13.93	14.07	10.53	12.84
T_7	106.00	109.07	90.60	101.89	4.13	3.93	3.40	3.82	13.60	13.73	9.20	12.17
T_8	120.00	119.67	97.40	112.35	4.80	5.00	4.53	4.77	14.20	14.67	10.53	13.09
T_9	109.33	115.60	94.40	106.44	3.07	4.53	3.93	3.84	13.87	14.07	9.73	12.55
T_{10}	107.67	113.33	89.87	103.62	3.93	3.93	3.67	3.84	13.40	13.80	9.20	12.13
T_{11}	117.67	114.73	93.27	108.55	4.13	4.26	4.60	4.33	13.80	14.07	10.53	12.80
T_{12}	116.67	114.07	88.20	106.31	3.40	4.47	3.87	3.91	13.53	14.20	9.53	12.42
T_{13}	110.00	107.67	84.80	100.82	3.20	3.87	3.13	3.40	13.20	13.60	9.13	11.97
T_{14}	92.00	96.67	81.07	89.91	3.27	3.07	3.00	3.29	12.93	12.73	8.67	11.44
SEM±	10.62	5.89	2.68	1.88	0.28	0.19	0.17	0.21	0.15	0.83	0.52	0.15
CD (P=0.05)	30.88	NS	7.80	5.48	0.81	0.54	0.49	0.61	0.44	NS	NS	0.44
CV (%)	16.91	9.14	5.17	3.16	12.03	7.80	7.70	9.38	1.92	10.39	9.47	2.15

The number of leaves tiller^{-1} was significantly promoted with all the treatments of organic manures as compare to absolute control and treatment i.e. vermicompost 20 q ha^{-1} alone (T_2) and vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_4). The highest number of leaves per tiller (13.53) was noticed with treatment of FYM 30 t ha^{-1} + vermicompost 20 q ha^{-1} + neem oil cake – 8 q ha^{-1} (T_5). The lowest numbers of leaves tiller^{-1} (11.79 & 11.44) were recorded with the organic treatment i.e. vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_4) and absolute control (T_{14}) respectively.

A crop should produce sufficient number of leaves to harness light energy and synthesize adequate photoassimilates for biomass production. In the present study, the number of leaves produced at various stage of growth and development from month after planting to harvest revealed that FYM 30 t ha^{-1} + vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_5) gave the maximum number of leaves tiller^{-1} followed by FYM 30 t ha^{-1} + vermicompost 15 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_8) manure helped in maintaining a higher number of leaves throughout the cropping period. This might be due to the adequate availability and supply of nutrients in proportion, which ultimately result in triggering the production of plant growth hormones viz. IAA and other hormones. The present findings are in consonance with the previous observation of Cosenova *et al.* (1990), Singh *et al.* (2009) and Sanker *et al.* (2009).

The average yield of turmeric plot^{-1} and projected yield were significantly increased with all the treatments of organic manures (Table 2) as compared to absolute control and treatments like vermicompost 20 q ha^{-1} alone (T_2) and FYM 10 t ha^{-1} + vermicompost 10 q ha^{-1} + neem oil cake 8 q ha^{-1} (T_{13}). The highest average yield 14.64 kg/3m^2 plot^{-1} and projected yield 48.82 t ha^{-1} were recorded with the treatment of FYM 30 t ha^{-1} + vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1}, followed by FYM 30 t ha^{-1} + vermicompost 15 q ha^{-1} + neem oil cake 8 q ha^{-1} i.e. average yield plot^{-1} 13.52 kg/3m^2 and average projected yield 45.07 t ha^{-1}. The lowest average yield plot^{-1} 9.74 kg/3m^2 and average projected yield 32.48 t ha^{-1} were recorded by organic farming treatment of vermicompost 20 q ha^{-1} alone (T_2).

When different doses of organic manure were compared for finding the best dose for highest yield and maximum profit, the treatment of FYM 30 t ha^{-1} + vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1} was recommended due to production of highest yield (48.82 t ha^{-1}) and more net return Rs. 3.64 unit cost^{-1} (1:3.64).

In the organic treatments, farmyard manure increases the soil organic matter and improve the soil structure and biological activity of soil. This would have reduced the loss of nitrogen by increased caution and enhancing the turmeric rhizome development and yield. Further, by improving the

Table 2. Response of different treatments of organic components on yield of turmeric

	Yield plot^{-1} (kg)			Pooled mean	Yield t ha^{-1}			Pooled mean	Cost : Benefit ratio
	2007-08	2008-09	2009-010		2007-08	2008-09	2009-010		
	14	15	16	17	18	19	20	21	22
T_1	10.30	13.27	7.83	10.46	34.33	44.22	26.11	34.88	1:3.05
T_2	10.37	12.70	6.17	9.74	34.56	42.33	20.56	32.48	1:2.85
T_3	10.67	12.00	8.67	10.54	36.56	40.00	28.89	35.15	1:2.88
T_4	11.00	13.07	6.67	10.24	36.67	43.55	22.22	34.14	1:2.80
T_5	15.20	18.07	10.67	14.64	50.67	60.22	35.57	48.82	1:3.64
T_6	13.87	15.17	9.00	12.68	46.22	50.57	30.00	42.26	1:3.25
T_7	11.67	14.40	6.83	10.96	38.89	48.00	22.78	36.55	1:2.90
T_8	14.73	15.83	10.00	13.52	49.11	52.78	33.33	45.07	1:3.44
T_9	12.00	15.07	8.17	11.74	40.00	50.22	27.22	39.14	1:3.08
T_{10}	11.77	13.83	6.50	10.70	39.22	46.11	21.67	35.66	1:2.90
T_{11}	12.67	14.73	9.50	12.30	42.22	49.11	31.67	41.00	1:3.20
T_{12}	11.23	14.53	7.17	10.97	37.44	48.44	23.89	36.59	1:2.95
T_{13}	9.73	13.73	6.17	9.87	32.44	45.77	20.56	32.92	1:2.74
T_{14}	10.33	11.17	4.67	8.72	34.45	37.22	15.55	29.07	1:2.85
SEM±	0.93	0.75	0.23	0.42	3.11	2.52	0.77	1.41	-
CD(0.05)	2.71	2.20	0.67	1.23	9.04	7.32	2.24	4.12	-
cv(%)	13.65	9.27	5.21	6.56	13.64	9.26	5.20	6.57	-

structure of the soil by more aggregation, water holding capacity and air permeability are increased. These comprehensive changes in soil might improve the rhizome development. This is in line with the finding of Mizuno (1996). All these comprehensive changes paved the way for greater fresh and average weight of rhizomes. Further, the reduced loss of Nitrogen by ammonia volatilization and narrower C : N ratio might have also contributed to the better performance of crop supplied with farmyardmanure (Kirchmann and Witter, 1992). Another possible reason for pronounced yield improvement in organic treatment might be due to sustained availability of nitrogen in the soil throughout the growing phase and also due to enhanced carbohydrate synthesis and effective translocation of this photosynthesis to the plant. Lower fertility level plants remain stunted resulting in decreased yield.

It was concluded that the combined soil application of FYM 30 t ha^{-1} + vermicompost 20 q ha^{-1} + neem oil cake 8 q ha^{-1} recorded the highest yield and more return as compared to other doses of organic treatments.

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Standardization of dose and time of soil application of Cultar on flowering and yield in mango cv. Banganpalli

A. Bhagwan, K. Vanajalatha, S.K. Sarkar, A. Girwani and A.K. Misra[1]

Fruit Research Station, Sangareddy, Medak - 502 001, Andhra Pradesh, India
[1]Central Institute of Subtropical Horticulture, Rehmankhera, PO. Kakori, Lucknow – 226 101, U.P., India

ABSTRACT

Excessive vegetative growth and poor fruit set at the time of blossoming of the mango (*Mangifera indica* L.) are identifiable cause of low yield in mango cv. Banganpalli (3-7 t ha^{-1}) in subtropical regions of India. Even though several experiments have confirmed the promotional effects of paclobutrazol on flower induction and yield of mango tree, little is known about the exact dose and time of application of Cultar (25% paclobutrazol) with reference to cv. Banganpalli, a prominent mango variety of south India. Hence, a five year study on the effect of soil application of Cultar with different dosages (i.e. 3 ml and 5 ml m^{-1} of canopy diameter) at different times of application before flowering (i.e. 120, 90 and 60 days before bud break) in 15 year old mango cv. Banganpalli was investigated. The experiment was conducted at Fruit Research Station, Sangareddy, A.P, India from 1999-2004, using randomized block design with 4 replications. Cultar was applied in the month of September, October and November every year corresponding to the 120, 90 and 60 days before bud break. Last week of December is the flower bud break time in southern India. Data on shoot growth, internodal length, per cent flowering, per cent perfect flowers, fruit set and yield was recorded. Pooled analysis of 5-year data revealed that 5 ml m^{-1} diameter Cultar application significantly reduced the vegetative growth, increased percentage of shoots flowered and per cent perfect flowers as compared to 3 ml m^{-1} diameter and untreated mango trees (cv. Banganpalli). Irrespective of the dosage of Cultar, soil application of Cultar at 120 days before bud break significantly reduced vegetative growth, increased perfect flowers, fruit set and yield over 90 and 60 days before bud break and untreated trees. Application of Cultar at either 3 or 5 ml m^{-1} canopy diameter at 120 days before bud break equally increased the yields upto 35% over control.

Key words: *Mangifera indica*, Cultar, fruit set, perfect flowers, paclobutrazol, internodal length, panicle length, flowering

Mango is one of the most widely cultivated tropical fruits in the world. In India it cultivated in about 1,963,000 ha. Excessive vegetative growth at the time of blossoming results poor fruit set and low yields (3-7 t ha^{-1}) in mango (*Mangifera indica* L.) in cv. Banganpalli. Under South Indian condition, the flower bud differentiation starts at the end of December and fruits come to maturity by May. However, temperature fluctuations often occur in December, resulting in poor and delayed flowering. The delay in flowering results in poor fruit set leading to low yield. Normal flowering is necessary to obtain consistent production of mango under subtropical climate conditions. Flowering from one season to the other is not normal and is unreliable because of the environmental signals for flower initiation. Understanding of the factors controlling flower bud initiation in mango trees has been very complex. An alterative to dependence upon environmental signals for flower initiation is the development of management strategies that can substitute these signals.

A number of diverse chemicals that have growth regulating properties in plants have been tested for promoting/inhibiting flower production in mango in different countries (Chacko, 1991). Paclobutrazol, a strong gibberellin biosynthesis inhibitor, has been reported effective in promotion of flowering in many fruit crops species including mango (Dissanayake, 1989; Tongumpai *et al*., 1989; Burondkar and Gunjate, 1991; Charnvichit *et al*., 1991; Winston, 1992; Tongumpai *et al*., 1997). Paclobutrazol as soil drench and foliar spray were effective for promotion of flowering in mango, however, soil drench was more significant, convenient and cost effective (Burondkar and Gunjate, 1991; Winston, 1992).

The effectiveness of the paclobutrazol in inducing flowering of mango depends upon cultivar and time of application (Hasdiseve and Togumpai, 1986; Tongumpai *et al*., 1989, Chacko, 1991). Therefore, growth regulator chemicals and application rates which can promote or induce flowering must be tested under local conditions for the locally available mango varieties. However, little work has been done in Andhra Pradesh, in controlling the vegetative growth of locally grown mango varieties (cv. Banganpalli) with the use of paclobutrazol, as there is no information on standard dose and time of application of Cultar under south Indian condition with reference to cv.

Banganpalli. This paper attempts to standardize the dose and time of application of the Cultar on commercial variety of mango cv. Banganpalli which is the most important mango variety under south Indian conditions.

MATERIALS AND METHODS

Fifteen year old mango trees, variety Banganpalli, of uniform vigour and size and growing at Fruit Research Station, Sangareddy, Andhra Pradesh, were used as experimental plant material. All experimental trees received similar cultural practices, namely fertilizers, irrigation and plant protection sprays. Cultar containing 250 g a.i l^{-1} paclobutrazol (Cultar ICI, Plant Protection Plc, U.K) was applied in two different dosages of 3 ml m^{-1} diameter and 5 ml m^{-1} diameter of canopy diameter and at 120, 90 and 60 days before flower bud break (September, October and November months). The experimental layout was a randomized block design, of single tree plots in four complete replications. Cultar was applied as soil collar drench. Under south Indian conditions, the flower bud differentiation takes place in last week of December. The annual growth of shoot and internodal length (average of 25 tagged shoots $tree^{-1}$) were recorded one year after the treatments. The data on flowering was assessed as percentage of the shoots flowered using randomly selected 50 tagged shoots. The length of panicle, percentage of perfect flower, (average of 20 tagged panicles $tree^{-1}$) at full bloom stage and fruit set at peanut stage were recorded 2 weeks after full bloom (average of 20 tagged panicles $tree^{-1}$). Fruit yield was recorded in kilograms and number of fruits $tree^{-1}$. Data on above parameters were recorded for five years (1999 to 2004) and pooled analysis was done using randomized block design with factorial concept of two levels of dosage and three different times of application of Cultar as factors.

RESULTS AND DISCUSSION

Vegetative growth

The application of Cultar significantly reduced the vegetative growth in mango trees in cv. Banganpalli in terms of reduction in annual shoot growth and internodal length of terminal shoot at bud break (Table 1). Irrespective of time of application, 5 ml m^{-1} diameter application of Cultar significantly lowered the annual shoot growth and internodal length. Application of Cultar at 120 days before bud break, irrespective of dosage, significantly lowered the vegetative growth. Least reduction in vegetative growth was observed with application of 3 ml m^{-1} diameter Cultar at 60 days before bud break (Table 1). The major biochemical effect of Cultar is suppression of gibberellin production by inhibiting the oxidation of kaurene to kaurenoic acid in gibberellin biosynthetic pathway (Jones, 1973). The reduction of gibberellin level reduces cell division and expansion and as a direct morphological consequence, vegetative growth is reduced (Dalziel and Lawrence, 1984). In the present study reduction in the vegetative growth in mango cv. Banganpalli indicates that the trees have taken up the Cultar. Further, the reduction was more pronounced at 120 days than at 60 days before bud break, indicating Cultar uptake and its anti-gibberellin effects. Cultar inhibits the gibberellin production and so reduces vegetative growth (Tongumpai *et al*., 1997). Soil application of Cultar was also found to be more effective in reducing the tree vigour in mango (Kulkarni, 1988; Rowley, 1990; Voon, *et al*., 1991) and other fruit crops (Lever, 1986).

Table 1. Effect of different dosage and time of application of Cultar on vegetative growth of mango in cv. Banganpalli

Treatment	Annual growth (cm) of the shoot				Internodal length (cm) of terminal shoot at bud break			
	Days before bud break				Days before bud break			
Dose	120	90	60	Mean	120	90	60	Mean
3 ml m^{-1}	23.50	25.25	30.25	26.33[a]	15.48	16.26	16.40	16.04[a]
5 ml m^{-1}	18.95	21.47	28.50	22.97[b]	12.55	15.91	15.90	14.78[b]
Mean	21.22[a]	23.37[b]	29.37[c]	24.65	14.01[a]	16.08[b]	16.15[b]	15.41
Control				30.20				18.08
	Dose	Time of application	Interaction	Control vs. Rest	Dose	Time of application	Interaction	Control vs. Rest
SEM	0.494	0.606	2.846	1.210	0.64	0.78	1.11	1.58
CD 5 %	1.078	1.320	NS	2.640	1.55	1.71	2.85	3.45

*Each data point is a pooled mean of 5 years data. *Means followed by the same letter did not differ significantly at 5% level.

Flowering parameters

The application of Cultar significantly promoted flowering and reduced the panicle length. Flowering intensity in terms of percentage of shoots flowered and length of flower panicles is depicted in Table 2. Irrespective of the different dosage, application of Cultar at 120 days before bud break increased the per cent shoots flowered and reduced the panicle length. However, there is no significant difference between application of Cultar at 90 and 60 days before bud break. Regarding the dosage, application of 3 and 5 ml m^{-1} of canopy diameter equally increased the percentage shoots flowered and reduced the panicle length as compared to control. The promotion in flowering may be ascribed either due to the increased synthesis of the floral stimulus in an inductive cycle or by affecting the balance between the flower inhibiting and flower promoting phytohormones. There are considerable evidences that gibberellins either exogenously applied or endogenously produced in plant, play inhibitory role in mango flowering. Kachru *et al.* (1971) and Nunez and Davenport (1991) reported that foliar spray of gibberellins is effective in suppression of flowering even under inductive conditions. In the present study application of Cultar probably promoted flowering in mango because it is a potent and highly specific inhibitor of gibberellin biosynthesis (Dalziel and Lawrence, 1984). When gibberellin synthesis is affected, flower bud is stimulated (Sergent *et al.*, 1996). The pronounced effect of application of Cultar at 120 days before bud break on flowering can be ascribed to the fact that the reduced vegetative growth (Table 1) in turn gave more time for the shoots to mature. In earlier studies, mango trees treated with Cultar showed early bud break about two weeks before the control trees (Peiris, 2000). It may also be argued that the reduced vegetative growth may have contributed to the promotion of flowering, because vigorous growth is known to antagonize the flowering in mango (Chacko, 1991).

Cultar shortened panicle length significantly (Table 2) and the compacted flower are visibly noted in treated trees. However, higher dosage (5 ml m^{-1} diameter) did not compact the flower panicles too much. Shorter flower panicles give an indication of the fact that Cultar has been taken up by the trees. Winston (1992) showed that panicle size reduction is due to effect of Cultar, but not caused by an increase in number of panicles.

Perfect flowers and fruit set

The application of Cultar significantly increased the number of perfect flowers and subsequent fruit set as compared to control. The effect of Cultar on percentage of perfect flowers and fruit set is shown in Table 3. Irrespective of the dosage, application of Cultar at 120 days before bud break significantly increased the percentage of perfect flowers and subsequent fruit set over 90 and 60 days before bud break, which are at par. Higher doses of Cultar (5 ml m^{-1}) significantly increased the percentage of perfect flowers over lower doses (3 ml m^{-1}). However, the increase in perfect flowers did not reflected significant increase in fruit set with higher doses of Cultar used (Table 3). Gibberellin has been shown to be powerful modifiers of sex expression,

Table 2. Effect of different dosage and time of application of Cultar on flowering parameters of mango in cv. Banganpalli

Treatment	No. of shoots flowered (%)				Panicle length (cm)			
	Days before bud break				Days before bud break			
Dose	120	90	60	Mean	120	90	60	Mean
3 ml.m^{-1}	79.73(67.14)	46.65(43.07)	51.55(45.88)	59.31(52.03)	21.99	22.42	22.03	22.15
5 ml.m^{-1}	74.88(64.70)	50.52(44.80)	48.20(43.96)	57.86(51.15)	18.95	22.50	23.20	21.55
Mean	77.30(65.92)^{a}	48.58(43.93)^{b}	49.87(44.92)^{b}	58.58(51.59)	20.47^{a}	22.47^{b}	22.61^{b}	21.85
Control				40.74(45.77)				24.47
	Dose	Time of application	Interaction	Control vs. Rest	Dose	Time of application	Interaction	Control vs. Rest
SEM	2.50	3.06	4.33	6.13	0.75	0.91	1.28	1.83
CD 5 %	NS	6.64	NS	13.36	NS	1.98	2.79	3.98

*Each data point is a pooled mean of 5 years data. *Means followed by the same letter did not differ significantly at 5% level.
Figures in parentheses indicate transformed angular values of percentage.

Table 3. Effect of different dosage and time of application of Cultar on perfect flowers and fruit set of mango in cv. Banganpalli.

Treatment	Perfect flowers panicle^{-1} (%)				Fruit set panicle^{-1} at pea nut stage			
	Days before bud break				Days before bud break			
Dose	120	90	60	Mean	120	90	60	Mean
3 ml.m^{-1}	10.13	8.23	9.21	9.19^{a}	11.33	15.18	9.24	11.92
5 ml.m^{-1}	12.21	9.78	9.39	10.46^{b}	15.95	8.95	12.44	12.44
Mean	11.17^{a}	9.00^{b}	9.30^{b}	9.82	13.64^{a}	12.06^{b}	10.84^{b}	12.18
Control				6.11				6.00
	Dose	Time of application	Interaction	Control vs. Rest	Dose	Time of application	Interaction	Control vs. Rest
S.EM	0.50	0.621	0.87	1.24	0.76	0.93	1.32	1.86
CD 5 %	1.10	1.35	NS	2.70	NS	2.03	2.88	4.07

*Each data point is a pooled mean of 5 years data *Means followed by the same letter did not differ significantly at 5% level.

particularly in cucurbits by favoring the production of staminate flowers. It has been demonstrated that by careful manipulation of gibberrellin using inhibitors can change sex ratio (Jones, 1973). In the present study, Cultar by specifically inhibiting gibberrellin might have increased the per cent perfect flowers as compared to control. Further, the increase in the fruit set with the application of Cultar may be attributed to the increased percentage of prefect flowers as compared to control (Table 3). Cultar was found to increase the per cent prefect flowers and fruit set in Dashehari mango (Singh, 2000) and Alphonso mango (Burondkar *et al.*, 1997) under north Indian conditions.

Yield

The yield of the mango trees were measured in terms of number of fruits tree^{-1} and weight (kg tree^{-1}). The application of Cultar significantly increased the yield of the mango in cv. Banganpalli over untreated trees (Table 4). Irrespective of the dosage, application of Cultar at 120 days before bud break significantly increased the fruit number tree^{-1} and yield (kg tree^{-1}) over 90 and 60 days before bud break, which were at par. However, there was non singinficant difference in the yields of mango between 3 and 5 ml m^{-1} canopy diameter application of Cultar irrespective of the time of application. Even though 5 ml m^{-1} canopy diameter application of Cultar significantly increased the per cent perfect flowers the same pattern was not reflected in the yields. The reduction in the vegetative growth of mango (Table 1) with the application of Cultar has given more time for the shoots to mature, which in turn enhanced the flower bud formation as reflected in the increase in the percentage of the flowering shoots (Table 2). The extended flowering period with the application of Cultar enhanced the percentage of perfect flower and fruit

Table 4. Effect of different dosage and time of application of Cultar on yield of mango in cv. Banganpalli

Treatment	Fruit number (tree^{-1})				Fruit yield (kg tree^{-1})			
	Days before bud break				Days before bud break			
Dose	120	90	60	Mean	120	90	60	Mean
3 ml m^{-1}	239.70	200.75	182.65	207.70	82.40	69.64	66.29	72.77
5 ml m^{-1}	246.40	205.40	184.75	212.18	92.07	58.24	65.35	71.88
Mean	243.05^{a}	203.07^{b}	183.70^{b}	209.94	87.23^{a}	63.94^{a}	65.82^{a}	72.36
Control				162.05				55.20
	Dosage	Time of application	Interaction	Control vs Rest	Dosage	Time of application	Interaction	Control vs Rest
S.EM	12.69	15.39	21.70	31.00	4.53	5.49	7.75	11.00
CD 5 %	NS	33.55	47.30	67.27	NS	11.96	16.82	23.98

* Each data point is a pooled mean of 5 years data * Mean followed by the same letter did not differ significantly at 5% level

set (Table 3). Application of Cultar at either 3 or 5 ml m^{-1} canopy diameter at 120 days before bud break increased the yields of mango in cv. Banganpalli upto 35% over control. Cultar appeared to favorably alter the source-sink relationship of mango to support increased yield and fruit growth with lower leaf number and area (Kurian *et al.,* 2001). Cultar similarly increased the yields of Dashehari mango (Singh, 2000) and Alphonso mango (Burondkar *et al.,* 1997) under north Indian conditions.

The results of the present study revealed that the Cultar application at 120 days before bud break at either 3 ml m^{-1} or 5 ml m^{-1} of canopy diameter is best for increasing the yields of mango up to 35 per cent over control. However, based on the cost benefit ratio, application of 3 ml m^{-1} canopy diameter at 120 days before bud break (September under south Indian conditions) proved to be effective and cost effective in increasing the yield of mango in cv. Banganpalli.

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Genetic variability and character association studies on post-flowering drought tolerance in Sorghum

Suvarna Patil, B. Fakrudin, Yemane Girma, Addissu Gabre, Rajkumar, Anand Khot, Y. D. Narayana and P. U. Krishnaraj

Institute of Agri-Biotechnology, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India
E-mail: bfakrudin@gmail.com

ABSTRACT

Two sorghum Recombinant Inbred Populations (RIPs) were evaluated to estimate the extent of genetic variability for the post-flowering drought tolerance at Dharwad (Karnataka) over seasons. Significant mean sum of squares were obtained in the pooled analysis of variance suggesting that, the RIPs were highly variable, therefore, would respond to selection. Pooled analyses indicated that most characters had higher phenotypic than genotypic variance estimates. Higher PCV and GCV were noticed for traits such as per cent GLA 30 DAF, per cent GLA 45 DAF and grain yield per plant, indicating selections could be made in the populations. Characters such as per cent GLA 30 DAF, per cent GLA 45 DAF, plant height and grain yield per plant responded positively to selection because of high broad sense heritability estimates. Per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area recorded higher genetic advance and it was moderate for grain yield per plant. As the economic end product was grain yield, which was positively and significantly correlated with traits per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF and total GLA. This indicates that stay-green trait have a significant grain yield advantage and can be successfully manipulated and incorporated into high yielding genetic backgrounds under post-flowering drought stress conditions.

Key words: Sorghum, recombinant inbred lines, post-flowering drought, genetic variability.

Sorghum (*Sorghum bicolor* L.) Moench is one of the most important staple food and fodder crops in parts of the arid and semi arid region of the world. Drought is a major limitation to crop productivity worldwide and possible global climate change scenarios suggest a future increase in the risk of drought (Ali *et al.*, 2009). Drought has diverse effects on yield depending on the development stage at which it occurs. Drought at both pre-flowering and post-flowering stages of development has the most adverse effect on yield (Kebede *et al.*, 2001). In sorghum, the best characterized form of tolerance to drought stress during post-flowering stage of crop growth is 'stay-green' trait. Stay-green is the ability of plant to withstand premature leaf and plant death, develop grain normally and resist charcoal rot and lodging when exposed to moisture stress during the late stages of grain development (Mahalakshmi and Bidinger, 2002).

The efficiency of improvement of genotypes may be enhanced by the identification of morphological traits associated with better yield response which in turn depends upon the nature and amount of variability present in the genetic stock and the extentd to which the desirable traits are heritable. Assessment of genetic diversity therefore becomes an essential prerequisite for identifying potential parents for hybridization. From reliable estimates of genetic parameters including heritability, genotypic and phenotypic coefficient of variations, and genotype × environment interaction, inferences can be made as to the type of gene action and appropriate breeding strategies could be formulated with respect to yield stability and cultivars adaptation across environments. The present investigation was conducted to know the extent of genetic variability and nature of interrelationship between grain yield and its contributory characters.

MATERIALS AND METHODS

The two RIPs each consisting of 226 F_{10-11} lines, were developed from the crosses IS 9830 x E 36-1 (RIP1) and N 13 x E 36-1 (RIP2). The F_5 seeds of mapping populations were obtained from the International Crops Research Institute for Semi Arid Tropics (ICRISAT), Patancheru, Hyderabad, India and were further advanced through single seed descent (SSD) method and maintained at Institute of Agri-Biotechnology (IABT), University of Agricultural Sciences (UAS), Dharwad, India. The E 36-1, a donor for stay-green trait and IS 9830 and N 13 are non stay-green types.

The two RIPs were evaluated at the Main Agricultural Research Station, Dharwad, during *rabi* season of 2007, 2008 and 2009 for post-flowering drought tolerance and yield

related traits. A set of 228 entries comprising of 226 RILs, two parents and checks were taken up for phenotyping. Simple lattice design was adopted on an area of 50 x 55 m^2 with four replications. Each replication consisted of 19 blocks, each with 12 entries planted randomly to accommodate 15 plants in a single row. The trial was laid out with a spacing of 45 x 15 cm^2 and all recommended package of practices were followed. In order to eliminate the border effects, three rows of M 35-1 were sown on all sides of the experimental plot.

Stay-green refers to genotypes with delayed leaf senescence during grain ripening under drought conditions. This was measured through an estimation of senescence from time of emergence of flag leaf and is visually estimated at weekly intervals according to Mahalakshmi and Bidinger (2002). Per cent GLA 15 DAF: mean green leaf area after 1st and 2nd week of flowering; per cent GLA 30 DAF: mean green leaf area after 3rd and 4th week of flowering; per cent GLA 45 DAF: mean green leaf area after 5th and 6th week of flowering. Total green leaf area at the time of flowering was computed by multiplying the per cent green leaf area with measured area (length x bredth) of each leaf and summing across the six measured leaves expressed in cm^2, plant height (cm), days to 50 per cent flowering and grain yield per plant (g) were also recorded.

Statistical analysis was carried out by pooling of the phenotypic data for all the three years. Analysis of variance for different characters was performed by applying the formula suggested by Burton and Devane (1953). Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated as suggest by Singh and Chaudhary (1977); heritability was estimated for all components as the ratio of total genotypic variance to the phenotypic variance (Falcanor, 1989). The extent of genetic advance expected through selection for each of the character was calculated as in Johnson *et al.* (1955). Phenotypic correlation coefficients and genotypic correlation coefficients were estimated by using the following formula suggested by Singh and Choudhary (1977).

RESULTS AND DISCUSSION

The pooled analysis of variance and the estimates of variability parameters such as PCV, GCV, heritability and genetic advance over per cent of mean for stay-green and yield related traits were presented (Table 1). The analysis of variance revealed highly significant difference among the RILs for all the traits in both populations and at both locations.

Phenotypic variability expressed by a genotype or a group of genotypes in any species can be partitioned into GCV and PCV. In present study, pooled analyses indicated that most characters had higher phenotypic than genotypic variance estimates in both RIPs. High PCV and GCV noticed for per cent GLA 30 DAF and per cent GLA 45 DAF indicates that these traits contributed markedly to the total variability and there is greater scope for improvement for these traits. Similar results were obtained by Haussmann *et al.* (2002) and Gebre (2009). Delaying the onset of leaf senescence and reducing its rate offer an effective strategy for increasing grain production, fodder quality and grain crop residues particularly under water limited conditions (Reddy *et al.*, 2007). The ability of sorghum plant to maintain green leaf area is positively associated with yield. High variability noticed for total green leaf area, extended greenness of leaves is expected to allow continued photosynthesis and better translocation from source to sink, which is likely to result in better grain fill and increased grain yield. In sorghum similar trend between stay-green and grain yield was noticed in

Table 1. Pooled analysis of variance and magnitude of variabilities of stay-green and yield related traits in the recombinant inbred populations.

		Mean sum of squares													
		RIP1							RIP2						
Component	d.f.	(%) GLA 15 DAF	(%) GLA 30 DAF	(%) GLA 45 DAF	Days to 50 (%) flowering	Total GLA	Plant height	Grain yield plant^{-1}	(%) GLA 15 DAF	(%) GLA 30 DAF	(%) GLA 45 DAF	Days to 50 (%) flowering	Total GLA	Plant height	Grain yield plant^{-1}
Replication	3	548.92	3100.60	1288.49	748.18	3653248.51	29228.68	1032.38	10545.65	1426.60	969.04	5247.24	3993215.86	21376.66	365.77
Environment (E)	2	12478.39**	4091.01**	26966.34**	13515.65**	28796554.29**	95189.66**	11286.76**	35326.40**	126842.15**	278069.05**	1703.93**	12126719.05**	91997.77**	7358.34**
Genotypes (G)	225	182.05**	908.59**	2044.96**	285.89**	544525.24**	6720.06**	941.65**	709.15**	1552.97**	2139.75**	223.46**	363227.71**	6866.50**	1059.09**
E x G	450	46.90**	381.89**	369.58**	97.48**	124000.27**	1284.82**	114.04**	158.49**	414.38**	749.44**	82.76**	176518.76**	1874.57**	92.90**
Error	2034	16.52	7.18	45.77	8.11	52295.03	603.34	48.01	98.14	9.11	31.35	3.58	47806.85	1493.00	50.62
SEm±		1.12	1.58	2.09	0.33	41.63	6.25	5.29	2.98	0.43	2.89	1.05	99.10	17.58	1.00
CV (%)		4.51	4.20	18.28	4.87	19.35	13.54	20.13	11.86	4.61	13.06	2.98	16.29	19.39	17.82
PCV		6.02	21.75	32.48	11.34	24.21	18.36	40.49	7.01	17.51	48.01	12.03	21.57	15.81	35.24
GCV		5.39	21.27	31.00	11.29	23.01	16.90	26.72	4.74	17.45	44.93	11.01	17.32	12.31	34.07
H		80.20	94.73	91.14	99.05	90.29	84.73	43.56	37.01	97.54	82.32	86.98	34.12	21.13	94.44
GAM		9.50	42.45	73.57	24.56	41.96	30.76	13.51	6.62	29.60	54.06	18.20	12.36	8.80	20.05

* - Significance at 5% probability level, ** - Significance at 1% probability level, GCV-Genotypic coefficient of variation, PCV-Phenotypic coefficient of variation, H- Heritability, GAM-Genetic advance over per cent of mean

many studies (Haussmann *et al.,* 2002; Reddy *et al.,* 2007). For grain yield per plant high variability was noticed and these observations were also in agreement with the studies of Arunkumar *et al.* (2004); Kachapur and Salimath (2009).

Lush (1949) defined heritability in broad sense as the ratio between the genotypic variance as a whole that is due to phenotype. In both RIPs, high heritability recorded for traits such as per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF and total green leaf area from this it is evident that these traits are less influenced by environment. Similar observations have been made in same populations in sorghum by Haussmann *et al.* (2002); Sundresha (2006) and Gebre (2009). High heritability also noticed for the traits such as days to 50 per cent flowering and plant height. Similar observations have been made in sorghum by Kachapur and Salimath (2009) for days to 50 per cent flowering and plant height.

Expected genetic advance over per cent mean is a parameter that considers both variability and heritability and provides a realistic estimate of genetic gain achievable through following selection. In both RIPs, high genetic advance over per cent mean for per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area (Haussmann *et al.,* 2002; Gebre, 2009), days to 50 per cent flowering, plant height and grain yield per plant and similar observations have been made in sorghum germplasm by Arunkumar *et al.* (2004). High heritability coupled with genetic advance indicates that additive gene effects are operating and selection for superior genotype is possible (Arunkumar *et al.,* 2004).

The correlation coefficient analysis helps to determine the nature and degree of relationship between any two measurable characters. It resolves the complex relationships between events into simple forms of association. Grain yield being dependent on physico-morphological features of the plant, it is desirable to know the individual contribution of these characters for developing effective selection strategies. Phenotypic and genotypic correlation coefficients in both RIPs (Table 2) revealed that, among the post-flowering drought parameters, per cent GLA 30 DAF had significant and positive association with total GLA, per cent GLA 45 DAF but had found negatively correlated with days to 50 per cent flowering. Highly significant positive association was found between per cent GLA 45 DAF and total GLA. Similar findings were reported (Haussmann *et al.,* 2002; Sundresha, 2006; Gebre, 2009) in RIPs developed from same crosses.

In both RIPs, a negative correlation between days to 50 per cent flowering and stay-green feature indicated by per cent GLA 15 DAF and per cent GLA 45 DAF under drought conditions clearly indicates the importance of GLA as a means to minimize the effects of post-flowering moisture stress. Correlations revealed that the genotypes with early flowering produced more grain yield. Highly significant positive associations were observed between yield and components of stay-green in both RIPs. Similar observations made in set of stay-green sorghum genotypes (Reddy *et al.,* 2007). Among yield related traits, yield shown positive correlation with total grain leaf area, but had negative association with days to 50 per cent flowering and plant height, which may be obvious when the plant possess more than expected height, the food allocation from source to sink of different part will be more. Less amount of processed food reaches to seed development and result in yield reduction (Haussmann *et al.,* 2002).

Table 2. Pooled phenotypic and genotypic correlation coefficients for stay-green and yield related traits in recombinant inbred

Traits		RIP1							RIP2						
		(%) GLA 15 DAF	(%) GLA 30 DAF	(%) GLA 45 DAF	Days to 50 (%) flowering	Total GLA	Plant height	Grain yield plant^{-1}	(%) GLA 15 DAF	(%) GLA 30 DAF	(%) GLA 45 DAF	Days to 50 (%) flowering	Total GLA	Plant height	Grain yield plant^{-1}
X_1	P	1	0.135*	0.362**	-0.015	0.089	0.032	0.004	1	0.558**	0.463**	-0.005	0.021	0.089	0.064
	G	1	0.158*	0.408**	-0.015	0.106	0.043	0.012	1	0.628**	0.507**	-0.006	0.023	0.118	0.162*
X_2	P		1	0.035	-0.03	0.067	0.086	0.044		1	0.667**	-0.021	0.051	0.127	0.158*
	G		1	0.037	-0.031	0.077	0.094	0.06		1	0.733**	-0.029	0.062	0.181*	0.329**
X_3	P			1	-0.026	0.225**	0.11	0.073			1	-0.055	0.077	0.064	0.148*
	G			1	-0.028	0.248**	0.006	0.113			1	-0.06	0.081	0.096	0.308**
X_4	P				1	-0.248**	0.083	-0.008				1	-0.116	0.026	-0.014
	G				1	-0.262**	0.088	-0.006				1	-0.127	0.015	-0.007
X_5	P					1	0.22**	0.061					1	0.121	0.056
	G					1	0.244*	0.085					1	0.186**	0.098
X_6	P						1	-0.001						1	-0.035
	G						1	-0.026						1	-0.11
X_7	P							1							1
	G							1							1

P – Phenotypic correlation G – Genotypic correlation ** - Significant at 1% level of probability* - Significant at 5% level of probability

In conclusion, higher PCV and GCV were noticed for traits such as per cent GLA 30 DAF, per cent GLA 45 DAF and grain yield per plant, indicating selection could be made in the population. Higher heritability was recorded for per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF, days to 50 per cent flowering, plant height and total green leaf area indicating additive genes are acting. Trait such as per cent GLA 30 DAF, per cent GLA 45 DAF, total green leaf area recorded higher genetic advance. In both RIPs, grain yield per plant was highly significant and positively correlated with total green leaf area, per cent GLA 15 DAF, per cent GLA 30 DAF, per cent GLA 45 DAF. It indicates that genotype possessing the stay-green traits have a significant yield advantage under post-flowering drought stress conditions. The parents used in this study were diverse in nature for stay-green trait and trait can be used in marker assisted selection for development of improved stay-green genotypes which tolerate post-flowering drought tolerance.

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Species composition and abundance of natural enemies of *Myzus persicae* (Sulzer) in potato agro-ecosystem in Shimla hills

Meena Thakur and V.K. Chandla

Division of Plant Protection, Central Potato Research Institute, Shimla - 171 001, Himachal Pradesh, India
E-mail: meenauhf@gmail.com

ABSTRACT

Aphids *M. persicae* (Sulzer) are one of the most serious pests of potato crop worldwide, causing major yield and economic losses. Despite advances in integrated pest management, and frequent use of insecticides, the industry is still plagued by the insect. Adding to the problem is the fact that the peach aphid has proved to be resistant to various insecticides so there is a need to shift emphasis on biological control agents. Natural enemies could be a good option therefore, the present studies were carried out to observe the occurrence and diversity of natural enemies of green peach aphid, *M. persicae* in Shimla hills in potato and surrounding vegetation and the effect of weather parameters on their seasonal abundance. The periodical sampling of natural enemies (predators and parasitoids) associated with green peach aphid revealed the occurrence of four species of hoverflies (Hymenoptera) viz. *Episyrphus balteatus*, *Eupeodes* sp., *Scaeva pyrastri*, four species of coccinellids (Coleoptera) viz. *Coccinella septempunctata*, *Menochilus sexmaculata*, *Adalia quadrispinolata*, *Adalia decempunctata*, a neuropteran *Chrysoperla carnea* and two species of parasitoids (Hymenoptera) viz, *Aphelinus abdominalis* and *Aphidius* sp. Seasonal abundance of predators synchronized with the pest activity, maximum being during July to August. The mummification rate showed a specific increasing trend late in the season (August–September). Correlation between the natural enemy and abiotic factors revealed a positive effect on their population dynamics (except for relative humidity w.r.t parasitoids, showing negative non-significant correlation). Among the predators *C. septempunctata* and *M. sexmaculata* were most abundant therefore their feeding potential was evaluated under controlled conditions which revealed that different larval stages of *C. septempunctata* and *M. sexmaculata* fed an average of 372.1 ± 6.25 and 316.71 ± 8.60 aphids during the complete larval period, whereas the feeding potential of adults were 66.3 ± 15.2 and 57.0 ± 10.0 aphids per day, respectively.

Key words: Natural enemy, *Myzus persicae*, predation potential, seasonal occurrence

Potato has emerged as an important cash crop and one of the leading vegetables in India. With an average annual production of 37.3 million tones, India ranks second in world potato production (Anonymous, 2010). It is attacked by many insects among which, sucking insects especially green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera : Aphididae) is the most important (Shah, 1988). Aphids are pests that, by their rapid growth and their ability to spread virus, constitute major problems for crop production (Van *et al*., 2007). In potato heavy infestation of green peach aphid causes considerable damage to the crop by severely dwarfing and curling the leaflets and by dwarfing the tops. In extreme cases, the whole plant may be killed (Painter, 1951).

The use of chemical treatments, unfortunately still widespread, leads to the selection of resistant individuals whose eradication becomes increasingly difficult (Field *et al*., 1998; Kerns and Gaylor, 1992; Devonshire, 1998; Foster *et al*., 2000). Furthermore, the extensive and repeated use of hard chemicals has disrupted the natural balance between the pests and their natural enemies (Amer and Marei, 2001).

Fortunately for farmers aphids are attacked by several natural enemies including predators such as hoverflies, coccinellids, lace wing bugs, and hymenopteran parasiticides. These natural enemies have been reported as a potential governing factor of aphid population (Van *et al*., 1996; Dixon, 1998). When feeding on the plant phloem sap, aphid excretes large amounts of honeydew, a complex mixture of sugars, organic acids, amino acids and some lipids (Lefroy *et al*., 2011). This excretory product is considered as a food complement for many aphid natural enemies (Hogervorst *et al*., 2007) and the volatiles that are released are supposed to act as kairomones for parasitoids and predators (Verheggen *et al*., 2008; Almohamad *et al*., 2009).

In India, *M. persicae* is attacked by over 24 predators (Syrphids, Chrysopids, Coccinellids, Chamaemyiids), 22 parasitoids (Aphidiids, Aphelinids) and 5 entomopathogenic fungi. But only very few of these predators, *viz*., *Allograpta javana* (Wiedemann) (Pruthi, 1946); *Episyrphus balteatus* (De Geer) (Ghosh *et al*., 1985); *Ischiodan scutellaris* (Fabricius), *Sphaerophoria scripta* (Linnaeus) (Ghosh *et al*.,

1985); *S. indiana* Bigot (Pruthi, 1946); *Leucopis fumidilarva* Tanas (Saxena, 1981); *Cheilomenes* (*Menochilus*) *sexmaculata* (Fabricius) and parasitoids like *Aphelinus* sp. (Verma *et al*., 1976) and *Aphidius colemani* Viereck (Trivedi, 1988) have been recorded attacking *M. persicae* on potato crop. There is little information on the seasonal occurrence and abundance of natural enemy fauna of *M. persicae* in Shimla hills and with the changing climatic conditions it becomes imperative to study the effect of abiotic factors on their survival and activity. Therefore, the present study aimed at studying the natural enemy fauna of green peach aphid in Shimla hills and working out the feeding potential of most abundantly found coccinellids under polyhouse conditions in potato.

MATERIALS AND METHODS

The study was conducted at Central Potato Research Institute, Shimla, situated at 37^0 North latitude, 77^0 East longitude and at about 2200m above mean sea level (amsl). The feeding potential of coccinellids was studied under laboratory and polyhouse conditions during the months of March-May and the natural enemy fauna was observed throughout the cropping period in open conditions in potato fields.

Potato var. Kufri Jyoti was sown in two cropping seasons i.e. during the month of April as kharif crop and during October as *rabi* crop to study the population fluctuation and diversity and density of natural enemies of *Myzus persicae* on potato and surrounding agro-ecosystem.

A field survey was conducted from the month of March 2011 to April 2012 in selected potato fields and adjacent area to record the abundance of different natural enemies of *M. persicae*. The experimental site was kept free from pesticide application. No chemical control was done in the selected fields. The number of adults of different predators and parasitoids were counted per 10 m^2 area randomly on the basis of visual observation at a given space and time (per 15 minutes) and their relative abundance were worked out. The mummified aphids were also collected from infested potato plants to assess the parasitoid species.

Correlation of population fluctuation of natural enemies with abiotic factors

Temperature and relative humidity (RH) have most important governing influence on insect population therefore minimum and maximum temperature and RH were recorded during different months and was subjected to correlation with the population fluctuation of natural enemies.

Feeding efficiency of coccinellids

During the study on abundance of natural enemies coccinellids were found active throughout the study period therefore two coccinellid species i.e. *Coccinella septempunctata* and *Menochilus sexmaculata* were selected to study the predation potential of coccinellids on *M. persicae* to assess their role in regulating the aphid population under conditions. The experiment was laid out in completely randomized design (CRD). Adults of both the species (both male and female) were collected from field and feeding efficiency of the larval instars was assessed by rearing individuals from their early stage separately on plastic Petri-dish. Counted number of aphids was provided to them daily and prey consumption was quantified by counting the left over aphids in each twig. The newly formed adults were released under polyhouse conditions on highly infested potted potato plants. The selected plants were covered with nylon net after releasing the adult beetle (one beetle $plant^{-1}$). The number of aphids per plant was recorded before and after the release of predators daily upto one week. Totally five replications were maintained for each coccinellid species. Five plants were kept covered without any adult beetle as control to assess the rate of change of aphid population (increase or decrease) under natural conditions.

The per day predation potential of adults was calculated by using the following formula given by:

$X = R-(T-C)$

X= Actual no. of aphids consumed by predator

R= Total no. of aphids $plant^{-1}$ before predator release or prior to subsequent observation

T= Actual aphids $treatment^{-1}$

C= Average increase in no. of aphids day^{-1} $plant^{-1}$ in control (including natural mortality)

Experimental data of feeding potential of beetles was subjected to analysis of variance (ANOVA) in Completely Randomized Design (CRD) using statistical software AGRIS.

RESULTS AND DISCUSSION

Field assessment indicated the presence of three different groups of predatory insects like Syrphids, Coccinellids and a Neuropteran and one parasitoid group. Of the three predatory groups, neuropteran (*Chrysoperla carnea*) occurred sporadically, while syrphids and coccinellids were observed throughout the period of aphid infestation. The relative per cent abundance of each group was worked out from the total count of natural enemies recorded per 10 m^2 which revealed coccinellids (33%) as the most abundant and active natural enemies followed by syrphids (31%), parasitoids (27%) and neuropteran (9%)

Table 1. Relative abundance and occurrence of predators and parasitoids on *Myzus persicae* in Shimla hills

Species Predators		Relative abundance (%)	Active period
Coccinellids	*Coccinella septempunctata*	33	Throughout cropping period
	Menochilus sexmaculata		Feb.–March & July-Oct.
	Adalia quadrispinolata		July-Sept.
	Adalia decempunctata		July- August
Syrphids	*Episyrphus balteatus*	31	Feb.–March & June-Sept.
	Eupeodes sp.		Feb.–March & June- Aug.
	Scaeva pyrastri		Feb.–March & July- Sept.
	Metasyrphus confrater		Feb.–March & July-Aug.
Green lacewing bug	*Chrysoperla carnea*	9	Throughout cropping period
Parasitoids	*Aphelinus abdominalis*	27	July-October
	Aphidius sp.		Feb.-April & Aug.- Nov.

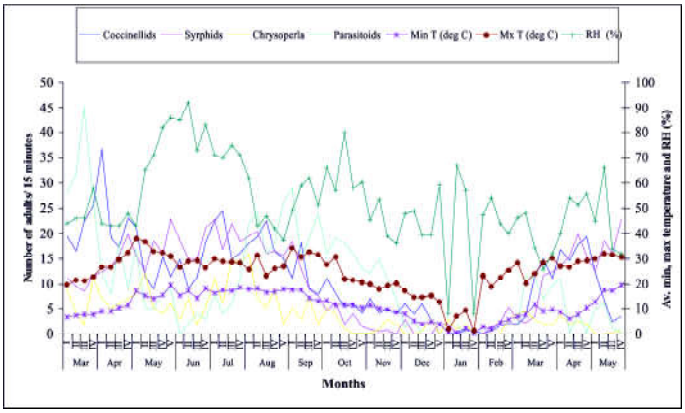

Fig. 1. Population fluctuation of predators and parasitoids w.r.t. aphids in potato in Shimla hills

(Table 1 and Figure 1). Among coccinellids *Coccinella septempunctata* was the predominant species both in case of density and frequency of occurrence followed by *Menochilus sexmaculata*, *Adalia quadrispinolata* and *Adalia decempunctata*. Similarly among syrphids *Episyrphus balteatus* outnumbered the other species like *Eupeodes* sp., *Scaeva pyrastri* and *Metasyrphus confrater*. Among the parasitoids *Aphidius* sp. and *Aphelinus abdominalis* were observed in field collected mummified aphids. Among aphids' enemies, the hoverfly *E. balteatus* (Diptera: Syrphidae) is recognized as a very efficient aphidophagous predator. This syrphid is considered as the most abundant natural enemy in agroecosystems and natural habitats (Tenhumberg and Poehling, 1995, Colignon *et al*., 2001) as the most efficient aphid predator and is associated with many different aphid-plant complexes (Bargen *et al*., 1998).

The correlation between the density of natural enemies and abiotic factors revealed a positive effect on their population fluctuation, except for relative humidity which showed a negative but non-significant correlation with the abundance of parasitoids. Temperature had a highly positive significant effect on population fluctuation of coccinellids, syrphids and chrysoperla with 'r'= 0.441 and 0.493, 0.757 and 0.717 and 0.458 and 0.380 with minimum and maximum temperature, respectively (Table 2).

Table 2. Correlation ('r') of population fluctuation of natural enemies with abiotic factors

	Min. temp.	Max. temp.	R.H.
Coccinellids	0.441**	.493**	.173
Syrphids	0.757**	.717**	.345**
Chrysoperla	0.459**	.380**	.313*
Parasitoids	0.141	.135	-.129

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).

Comparative study on the feeding efficiency of *C. septempunctata* and *M. sexmaculata* revealed no significant variation in their voracity. The different larval stages of *C. septempunctata* and *M. sexmaculata* consumed a total of 304.1 ± 6.25 and 279.71 ± 8.60 aphids/larva respectively during their development whereas the adults consumed an average of 66.2 ± 15.2 and 57 ± 10.0 aphids per day (Table 3). The per day aphid consumption of adult coccinellids was studied upto a week and is presented in Figure 2. Considering the occurrence, diversity and feeding potentials of coccinellids, it becomes clear that they limit the density of the aphids under field condition to some extent. Variation in consumption rate could be attributed to variation in size of the nymphs, temperature and other rearing conditions (Veeravel and Bhaskaran, 1996). Therefore, considering the occurrence frequency, periodicity, diversity and feeding

Table 3. Predation potential of *Coccinella septempunctata* and *Menochilus sexmaculata*

Developmental stages	No. of aphids consumed	
	Coccinella septempunctata	*Menochilus sexmaculata*
1st instar	33.33±1.15	26.22±0.95
2nd instar	59.95±2.85	44.99±1.11
3rd instar	111.22±1.42	96.9±1.33
4th instar	167.6±0.98	148.6±0.56
Total larval period (days)	30.0± 0.54	26.4± 0.77
Total larval consumption	372.1±6.25	316.71±8.60
Mean larval consumption	76.0±1.56	69.93±2.15
Adult consumption day $^{-1}$	66.2±15.2	57±10.0

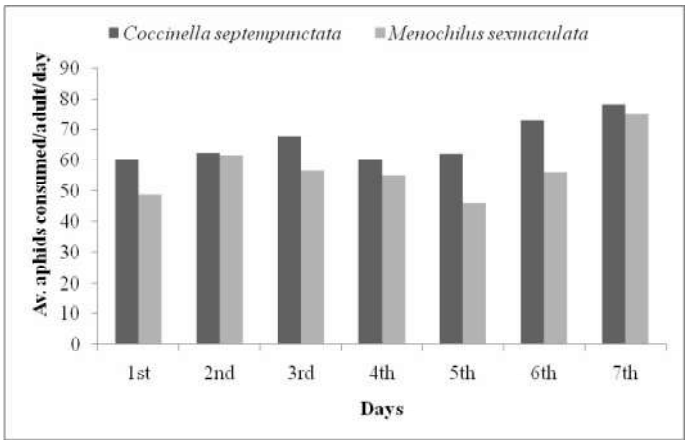

Fig. 2. Per day feeding potential of adult coccinellids on *M. persicae*

potentials of various predators, it becomes clear that they limit the density of the aphids under field conditions to some extent.

In the present study, predation rate of larvae was found to be higher than adults. Doghairi (2004) observed 2.0 fold greater predation rates in larvae than those of adults. High predation rate by larvae may be because of the need of the larvae to cumulate high quantity of its food requirement to be able to proceed with its development and metamorphose to the pupal stage. Findings in present study indicate that larvae of coccinellids may have a stronger impact on pest populations than the adults as suggested earlier (Khan and Suhail, 2001; Pervez and Omkar, 2005).

Laboratory studies may have little resemblance to those that could be measured in the field (Wang *et al*., 1984; Hussain and Ahmed, 2003). However such studies can be used to infer basic mechanisms underlying predator-prey interactions. Further studies on predation rate of coccinellids on homopteran pests both in the laboratory and field need to be done to successfully incorporate them into IPM programs and enhance the ecological functions of agro-ecosystems.

Based on these studies it can be concluded that the presence of natural enemies in the field can keep the aphid population below economic threshold level by preventing the aphids from becoming a serious pest.

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Fluctuation of insect pest population in rice-rice system in Tungabhadra Project area of Karnataka

Mahabaleshwar Hegde and R. A. Balikai[1]

AICRP on Groundnut, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India
[1]College of Agriculture, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India
E-mail: mghegdesrg@yahoo.co.in

ABSTRACT

A rowing survey was undertaken from 1998 to 2006 *kharif* (June to November) season to study the fluctuating insect pest population on rice crop at Tungabhadra Project area of Karnataka, India. The surveying time was so adjusted to coincide with the peak activity period of insects. The observations were recorded on 100 hills in five fields of each village and such five villages were surveyed during every season. The virtual population count was restricted to planthoppers (brown planthoppers and white backed planthoppers), leaf folders, yellow stem borer and green leafhoppers, which are considered as devastating in rice ecosystem. The population of planthoppers was above economic threshold level in all the surveyed seasons and the very high population was recorded during 2004. The maximum population of green leafhopper was recorded during the year 1998. The higher population of leaf folder and yellow stem borer were recorded during 2006 among the surveyed years.

Key words: Planthopper, leaf folder

Mono-cropping coupled with dominance of single variety over years may promote build up of particular insect pest/disease and may lead to failure of a crop/cropping system which is an age old concept in crop husbandry. Tungabhadra Project area of Karnataka, India is known for its irrigated rice–rice system. BPT-5204 is the ruling rice variety from 1980's onwards in the kharif season (from June to November) due to its quality and substantial yielding potential. The variety is susceptible to insect pests and diseases. Rajendra Prasad *et al.* (2011) reported that brown planthoppers, white backed planthoppers, green leafhoppers, yellow stem borer and leaf folders are economic pests of rice in Karnataka. However, during the rabi/summer season (From November to April) many short duration varieties are being grown from 1990's due to availability of water for limited period of time. The insect pest problem is also comparatively low irrespective of the variety during rabi/summer season. After 1970's during *kharif* season insect population, especially the planthopper has been consistently appearing in destructive form in the entire project area on rice crop and has become unmanageable. However, insect pest population level varied a lot over the years although the cropping system, cultivation practices including varietal composition remained the same. Ascertaining their population fluctuation over the years may help in decision making process and also make arrangements to take up right management practices in advance to reduce the yield loss. A trial was undertaken to study the fluctuation in the population of major insect pests in the Tungabhadra Project (TBP) area.

MATERIALS AND METHODS

A rowing survey was undertaken from 1998 to 2006 during kharif (June to November) season at peak insect incidence time. During every year survey was conducted in five villages under the TBP area. The observations were recorded on 100 hills in five fields of each village and such five villages were surveyed during every season. Population of four major insect pests *viz*., brown planthopper (BPH), *Nilaparvatha lugens* (Stal), white backed planthopper (WBPH), *Sogatella furcifera* (Hovarth) and green leaf hopper (GLH), *Nephotettix nigropictus* (Stal) and *Nephotettix virescens* (Distant)) was recorded. Damaged leaves (DL) and white ears were observed to ascertain the population level of rice leaf folder (RLF), *Cnaphalocrosis medinalis* (Guenee) and yellow stem borer (YSB), *Scirapophaga incertulas* (Walker). The observations were averaged per hill in case of population count and damaged leaves. The percentage of white ears was also computed. The observations from each village were taken as separate unit and subjected to a standard deviation. Results of all the five observations were averaged and used for interpretations.

RESULTS AND DISCUSSION

The average population of brown planthopper was more than the economic threshold during the study period

in every year. The population of BPH was 32.6 ± 15.15 hill^{-1} in the year 1998, increased to 57.8 ± 21.9 hill^{-1} during 1999 and remained low (ranged from 23 to 18 hill^{-1}) till 2003. The population averaged to 128.6 ± 22.2 hill^{-1} in the year 2004 and became unmanageable throughout the TBP area. However, the very next year the population averaged to 82.5 ± 18.6 and reduced further to 43.6 ± 9.7 hill^{-1} in the year 2006 (Table.1). White backed planthopper population started to buildup from the year 2003 (9.6 ± 1.8), reached its peak in the year 2005 (28.7 ± 8.1) and reduced to 19.8 ± 3.9 hill^{-1} during 2006. Green leafhopper population remained low throughout the study period. Maximum population of 3.7 ± 1.7 was noticed in the year 1998 and dwindled thereafter until 2005 and recovered to 1.28 ± 0.7 during 2006. The leaf folder damage was comparatively low from 1998, up to 2001 (ranged from 0.2 ± 0.2 to 1.6 ± 0.5 damaged leaves hill^{-1}). The damaged leaves were 3.2 ± 0.6 and 4.6 ± 1.3 hill^{-1} in the year 2002 and 2003, respectively. However, it was comparatively low for the next two consecutive years. The maximum damaged leaves of 6.4 ± 2.1 were noticed in 2006. There was no definite trend in the population of leaf folder. However, there was overall increase in the population after 2001. Yellow stem borer incidence during the period ranged from 0 to 5.3. From 1998 to 2002, the per cent white ears was very low and reduced to nil in the year 2003 and 2004. The incidence reappeared during the year 2005 (2.3%) and reached its peak of 5.3 per cent during the year 2006. A peak incidence of 5.3 ± 2.2 was observed in 2006. However, the population of all the major insects was low during rabi/summer season baring few patches of higher incidence of yellow stem borer. It is well established that the relative humidity and environmental factors decide the population of planthopper (Somchai Isichaikul and Toshihide Ichikawa, 1993). However, higher planthopper populations were recorded during the year 2004 and 1999 (Fig. 1). But the maximum rainfall and more number of rainy days were noticed a year before (1998) and a year after (2005) planthopper population attained its peak status. It was known that the stress (scarcity of moisture) enhances incidence of yellow stem borer. However, yellow stem borer incidence remained low. Syunro Utida (1958) opined that the fluctuation in population density of insect pests from one season to another will occur with a wider range of amplitude, the outbreak periods occur at intervals of several years, supports the results of the present study. It appears that intensive studies on the relationship between all the organisms of rice ecosystems and influences of environmental factors on them may explain the population fluctuation of insect population in a particular ecosystem. Similar opinions are also expressed by Kishimoto (1981), Kuno (1984) and Holt *et al*. (1987).

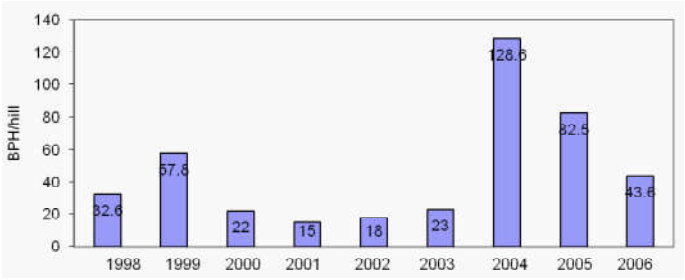

Fig. 1. Peak brown planthopper population hill^{-1} during kharif season (1988-2006).

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Table 1. Insect pest status and rainfall pattern at the Agricultural Research Station, Siruguppa, Karnataka (1998-2006)

Year	Brown planthoppers hill^{-1}	White backed planthoppers hill^{-1}	Green leafhoppers hill^{-1}	Damaged leaves hill^{-1}	White ears (%)	Rainfall (mm)	Number of rainy days
1998	32.6±15.5	-	3.7±1.7	1.2±0.5	1.9±1.3	921.6	55
1999	57.8±21.9	-	2.5±1.0	0.2±0.2	0.8±0.6	577.4	32
2000	22.0±3.9	-	1.5±0.7	1.1±0.4	0.5±0.5	576.0	42
2001	15.0±3.9	-	1.4±4.2	1.6±0.5	0.2±0.3	761.7	37
2002	18.0±4.8	-	0.9±0.4	3.2±0.6	0.6±0.6	249.8	18
2003	23.0±6.3	9.6±1.8	0.6±0.3	4.6±1.3	-	335.3	29
2004	128.6±22.2	13.8±2.2	0.7±0.2	2.8±1.2	-	611.6	34
2005	82.5±18.6	28.7±8.1	0.3±0.4	2.1±0.8	2.3±0.4	806.6	47
2006	43.6±9.7	19.8±3.9	1.3±0.7	6.4±2.1	5.3±2.2	441.3	37

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Weight loss in adult drone bees and their pupae (*Apis mellifera*) by *Varroa destructor* mite infestation levels in Kashmir Valley

Manzoor A. Paray and A.Q. Rather

Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences & Technology, Kashmir, Shalimar Campus-191 121, J & K, India

ABSTRACT

This study was carried out to determine the effect of different levels of *Varroa destructor* mite infestation (1-mite, 3-mite, 5-mite and without mite) on the body weight of adult honey bees (worker and drone bees) of *Apis mellifera* and their pupae in Kashmir Valley during the year 2008. Results revealed that uninfested drone bees weighed on average 135.14 mg and infested drone bees with 1, 3 and 5 mites weighed on an average of 127.03; 120.63 and 112.95 mg. We recorded a loss in the body weight of drone bees to the tune of 7.96; 11.07 and 160.32 per cent. Uninfested drone pupae weighed on average 96.09 mg and infested drone pupae with 1,3 and 5 mites weighed on an average 90.97; 83.58 and 76.93 mg with loss in the body weight to the tune of 5.10; 11.69 and 19.89 per cent. Studies indicated that weight loss increased with multiple infestation of an individual brood cell, especially from the red eyed pupal stage on. A significant weight loss from the brown-eyed stage on wards, and a considerable reduced body weight in the emerging adults was recorded

Key words: *Varroa destructor,* drone bees, drone pupae, Kashmir

Honey bee is the most valuable creature in nature. The state of Jammu and Kashmir comprising of four agro-climatic zone. Such a diversity of geographical features play a dominant role in determining the topography, climate and plant species present in the region. It offers great potential for both migratory and non-migratory bee-keeping. This low production of honey is attributed to mainly to the sick colonies by recent epidemic of *Varroa destructor* in the state. Since last few years, losses to the tune of 60-100 per cent to the brood in some apiaries have been reported (Paray *et al*., 2008). Honey bee species are reported to have many diseases, predator and enemies. The enemies of honey bees are wax moths, nectar hawk moths, predatory wasps and bee-eater birds. Among the most serious enemies are parasitic mites viz., *Varroa jacobsoni, Tropilaelaps elareae, Varroa destructor Varroa underwoodi, Euvarroa sinhai, Varroa rendereri* (De Jong *et al*., 1982). Among all these species of ectoparasitic mites *Varroa destructor* is posing a threat to bee-keeping industry throughout the world which can destroy almost entire colony within a few months. The breakdown of *Apis mellifera*, L. colonies due to *Varroa destructor* results from mainly from worker brood infestation. Mite females can reproduce in worker cells of the western honey bees and their feeding leads to pupal weight loss (De Jong *et al*., 1982). The parasitized adult bee emerges injured and with a reduced life expectancy (De Jong *et al*., 1982). The effects of parasite load have been studied mainly in worker bees (Schatton-Gadelmayer and Engels, 1988). In comparison, little has been recorded on drone injury. While *Varroa destructor* females are rare found in queen cells (De Jong, 1990), drone brood of *A. mellifera* is much more heavily infested than worker brood. The resulting damage of adult drones (Weinberg and Madel, 1985) and the decrease in the number of males from infested colonies available fro mating (Rinderer, *et al*., 1999) have been described. *Varroa destructor* mite feeds on the haemolymph of adult and immature honey bees causing a reduction upto 60 per cent in protein content of haemolymph, 30 per cent reduction of haemocytes, 25 per cent weight loss and wing and limb deformity in adult, a reduction in colony development and production activity (Shimanuti, *et al*., 1992). *Varroa* mites prefer to parasite drone pupae because of the lengthened time in the pupal stage compared to workers and queens (14.5 vs. 12 and 7.5 days, respectively). As a result of *Varroa's* preference for drone pupae, a number of studies have been conducted by Renderer, *et al*., 1999, looking for adherer effects of *Varroa* on drones. *Varroa* infestation was also found to have negative effect on drone weight, mucus gland and seminal vesicle weight and the number of spermatozoa produced by parasitized drones (Renderer, *et al*., 1999). Pedro *et al*. (2003) reported that drones emerge with significantly diminished weight even if only one female mite has invaded the brood cell. We studied the weight loss in the colonies where different mite inoculations (1-mite, 3-mite and 5-mite) were given to the adult been. The result

exhibited that any parasitism affects the weight of adult drones and their pupal stages and many reduce their fitness. However, drones often emerge even from heavily infested brood cells (Pedro, *et al*. 2003).

MATERIALS AND METHODS

Experiments were conducted to determine the effect of different infestation levels of *Varroa destructor* mite (1-mite, 3-mite and 5-mite) on the body weight of adult drone bees and drone pupae of *A. mellifera* honey bees in Kashmir valley. The experiments were conducted in an apiary near the vicinity of Shaer-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar, during spring season 2008. For this purpose, the experiments were laid out in honey bee hives. There were four treatments including control. Thirty bees were inoculated in each treatment replicated thrice. One frame of bees acted as one replication. Thirty (30) young emerging bees were collected randomly from the frame and were placed in separate plastic jars with perforated lids for respiration, together with a small lump of candy sugar. The bees were then inoculated with different levels of mites (1-mite, 3- mite and 5-mite). The maximum time the bees spent in the plastic jars was 1-hour. The inoculated bees were then released on the randomly chosen frames from where these bees have been collected. Then after the inoculation, adult stages of drone bees (3 drone bees frame^{-1}) and their pupal stage (3 drone pupae frame^{-1}) were randomly collected from the uninfested and infested frames and their weights determined both of uninfested and infested drone bees and their pupal stages with the help of a micro-balance. Per cent reduction in body weight of three groups of drone bees and their pupal stages with (bee with 1-mite, bees with 3-mite and bees with 5-mites) were determined.

RESULTS AND DISCUSSION

Average loss in the body weight of adult drone bees of *A. mellifera* and their pupal stage infested with different levels of *Varroa destructor* mite infestations during the year 2008, are summarized in the table 1 and 2.

Table 1. Average loss in body weight of adult drone bees of *Apis mellifera*, infested with *Varroa destructor* during the spring season 2008

Frame No.	Average body weight of drone bees without mites (mg)	Average body weight of adult drone bees with mite (mg)			Per cent loss in the body weight		
		1-mite	3-mite	5-mite	1-mite	3-mite	5-mite
1	142.45	131.74	125.82	115.30	10.71	11.67	19.05
2	136.00	130.57	121.74	112.37	5.43	10.48	17.37
3	134.65	125.50	117.32	112.25	9.15	12.87	15.89
4	125.27	118.14	112.00	109.35	7.13	10.59	12.70
5	137.35	129.97	123.93	114.50	7.38	9.57	16.63
Mean	135.14	127.03	120.63	112.95	7.96	11.07	16.32
CD at (P=0.05)			4.03			2.95	

Sample size: 3 drone bees per frame

Table 2. Average loss in body weight of drone pupae of *Apis mellifera*, infested with *Varroa destructor* during the spring season 2008

Frame No.	Average body weight of drone bees without mites (mg)	Average body weight of adult drone bees with mite (mg)			Per cent loss in the body weight		
		1-mite	3-mite	5-mite	1-mite	3-mite	5-mite
1	95.00	90.17	80.00	79.00	4.43	9.47	16.84
2	97.25	90.15	85.30	76.35	7.10	11.95	21.49
3	94.35	90.83	83.17	77.45	3.52	11.84	17.91
4	99.35	94.72	87.45	75.85	4.63	12.06	23.73
5	94.42	89.00	82.00	76.00	5.42	13.15	19.50
Mean	96.09	90.97	83.58	76.93	5.10	11.69	19.89
CD at (P=0.05)			4.04			3.01	

Sample size: 3 drone pupae frame^{-1}

The data recorded in the table-1 revealed that in case of adult drone bees without mites body weight ranged from 125.27 to 142.45 mg (average body weight 135.14 mg, whereas in case of drone bees infested with 1-mite, the body weight ranged from 118.14 to 131.14 mg (average body weight 127.03 mg) the average loss in the body weight was 7.96 per cent (Table 1). Results also revealed that in case of drone bees infested with 3-mites, the body weight ranged from 112.00 to 125.82 mg, the average weight being 120.63 mg. The loss in the body weight ranged to 11.07 per cent (Table 1). Results indicated that in case of drone bees infested with 5-mites, the body weight ranged from 109.35 to 115.30 mg, which averaged to 112.95 mg, thus resulting in the loss from 12.70 to 19.05 per cent (average 16.32%). Study indicated that the body weight of drone pupae (without mites) ranged from 94.36 to 99.35 mg, while the average body weight was 96.09 mg (Table-2). In case of drone pupae infested with 1-mite, 3- mite and 5-mites, the body weight came in the range between 89.00 to 94.72; 80.00 to 87.45 and 61.00 to 66.75 mg, respectively, resulting in the loss ranging from 5.1, 11.09 and 14.53 per cent, respectively (Table 2).

Studies indicated that all the mite infestation levels were deleterious to adult drone bees and their pupal stages as compared to control (non-infested). The treatment 5-mite infestation levels were highly harmful to adult drone bees and drone pupae followed by 3 and 1-mite infestation levels. The result indicated that as the level of mite infestation increased there was corresponding increase in the per cent loss in the body weight of adult done bees and drone pupae. Our results indicated that weight loss increased with multiple infestations of adult drone bees and their pupal stages. The drone bees that emerged from the cells infested by 5-adult female mites and their pupae showed a tremendous loss in the body weight. In all the three groups (1-mite, 3- mite and 5-mite), found significant weight loss from the brown eyed stage onwards and a considerable reduced body weight in the emerging adult bees. Our results are in agreement with De Jong *et al*. 1982; Pedro. *et al*. 2003; Renderer *et al*. 1999; Weinberg and Madel, 1985; and Shimanuki, *et al* 1992.

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Seasonal incidence and eco-friendly management of epilachna beetle, *Henosepilachana vigintioctopunctata* in brinjal

N. R. Koushik and M. Manjunatha

Department of Entomology, College of Agriculture, Navile, Shimoga - 577 204, Karnataka, India
E-mail: koushik.nr@gmail.com

ABSTRACT

A field trail was conducted to study the incidence and management of *Henosepilachana vigintioctopunctata* (Fab.) in brinjal. The peak population beetle (35.33 beetles plant^{-1}) was observed during August first fortnight, while lowest (0.53 beetles plant^{-1}) was noticed during May second fortnight. The incidence of *Epilachna* beetle population exhibited significant positive correlation with rainfall (0.48), while it was significantly negative with minimum (-0.42) and maximum temperature (-0.53). Among the nine treatments evaluated under field conditions, higher reduction in population of epilachna beetle was observed in NSKE (5%) (62.77%). The next best treatments included NSKE (5%) + Spinosad 45SC (15 g ai ha^{-1}) and recommended package of practices (Dimethoate 30 EC @ 1.7 ml l^{-1} – Endosulfan 35 EC @ 2 ml l^{-1}) with 56.13 and 51.28 per cent mortality, respectively.

Keywords: *Henosepilachana vigintioctopunctata,* Seasonal incidence, NSKE.

Brinjal (*Solanum melangina,* L.) is an important vegetable crop grown all over the country. *Epilachna* beetle, *Henosepilachana vigintioctopunctata* (Fab.) attacks various solanaceous vegetables like brinjal, tomato and potato in different parts of country. The losses caused by brinjal pests vary from season to season depending upon environmental factors as reported by Gangawar and Sachen (1981). The interaction between pest activity, biotic and abiotic factors helps in deriving at predictive models that aids in forecast of pest incidence. The damage is caused by the beetles and grubs by feeding on the upper surface of leaves, which in turn presents a lace like appearance, turn brown, dry and fall down. This pest has been successfully managed by the frequent use of insecticides (Mall *et al*., 1992). But its indiscriminate use to control this pest, particularly on vegetables, results in unpredictable side effect on non-target organisms and pest resurgence. So, it is necessary to develop an eco-friendly strategy for managing this pest with botanicals, mechanical methods and biological agents to safeguard the ecosystem. Keeping this in view, the present studies were carried out on the seasonal incidence and management of *Epilachna* beetle.

MATERIALS AND METHODS

The incidence of *Epilachna* beetle, *H. vigintioctopunctata* was recorded at fortnightly intervals starting from 15 February, 2009 to 1 February, 2010. In each plot five plants were selected randomly. On each plant, the total number of active forms of beetles and coccinelled beetles and spiders were counted and expressed in terms of numbers plant^{-1}. The pest incidence and natural enemy population was correlated with meteorological data for establishing the relationship between biotic and abiotic factors with pest and their natural enemies.

A field experiment was carried out during *kharif* season of 2009-10 at the Organic Farming Research Center, Zonal Agricultural Research Station and College of Agriculture, Shimoga, Karnataka, India. The experiment was laid out in randomized block design using Mysore green long variety of brinjal with three replications and nine treatments (Table 4). The 30 days old age seedlings were transplanted in a plot of 25 m^2 by adopting 60 x 60 cm spacing. All the treatments were imposed by using high volume knapsack sprayer @ 500 liters of spray solution ha^{-1}. The crop received totally four sprays. First spray was given at 30 days after transplanting and the remaining three sprays were given with an interval of 15 days between each spray. In case of recommended package of practices, Dimethoate 30 EC @ 1.7 ml l^{-1} and Endosulfan 35 EC @ 2 ml l^{-1} were sprayed alternatively twice. The population was recorded one day before spraying as pre count and at 3, 7 and 15 days after spraying as post treatment counts. The counts of *Epilachna* beetle was taken from five plants selected randomly. In each plant, the total number of active stages of *Epilachna* beetles were counted and per cent mortality was computed.

RESULTS AND DISCUSSION

The seasonal incidence data on *H. vigintioctopunctata* reveals that an incidence ranged from 0.53 to 35.33 beetles

plant^{-1}. The peak population was 35.33 beetles plant^{-1} during August first fortnight, while lowest population of 0.53 beetles plant^{-1} was recorded during May second fortnight. Steady decline in population was noticed from July second fortnight to November second fortnight. After that the population fluctuated without a definite pattern depending on temperature (Table 1). The incidence of *Epilachna* beetle population showed significant positive correlation with rainfall (0.48), while it was significant and negative with minimum (-0.42) and maximum temperature (-0.53) (Table 2). The coefficient of determination (R^2) for Epilachna beetle population was 0.50, which indicates that the abiotic factors together were able to explain the variation in the population of Epilachna beetle to the extent of 50 per cent (Table 3). The present findings are in close conformity with the findings of Sunil and Senapati (2001) who reported that the beetle population was found active from April to middle October. The present findings indicated significant and negative correlation of epilachna population with maximum and minimum temperature which is in agreement with the findings of Masarrat *et al.* (2009).

It is evident from the data that highest mortality of beetle was noticed in NSKE (5%) (62.77%). The next best treatments were NSKE (5%) + Spinosad 45SC (15 g ai ha^{-1}) and recommended package of practices (Dimethoate 30 EC @ 1.7 ml l^{-1} – Endosulfan 35 EC @ 2 ml l^{-1}) with 56.13 and 51.28 per cent mortality, respectively (Table 4). The present

Table 1. Seasonal incidence of *Epilachna* beetle, *Henosepilachna vigintioctopunctata* on brinjal at the College of Agriculture, Shimoga

Fortnight	Mean number of beetles plant^{-1}	Temperature (°C)		RH (%)	Rainfall (mm)
		Maximum	Minimum		
Feb II,2009	21.53	32.88	16.21	79.55	0.0
Mar I	20.20	34.96	18.62	76.77	0.0
Mar II	2.47	36.02	19.72	76.48	0.0
April I	1.27	37.16	21.57	75.66	12.2
April II	0.87	37.72	21.91	72.21	7.4
May I	0.80	38.46	22.29	74.41	12.4
May II	0.53	36.78	21.22	68.79	171
June I	1.73	36.08	21.24	66.19	18.4
June II	1.27	37.62	21.49	66.44	53.8
July I	35.07	34.82	20.4	66.75	211.8
July II	31.53	32.37	20.06	72.12	148.8
Aug I	35.33	32.49	20.74	77.33	14.6
Aug II	23.07	32.44	20.06	77.4	124.5
Sep I	20.13	31.97	20.02	77.79	69.7
Sep II	16.40	32.33	20.13	77.6	83.4
Oct I	11.27	31.39	19.73	77.7	65.6
Oct II	9.13	33.28	20.82	77.65	20.4
Nov I	5.03	33.66	20.81	77.67	27.7
Nov II	1.80	32.52	20.45	77.66	12.1
Dec I	2.87	33.34	21.05	77.67	0.0
Dec II	7.07	34.7	20.58	77.66	0.0
Jan I, 2010	1.53	33.5	19.84	77.66	16.5
Jan II	2.73	34.29	19.86	77.65	0.0
Feb I	10.73	36.38	20.02	77.66	0.0

Table 2. Correlation between *Epilachna* beetle, natural enemies and weather parameters

Pest/ natural enemy/weather parameters	Max. temp	Min. temp	Mean RH (%)	Rainfall	Coccinellids	Spiders
Epilachna beetle	-0.53*	-0.42*	0.05	0.48*	-0.18	-0.46

*Significantly correlated at 5%

Table 'r' value = 0.404

Table 3. Multiple linear regression coefficients between Abiotic factors and *Epilachna* beetle population on brinjal

Pests	Regression Equation	R^2
Epilachna beetle	$Y=-140.99-1.82X_1-2.95X_2-0.14X_3+0.81X_4$	0.50

Where; X_1- Maximum temperature, X_2- Minimum temperature, X_3- Relative humidity, X_4- Rain fall

Table 4. Effect of different management treatments on mortality of the Epilachna beetle, *Henosepilachna vigintioctopunctata*

Treatment	Number of beetles plant^{-1} (1 DBT)	Mean mortality (%)			Mean
		3 DAS	7 DAS	15 DAS	
T_1-Recommended package of practices (Dimethoate 30 EC @ 1.7 ml l^{-1} followed by Endosulfan 35 EC @ 2 ml l^{-1})	15.2	62.72 (52.35)^{ab}	50.44 (45.23)^{cd}	40.68 (39.61)^{bcd}	51.28 (45.72)^{bc}
T_2-*Bt* spray (5%)	14.4	14.41 (21.00)^{de}	24.50 (29.63)^{e}	25.46 (30.18)^{de}	21.46 (27.53)^{de}
T_3-Spinosad 45 SC (15 g ai ha)	13.2	14.10 (20.33)^{de}	22.21 (26.97)^{e}	25.00 (29.92)^{e}	20.43 (25.58)^{e}
T_4-NSKE (5%)	15.1	64.68 (53.52)^{a}	68.65 (56.01)^{a}	54.97 (47.84)^{a}	62.77 (52.39)^{a}
T_5-NSKE (5%)+ Spinosad 45 SC (15 g ai/ha)	15.4	55.84 (48.34)^{ab}	65.37 (53.93)^{ab}	47.19 (43.37)^{b}	56.13 (48.50)^{b}
T_6-Neemoil (4%) +Spinosad 45 SC (15 g ai/ha)	13.5	51.60 (45.93)^{b}	56.54 (48.79)^{bc}	36.79 (37.32)^{c}	48.31 (44.01)^{c}
T_7-Panchagavya (5%)	11.3	30.97 (33.78)^{c}	27.25 (31.45)^{e}	19.59 (26.25)^{f}	25.93 (30.30)^{d}
T_8-Panchagavya (5%) + Neemoil (4%)	12.7	50.13 (45.06)^{b}	55.38 (48.07)^{bc}	36.87 (37.37)^{cd}	47.46 (43.53)^{c}
T_9-Untreated Control (Water spray)	12.6	7.41 (14.79)^{e}	20.19 (26.35)^{e}	14.25 (22.11)^{f}	13.95 (21.75)^{f}
S.Em.±		2.54	2.26	1.45	1.19
C.D. (p=0.05)		8.12	7.23	4.64	3.79
C.V. (%)		11.93	9.84	7.20	5.50

DBT-Day before treatment, DAS-Days after spraying,
Values in parentheses are angular transformed values, Means in the same column showing similar alphabets are at par

findings are in accordance with Misra and Singh (2008) who reported that leaf and seed kernel extract of neem and its active compound azardirachtin had potent antifeedant activity against *E. vigintioctopunctata* with highest feeding deterrence (94.20% mortality). Jacobson (1989) ascribed the higher potentiality of neem seed kernel extract against *E. vigintioctopunctata* to highest concentration of biologically active compound in the seed. Zadda *et al.* (2006) stated that the antifeedant property of neem cake against the Epilachna beetle. Bomford and Isman (1996) and Isman (1997) also reported that neem formulation affected the feeding activity and reduced the consumption of food in various phytophagous insects, which supports the present findings regarding the feeding deterrent activity of neem products against Epilachna beetle.

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Screening of some indigenous plants for their insecticidal activity against stored grain pest *Callosobruchus chinensis* Linn.

S.S. Patole

Department of Zoology, V.V. M's. S. G. Patil ASC College, Sakri - 424 304, Dist- Dhule, M. S., India
E mail: sspatole63@gmail.com

ABSTRACT

In present study, the effectiveness of 50 locally available indigenous plant species from 29 families have been tested against stored grain pulse beetle, *Callosobruchus chinensis* Linn. for the period of six months. The result indicted that plants powder viz., *Acorus calamus, Adhatoda vasica, Azadirachta indica, Melia azedarch, Sphaeranthus indicus* and *Vitex nigundo* were the most effective plants in controlling pulse beetles followed by powders of *Anagallis arvensis, Annona squamosa, Duranta repens, Nicotiana tabacum* and *Randia dumetorum*. In contrast, rest of plants recorded least protection and was on par with untreated control.

Key words: *Callosobruchus chinensis*, indigenous plants, stored grain pests, pulse beetle.

Farmers, through a long history of battle against stored grain pest have learnt to exploit natural resources like botanicals (Gujar and Yadav, 1978). Traditional method usually provides cheap and feasible ways of post-harvest handling of crops by addition of some plant products, which contribute to the reduction of pest activity (Srivastava and Srivastava, 2003; Patole and Mahajan, 2008a). Botanicals, could, therefore, have advantages over synthetic as a cost-effective and environmentally sustainable alternative for protecting stored food against insect attack (Shivanna *et al*., 1994).

The pulse beetle, *Callosobruchus* species is most destructive pest of stored pulse seed. They cause 55 to 60 per cent loss in seed weight (Singh *et al*., 2001), seeds also lose their viability rendering them unfit for sowing and human consumption (Aslam *et al*., 2002; Patole and Mahajan, 2008b). Among the various methods, use of plant products as grain protectant has been traditional methods in villages and this has revived interest in recent years (Kalita *et al*., 2002; Singh, 2003; Patole and More, 2011). Various workers have reported mixing of plant material with pulse seeds which completely checked the population of pulse beetle. On other hand botanicals are relatively safe, low cost, easily availability and ecofriendly. Considering above facts in present piece of research, 50 indigenous plant species were screened against pulse beetle, *Callosobruchus chinensis* for six months of storage.

MATERIALS AND METHODS

The various parts of plant material mentioned in table 1 were collected locally, identified and preserved in herbarium of the botany departmental laboratory. The material was dried naturally under ambient environmental condition (i.e. room temperature, 24-30^{0}C and RH 70-85 %) for one week. The dried material were prepared by grinding in mixer grinder and sieved through mesh size 0.25 mm. The powder was preserved in airtight polythene container.

The fresh green gram seeds (*Vigna radiate* L.) were procured immediately after harvesting. They were dried in sunlight and stored in airtight container until required.

The test insect, *Callosobruchus chinensis* L. was obtained from laboratory maintained culture (Department of Life Science, North Maharashtra University, Jalgaon) and reared on whole grain of green gram seeds at 30 ± 2^{0}C and 75 per cent RH in BOD (Remi) chamber.

In plastic jar (Pearlpet) of 500 g capacity, plant power was applied @ 1 g 100g^{-1} green gram seeds. The seeds were infested deliberately with 10 freshly emerged 1-2 days old adults of *C. chinensis* of either sex in both treated and untreated control jars in triplicate, and stored under ambient conditions. The top of jar was covered with double muslin cloth and tied with rubber band.

The stored green gram seeds were sampled at 2, 4 and 6 months of storage. All the insects i.e. alive and dead along with plant powder were removed every time from all these sets within period of 5 days and per cent mortality of pulse beetle were recorded by Abbott's (1925) formula. Same

Table 1. Screening of some indigenous plants for their insecticidal potential against pulse beetle, *C. chinensis*

Name of the plant	Family	Part used	Per cent mortality within months of exposure		
			02	04	06
Acacia concinna D.C.	Leguminoceae	Pod	16.66 (23.90)	00.00 (00.00)	13.33 (21.40)
Acorus calamus Linn.	Araceae	Rhizome	80.00 (63.40)	50.00 (45.00)	36.66 (37.30)
Adhatoda vesica Nees.	Acanthaceae	Leaves	50.00 (45.00)	26.66 (31.10)	23.33 (28.90)
Agave Americana Linn.	Amaryllidaceae	Leaves	10.00 (18.40)	26.66 (31.10)	13.33 (21.40)
Allium sativum Linn.	Liliaceae	Bulb	16.66 (23.90)	20.00 (26.60)	13.33 (21.40)
Anagallis arvensis Linn.	Primulaceae	Leaves	36.66 (37.30)	23.33 (28.90)	16.66 (23.90)
Andropogan schoenthus L.	Poaceae	Leaves	00.00 (00.00)	30.00 (35.40)	20.00 (26.60)
Annona muricata Linn.	Annonaceae	Leaves	33.33 (35.30)	26.66 (31.10)	10.00 (18.40)
Annona squmosa Linn.	Annonaceae	Leaves	40.00 (39.20)	33.33 (35.30)	16.66 (23.90)
Azadirachta indica A. Juss	Meliaceae	Leaves	50.00 (45.00)	36.66 (37.30)	13.33 (21.40)
Bacopa monnierri Linn.	Scrophulariaceae	Leave	23.33 (28.90)	10.00 (18.40)	00.00 (00.00)
Balanites roxburghii Planch	Simarubaceae	Fruit	23.33 (28.90)	00.00 (00.00)	16.66 (23.90)
Brassica nigra Linn.	Cruciferae	Seed	20.00 (26.60)	33.33 (35.30)	23.33 (28.90)
Cestrum diurnum Linn.	Solanaceae	Leaves	20.00 (26.60)	00.33 (01.70)	13.33 (21.40)
Cestrum nocturnum Linn.	Solanaceae	Leaves	23.33 (28.90)	13.33 (21.40)	10.00 (18.40)
Citrullus colocynthis Shrad.	Cucurbitaceae	Fruit	23.33 (28.90)	10.00 (18.40)	13.33 (21.40)
Clerodendrum phlomidis L.	Verbenaceae	Leaves	00.33 (01.70)	33.33 (35.30)	13.33 (21.40)
Cocculus hirsutus Linn.	Menispermeacea	Leaves	43.33 (41.20)	23.33 (28.90)	10.00 (18.40)
Coriandrum sativum Linn.	Apiaceae	Leaves	16.66 (23.90)	23.33 (28.90)	00.00 (00.00)
Croton tiglium Linn.	Euphorbiaceae	Seeds	36.66 (37.30)	30.00 (33.20)	00.33 (01.70)
Datura metal Linn.	Solanaceae	Leaves	30.00 (35.40)	23.33 (28.90)	13.33 (21.40)
Dodonaea viscose Linn.	Sapindaceae	Leaves	16.66 (23.90)	20.00 (26.60)	00.66 (02.30)
Duranta repens Linn.	Verbenaceae	Leaves	10.00 (18.40)	26.66 (31.10)	33.33 (35.30)
Eucalyptus globules Labill	Myrtaceae	Leaves	16.66 (23.90)	30.00 (35.40)	00.66 (02.30)
Jatropha curcus Linn.	Euphorbiaceae	Leaves	10.00 (18.40)	16.66 (23.90)	33.33 (35.30)
Lantena camera Linn.	Verbanaceae	Leaves	30.00 (35.40)	20.00 (26.60)	20.00 (26.60)
Lycopersicon esculentum Mill.	Solanaceae	Leaves	13.33 (21.40)	23.33 (28.90)	23.33 (28.90)
Madhuca indica J.F. Gmel.	Sapotaceae	Fruit	16.66 (23.90)	13.33 (21.40)	13.33 (21.40)
Melia azadirachta Linn.	Meliaceae	Leaves	53.33 (46.90)	36.66 (37.30)	10.00 (18.40)
Mentha arvensis Linn.	Lamiaceae	Leaves	16.66 (23.90)	16.66 (23.90)	13.33 (21.40)
Murraya kioenigii Spreng.	Rutaceae	Leaves	00.00 (00.00)	23.33 (28.90)	13.33 (21.40)
Nerium oleander Linn.	Apocynaceae	Leaves	30.00 (35.40)	33.33 (35.30)	13.33 (21.40)
Nicotiana tabacum Linn.	Solanaceae	Leaves	36.66 (37.30)	36.66 (37.30)	13.33 (21.40)
Ocimum sanctum Linn.	Lamiaceae	Leaves	00.00 (00.00)	23.33 (28.90)	26.66 (31.10)
Peganum harmala Linn.	Rutaceae	Seeds	20.00 (26.60)	23.33 (28.90)	00.66 (02.30)
Polygonum hydropiper Linn.	Polygonaceae	Leaves	30.00 (35.40)	16.66 (23.90)	13.33 (21.40)
Pongamia glabra Vent.	Leguminoceae	Seeds	26.66 (31.10)	00.33 (01.70)	30.00 (35.40)
Randia dumetorum Lam.	Rubiaceae	Fruit	30.00 (35.40)	36.66 (37.30)	10.00 (18.40)
Ricinus communis Linn.	Euphorbiaceae	Leaves	26.66 (31.10)	23.33 (28.90)	10.33 (21.40)
Santalum album Linn.	Santalaceae	Stem	13.33 (21.40)	33.33 (35.30)	13.33 (21.40)
Sapindus trifoliatus Linn.	Sapindaceae	Fruit	33.33 (35.30)	20.00 (26.60)	00.66 (02.30)
Sphaeranthus indicus Linn.	Asteraceae	Fruit	30.00 (35.40)	16.66 (23.90)	26.66 (31.10)
Spilanthus acmella Murr.	Asteraceae	Stem	10.00 (18.40)	13.33 (21.40)	13.33 (21.40)
Tagetes minuata Linn.	Asteraceae	Flower	00.00 (00.00)	13.33 (21.40)	16.66 (23.90)
Tephrosia purpurea Pers.	Leguminosae	R. bark	23.33 (28.90)	16.66 (23.90)	10.00 (18.40)
Thevetia neriifolia Juss.	Apocynaceae	Leaves	16.66 (23.90)	26.66 (31.10)	10.00 (18.40)
Trigonella foenum graecum L.	Leguminosae	Leaves	10.00 (18.40)	26.66 (31.10)	10.33 (21.40)
Vitex nigundo Linn.	Vernaceae	Leaves	10.33 (21.40)	43.33 (41.20)	36.66 (37.30)
Withania somnifera Dunal.	Solanaceae	Leaves	20.00 (26.60)	13.33 (21.40)	13.33 (21.40)
Zingiber officinale Rosc.	Zingiberaceae	Rhizome	16.66 (23.90)	10.33 (21.40)	00.66 (02.30)
Control	-		00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
S. E ±			13.10	10.00	08.93
CD (P = 0.05)	-		34.48	26.30	23.48

Values in parenthesis are the arc sin transformation of mean values, which are average of three replicas.

methods were followed for 4 and 6 months of treatment. The statistical analysis of data was done by Randomized Block Design (RBD) ANOVA-method. Whereas the Critical Difference (CD at 5 % level of significance) was calculated (Bansal *et al.*, 1991).

RESULTS AND DISCUSSION

The per cent mortality of pulse beetle, *C. chinensis* during 2, 4 and 6 months of treated seeds with plant powder is presented in table-1. It revealed that, among the 50 plant screened for their insecticidal activity the plants like, *Acorus calamus, Adhatoda vasica, Anagallis arvensis, Azadirachta indica, Annona squamosa, Duranta repens, Melia azedarch, Nicotiana tabacum, Randia dumetorum, Sphaeranthus indicus* and *Vitex nigundo* showed significant mortality than other remaining plants.

At two months of storage, green gram seeds treated with *Acorus* powder showed highest mortality i.e. 80 per cent. Other plants like *Melia, Adhatoda* and *Azadirachta* and *Annona* registered 53.33, 50.00 and 40.00 per cent mortality respectively. At four months of seed storage, again *Acorus* powder showed highest mortality i.e. 50 per cent, followed by Vitex i.e. 43.33 per cent. The powder of plants viz., *Azadirachta, Melia, Nicotiana* and *Randia* showed similar mortality i.e. 36.66 per cent. At six months of storage, plant powder like *Acorus* and *Vitex* showed similar mortality i.e. 36.66 per cent. Whereas 33.33 per cent mortality observed in seeds treated with *Duranta* powder and least mortality i.e. 26.66 per cent showed in seeds treated with *Sphaeranthus indicus* plant.

Plant material with insecticidal properties provides small-scale farmers with a locally available, biodegradable and inexpensive material for stored grain pest control. Many earlier workers like Shivanna *et al*., 1994; Singh *et al*., 2001; Srivastava and Srivastava, 2003 and Patole and Mahajan, 2008b etc. reported importance of plants as pulse seed protectants. The above results are corroborated with their findings.

The plant products are biodegradable and less persistence in environment and they are potential alternatives to synthetic pesticides. They are also safe to human beings and less toxic to non-target organisms. The use of botanicals has the potential to bring prosperity to rural areas. Hence, plant products should be exploited for their insecticidal properties.

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Evaluation of pearl millet hybrids and varieties for their reaction to *Peregrinus maidis*

R. A. Balikai[1], D. Lakshmana[2] and A. K. Guggari

AICRP on Pearl Millet, Regional Agricultural Research Station, Bijapur - 586 101, Karnataka, India. [1]College of Agriculture, University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India. [2]University of Agricultural Sciences, Bangalore - 580 005, Karnataka, India
E-mail: rabalikai@gmail.com

ABSTRACT

Two trials consisting of advanced hybrids (11 entries) and released hybrids and varieties (10 entries) were taken up during *kharif* 2007 at the Regional Agricultural Research Station, Bijapur, Karnataka, India to know their reaction to shoot bug, *Peregrinus maidis* (Ashmead). In the first trial, significantly less percentage of plants were infested by shoot bug in JKBH-704 (10.9%) followed by KDBH-1157, GHB-558 and Vasundhara-1157 with 15.6, 20.3 and 20.6 per cent, respectively as compared to rest of the entries. The hybrid JKBH-704 recorded lower shoot bug population of 10 to 25 plant^{-1}. The entries Vasundhara - 1157, KDBH-1157 and GHB-558 recorded medium population of 26 to 50 shoot bugs per plant. The black ant activity was significantly lowest in JKBH-704 (5.3 plant^{-1}) followed by KDBH-1157 and Vasundhara-1157 with 20.8 and 25.5 black ants plant^{-1}, respectively as compared to rest of the entries. Significantly highest grain yield was obtained from Vasundhara-1157 (48.44 q ha^{-1}) followed by P-104, P-105 and KDBH-1157 with 45.49, 42.15 and 40.97 q ha^{-1} of grain yield, respectively. In the second trial, significantly less percentage of plants were infested by shoot bug in PB-106 and ICTP-8203 (5.6%) followed by ICMV-155, Sharadha and ICMV-221 with 5.7, 5.9 and 6.4 per cent, respectively as compared to rest of the entries. The entries *viz*., GHB-558, Pusa-23, PB-106, Sharadha, ICMV-221 and ICTP-8203 recorded lowest shoot bug population of 10 to 25 plant^{-1}. The black ant activity was significantly lowest in Pusa-23 (10.6 plant^{-1}) and PB-106 followed by ICTP-8203, ICMV-221 and ICMV-155 with 15.5, 15.7 and 15.8 black ants plant^{-1}, respectively as compared to rest of the entries. Significantly highest grain yield was obtained from PB-106 (49.31 q ha^{-1}) followed by GHB-558 (43.06 q ha^{-1}) as compared to rest of the entries.

Key words: *Peregrinus maidis*, pearl millet, advanced hybrids, released hybrids and varieties

Pearl millet, *Pennisetum glaucum* (L.) R.Br. is a major staple crop of north Karnataka covering an area of 2.1 lakh hectares with production of 1.1 lakh tones and productivity of 531 kg ha^{-1}. In Bijapur district alone the crop is being cultivated in an area 1.12 lakh hectares with a productivity of 578 kg ha^{-1}. Insect pests are one of the important constraints in increasing pearl millet production. Over 100 species of insect pests have been reported to be associated with this crop. However, Prem Kishore and Solomon (1989) listed about 25 species of potential insect pests of cropping systems in India based on pearl millet. Balikai (2009) reported a total of 26 insect and 2 non-insect pests feeding on pearl millet in Karnataka. The adults and nymphs of shoot bug, *Peregrinus maidis* (Ashmead) suck the sap from peduncle and compact earheads leading to chaffyness of earheads. The activity of ant, *Camponotus compressus* Fb. can be seen attending shoot bugs for their honey dew excretion. Hence, the entries in All India Co-ordinated trials *viz*., Advanced Hybrids Trial and Released Hybrids and Varieties Trial were evaluated for their reaction to shoot bug, *P. maidis*.

MATERIALS AND METHODS

Two trials consisting of advanced hybrids with 11 entries (Table 1) and released hybrids and varieties with 10 entries (Table 2) were taken up during *kharif* 2007 at the Regional Agricultural Research Station, Bijapur, Karnataka, India. The experiments were conducted in a randomized block design with three replications. The plot size for each entry was 9.6 m^2. Recommended dose of fertilizer i.e. 50:25:0 kg NPK ha^{-1} was applied at the time of sowing which was done on 22-06-2007. Number of plants infested by shoot bug was recorded at 80 days after sowing and percentage of plants infested by shoot bug was worked out. The shoot bug population (both nymphs and adults) present on the peduncle portion of each plant was counted and rated using the following scale.

Category	Symbol	Number of shoot bugs plant^{-1}
Low population	L	10-25
Medium population	M	26-50
High population	H	>51

Number of black ants (*C. compressus*) attending the shoot bugs for their honey dew excretion was also recorded plant^{-1}. Finally earheads were harvested at the maturity state and grain yield plot^{-1} was recorded and computed to ha^{-1}.

RESULTS AND DISCUSSION

The results on reaction of advanced hybrid trial entries to shoot bug, *P. maidis* are presented in Table 1. The percentage of plants infested by shoot bug varied from 10.9 in JKBH-704 to 40.7 in Pusa - 23. Significantly less percentage of plants were infested by shoot bug in JKBH-704 (10.9%) followed by KDBH-1157, GHB-558 and Vasundhara-1157 with 15.6, 20.3 and 20.6 per cent, respectively as compared to rest of the entries. More than 25 per cent of plants were infested by shoot bug in case of JKBH-703 (27.0%), P-105 (30.4%), P-104 (30.5%), GHB-797 (30.8%), Saburi (35.7%) and Pusa-23 (40.7%). The hybrid JKBH-704 recorded lowest shoot bug population of 10 to 25 plant^{-1}. The entries Vasundhara-1157, KDBH-1157 and GHB-558 recorded medium population of 26 to 50 shoot bugs plant^{-1}. Rest of the entries *viz*., P-104, JKBH-703, P-105, GHB-785, GHB-797, Pusa-23 and Saburi attracted high population of more than 51 shoot bugs plant^{-1}. The black ant activity was significantly lowest in JKBH-704 (5.3 plant^{-1}) followed by KDBH-1157 and Vasundhara-1157 with 20.8 and 25.5 black ants plant^{-1}, respectively as compared to rest of the entries. The highest activity of black ants was recorded in P-104 (65.5 plant^{-1}) which sustained higher population of shoot bugs (>51 plant^{-1}). Significantly highest grain yield was obtained from Vasundhara-1157 (48.44 q ha^{-1}) followed by P-104, P-105 and KDBH-1157 with 45.49, 42.15 and 40.97 q ha^{-1} of grain yield, respectively. The lowest grain yield was recorded by GHB-558 (32.88 q ha^{-1}) (Table 1).

The results on reaction of released hybrids and varieties trial entries to shoot bug, *P. maidis* are presented in Table 2. The percentage of plants infested by shoot bug varied from 5.6 in PB-106 and ICTP-8203 to 40.4 per cent in ICMH-356. Significantly less percentage of plants were infested by shoot bug in PB-106 and ICTP-8203 (5.6%) followed by ICMV-155, Sharadha and ICMV-221 with 5.7, 5.9 and 6.4 per cent, respectively as compared to rest of the entries. The higher percentage of plants were infested by shoot bugs in ICMH-356 (40.4%). The entries *viz*., GHB-558, Pusa-23, PB-106, Sharadha, ICMV-221 and ICTP-8203 recorded lower shoot bug population of 10 to 25 per plant. The entries ICMH-356, Saburi, ICMV-155 and Raj-171 recorded medium population of 26 to 50 shoot bugs plant^{-1}. None of the entries attracted high population of more than 51 shoot bugs per plant. The black ant activity was significantly lowest in Pusa-23 (10.6 plant^{-1}) and PB-106 (10.9 plant^{-1}) followed by ICTP-8203, ICMV-221 and ICMV-155 with 15.5, 15.7 and 15.8 black ants plant^{-1}, respectively as compared to rest of the entries. The highest activity of black ants was recorded in ICMH-356 (25.8 plant^{-1}) which sustained medium population of shoot bugs (26-50 plant^{-1}). Significantly highest grain yield was obtained from PB-106 (49.31 q ha^{-1}) followed by GHB-558 (43.06 q ha^{-1}) as compared to rest of the entries. The lowest grain yield was recorded by Pusa-23 (27.22 q ha^{-1}) (Table 2).

Table 1. Reaction of advanced hybrid trial entries to shoot bug, *Peregrinus maidis*

Entry	Percentage of plants infested	Number of shoot bugs plant^{-1}	Black ants plant^{-1}	Grain yield (q ha^{-1})
P-104	30.5 (33.5)	H	65.5	45.49
Vasundhara -1157	20.6 (27.0)	M	25.5	48.44
JKBH-704	10.9 (19.3)	L	5.3	38.06
KDBH-1157	15.6 (23.3)	M	20.8	40.97
JKBH-703	27.0 (31.3)	H	61.0	38.89
P-105	30.4 (33.4)	H	50.7	42.15
GHB-785	24.8 (29.9)	H	50.2	36.11
GHB-797	30.8 (33.7)	H	61.2	38.89
Pusa -23	40.7 (39.7)	H	60.7	35.73
Saburi	35.7 (36.7)	H	61.8	38.47
GHB-558	20.3 (26.7)	M	40.4	32.88
S.Em.±	1.0	-	1.6	1.58
C.D. at 5%	2.9	-	4.8	4.66

Figures in the parentheses are arc sin transformations
L= 10-25, M= 26-50, H= >51

Table 2. Reaction of released hybrids and varieties to shoot bug, *Peregrinus maidis*

Entry	Percentage of plants infested	Number of shoot bugs plant^{-1}	Black ants plant^{-1}	Grain yield (q ha^{-1})
GHB-558	20.3 (26.8)	L	20.8	43.06
Pusa-23	21.5 (27.6)	L	10.6	27.22
PB-106	5.6 (13.7)	L	10.9	49.31
ICMH-356	40.4 (39.4)	M	25.8	27.78
Saburi	21.2 (27.4)	M	20.8	37.15
Sharadha	5.9 (14.1)	L	21.1	31.60
ICMV-221	6.4 (14.6)	L	15.7	30.21
ICTP-8203	5.6 (13.7)	L	15.5	31.39
ICMV-155	5.7 (13.8)	M	15.8	30.00
Raj-171	10.4 (18.8)	M	20.6	29.17
S.Em.±	0.9	-	1.4	1.32
C.D. at 5%	2.6	-	4.1	3.94

Figures in the parentheses are arc sin transformations
L= 10-25, M= 26-50, H= >5

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Bioassay methods to quantify toxicity of transgenic cotton against cotton bollworm, *Helicoverpa armigera* (Hübner)

Suneet Pandher[1] and V.K. Dilawari

[1]*Punjab Agricultural University, Regional Station, Faridkot-151 203, Punjab, India*
E mail: suneet@pau.edu

ABSTRACT

The study was carried out to evaluate the bioassay methods (leaf mush + diet and leaf disc) for their efficacy in recording mortality of *Helicoverpa armigera* neonates on bt cotton hybrid RCH 134. Mortality was more in leaf disc method (90%) than the leaf mush method (83.3%) at 7 days interval as evident from the amount of Cry 1Ac which was found out to be at higher level in leaf disc (9.74 µg g^{-1}) than the leaf mush (6.20 µg g^{-1}). The treatment of leaves at 50^0C to produce mush seemed to reduce toxin concentration and thus the leaf disc method proved more efficacious. Besides, sensitivity of larval instars decreased with age as supported by highest mortality in the first instars at different stages of growth of plant.

Key words: Transgenic cotton, *H. armigera*, bioassays, leaf disc, leaf mush

Genes from *Bacillus thuringiensis* var. *kurstaki* producing the *Cry* 1Ab or *Cry* 1Ac proteins that are toxic specifically to lepidopteran species were inserted into cotton plants by Perlak *et al.* (1990). The target pests were the heliothine species, *Helicoverpa zea* and *Heliothis virescens*, major pests of cotton and other crops in North America. Australian cotton varieties were also transformed using this gene construct to give protection against pests, *Helicoverpa armigera* and *H. punctigera.* The first Bt transgenic cotton varieties (Bt cotton) were commercially released in Australia (Ingard) and the United States (Bollgard) in 1996. Since then, there has been considerable increase in the number of countries adopting this technology to reduce pesticide load on the cotton crop. In India, three Bt-cotton hybrid varieties,

Bollgard MECH-12, MECH-162 and MECH-184 were released during 2002 by Mahyco India Ltd. for commercial cultivation after GEAC approval. Different aspects of Bt cotton have been worked upon since 1996 and in continuity with that, present study was taken up to investigate the temporal variation in efficacy of Bt toxin, bioassays done in lab to study the effective way to quantify the toxicity of Bt toxin.

MATERIALS AND METHODS

The study was carried out to evaluate the response of *H. armigera* larvae using the terminal leaves of bt and non bt hybrid RCH 134 at different plant growth stages. Larvae were reared on gram flour based artificial diet in the laboratory according to Armes *et al.* (1992). In the leaf mush method, terminal leaves were collected at different plant stages varying from 70 to 90 DAS and washed with distilled water. After air drying, leaves were put in vacuum dessicator for about three hours at 50^0C and then finely ground using pestle mortar to produce finely ground leaf mush. The mush (3 g) was added to the artificial diet (30 ml) before solidification and poured into vials to be used in bioassays. In leaf disc method, terminal leaves were used to cut out discs of about 4 cm diameters and placed over 2 ml of solidified agar (2 %) separated by filter paper disc of same size. The neonates were released singly into each vial and scored as dead or alive after probing with a brush. Each larva was considered as a single replication. Leaf mush and discs were analyzed for their Cry 1Ac content using ELISA kit of Envirologix 500 Marine, USA as per manufacturer's protocol. Determination of sensitivity of larval instars of *H. armigera* to terminal leaves was carried out using leaf disc method at different plant growth stages. Mortality data in different treatments was subjected to Z test for analysis.

RESULTS AND DISCUSSION

Efficacy of bioassay methods against neonates of *H. armigera* with bt cotton RCH 134:

Two bioassay techniques were developed to evaluate the toxicity of leaves of Bt cotton against neonates of *H. armigera.* These were leaf mush + diet method and leaf disc method. Per cent mortality of *H. armigera* neonates in these bioassays at two and seven days after treatment has been presented in table 1. Non Bt RCH 134 cotton leaves were used as control in the bioassays. Comparison between these two bioassays showed that mortality was more in leaf disc

method (90%) than the leaf mush method (83.3%) at 7 days interval. Amount of Cry 1Ac was measured in both leaf disc and mush and found out to be at higher level in leaf disc (9.74 µg g^{-1}) than the leaf mush (6.20 µg g^{-1}). The leaf disc method proved more efficacious than the leaf mush method as shown by z-test values which are significantly different for the mortality data. For each set of comparison between bt and non bt RCH 134 leaves in the two bioassay methods, bt RCH 134 was found to be more effective than non bt RCH 134 at different intervals of observations (i.e. 2 and 7 days). Per cent mortality was lower at two days after the treatments were laid out in both the methods. Per cent mortality data at seven days was significantly superior to that at two days after treatment.

In this experiment, two bioassay methods were evaluated for their efficacy in quantifying toxicity of Cry 1Ac toxin in bt cotton RCH 134 leaves. Results indicated that leaf disc method proved better in recording the mortality of *H. armigera* neonates than the leaf mush + diet method. Amount of Cry toxin detected was also lower in leaf mush as compared to leaf disc used in bioassays which could explain the differences in per cent mortality in both bioassays. Toxicity of Cry 1Ac protein is reduced depending on the source of toxin and bioassay method used (Olsen and Daly 2000). Handling of plant tissue was different for leaf mush and leaf disc bioassays. Physiological changes occur when plants are damaged and plant response also varies with the type of damage and the region of the plant. There is the potential for the toxins to be differentially bound among treatments as artifacts of the plant handling.

Olsen *et al.* (2005) reported that bioassays are not a direct measure of bt toxin, but rather reflect the combined effect of bt and that of naturally occurring plant compounds. This could explain the apparent discrepancy between the extent of the decline in efficacy in field bioassays and that determined for the bt toxin. The toxin may be sequestered or altered by the plant. Secondary metabolites such as phenolics and terpenoids have been implicated in this process.

Toxicity of bt toxin is influenced by the handling of plant tissue as reported earlier. Different bioassay methods have been developed since the introduction of bt cotton to improve the efficacy under the laboratory conditions. Stewart *et al.* (2001) detected the interactions between the cultivar effect and assay method which could indicate that one of the assay methods was more reliable in separating treatment effects. These interactions resulted from the recovery of larvae after they were transferred from bt cotton tissue to diet whereas recovery did not occur when insects were continuously fed plant material. Thus, maintaining insects on plant material rather than transferring to diet, appeared the best approach for increasing the sensitivity of fresh tissue assays. Assays using lyophilized plant tissue incorporated into diet have some advantages as reported by Stewart *et al.* (2001). Plant tissues could be stored until needed and allowed for comparison among plant tissues collected at different times. Further, dosages could be adjusted so that excessive mortality caused by toxic plant tissue didn't occur and hence neonates could be used in assays. Besides plant tissues, components of the artificial diet play a crucial role in determining toxicity of Cry1Ac toxin (Gunning and Moores, 2010). Detection of Cry1Ac resistance in *H. armigera* could be masked if the artificial diet contained raw soy bean flour which exposed the larvae to potent protease inhibitors compared to heat-denatured flour.

Studies conducted by Kranthi *et al.* (2005) clearly showed that bt cotton seeds were a good source of Cry 1Ac for bioassays on *H. armigera.* Bioactivity of the Cry 1Ac toxin did not decline despite the seeds having been stored at room temperature for two years as shown by the similarity in toxic effect of the two year old seed lot in comparison to the fresh lot. The results also showed that the influence of maximum initial heat of 64^0C during diet preparation had minimal detrimental effect on the bioactivity of Cry 1Ac on *H. armigera.* However, it was suggested to cool the mixture of agar-yeast for 3-4 minutes before adding to diet containing bt cotton seed flour because the average weight of the larvae surviving the diet prepared at initial maximum temperature of 64^0C was higher compared to that from the rest of the treatments. It was thus possible that the initial heat of 64^0C might have affected the bioactivity of Cry 1Ac, though to a minor extent.

Sensitivity of larval instars of *H. armigera* to terminal leaves of Bt cotton

Neonates, second instar and third instar larvae were evaluated for their sensitivity to terminal leaves of bt cotton RCH 134 cotton at two stages of plant growth i.e. 50 and 80 DAS (days after sowing) using leaf disc method. Older instars i.e. second and third were included in the experiment to study the sensitivity of larvae because bollworms may have the ability to survive on bt cotton plant parts expressing low levels of toxin (i.e. flowers) or immigrate from non-Bt cotton.

The results given in table 2 showed that terminals at 50 DAS were the most toxic to neonates followed by second and third instar larvae. Per cent mortality varied in the range from 91.1 to 40 in neonates and third instar respectively with intermediate mortality of 66.67 per cent in second instar larvae. Toxicity was more pronounced at 7 days of treatment as compared to 2 days among larvae of all instars. This shows results should be observed 6-7 days after the bioassay. Even

under control, mortality was recorded up to 4.44 per cent in neonates which might be due to plant chemistry.

Analysis of observations showed that the neonates were the most sensitive stage among the larval instars as there was significant difference between the per cent mortality of neonates and third instar larvae (Jackson *et al.* 2005).

In another study, sensitivity of larval instars was determined to terminal leaves of RCH 134 at 80 DAS. Per cent mortality was the highest in the neonates (82) as observed earlier and decreased as the larval age increased to third instar (50%). However, the difference in mortality found among the three instars was not statistically different. Marked effect had been noticed with the larval growth and the surviving first instar larvae continued to be in first instar even after four days while those on the non-transgenic lines molted to second instars on the day three of the feeding. In the case of second and third instar, the survival percentage was more on terminals of transgenic cotton compared to the first instar but the developmental impact of bt cotton was very much evident.

Murugan *et al.* (2003) observed that even though second and third instars recorded increased level of survival compared to first instar, the effect of bt cotton feeding on the final weight was very high for both the instars as seen by the poor weight gain of both these instars. Thus, despite causing mortality in the early instars, the bt cotton could disrupt feeding and development of *H. armigera* in later instars. Benedict *et al.* (1992) had observed that the feeding deterrent effect may cause larvae to rapidly leave transgenic cotton plants and seek other food sources as had been noticed with *H. virescens.* Weakening of the larvae and their continued presence over a longer period of time in the field may expose them to beneficial insects or other sources of mortality. Another factor also comes into play when the laboratory bioassays are conducted as mentioned by Chitkowski *et al.* (2003). Rearing of larvae on diet and then transferring them as second instars to bt cotton would make them more tolerant of the toxins than neonates in field situations that feed only on toxic plant tissue. This factor must be considered while comparing the bioassays results with the field studies on the survival of larvae. Similar observations were reported by Stewart *et al.* (2001) in which third instar bollworm survived and damaged young bolls from dual-toxin bt cultivars if they were fed relatively non toxic hosts or plant parts before exposure to bt cotton.

Table 1. Evaluation of bioassay methods against neonates of *H. armigera* with bt cotton leaves

Bioassay method	Per cent mortality*			
	Bt RCH 134		Non Bt RCH 134	
	2 DAT	7 DAT	2 DAT	7 DAT
Leaf mush + Diet (M_1)	16.67	83.3	3.33	10
Leaf disc (M_2)	26.67	90	3.33	6.67

DAT: Days after treatment; *Mean of 30 larvae treatment^{-1},

Avg. Amount of Cry 1Ac (mg g^{-1}): Leaf Disk = 9.74; Leaf Mush = 6.20, Z (M_1 & $M_{2)}$ = 4.73,

Bt and non Bt RCH 134 = 68.45; 2 and 7 DAT = 49.56

Table 2. Sensitivity of larval instars of *H. armigera* to terminal leaves of Bt cotton at 50 (Days after sowing)

Instar Tested	% Mortality*			
	Bt RCH 134		N Bt RCH 134	
	2 DAT	7 DAT	2 DAT	7 DAT
Neonates	22.22	91.1	2.22	4.44
IInd	13.33	66.67	0	0
IIIrd	3.3	40	0	0

DAT = Days after treatment *Mean of 30 larvae/treatment

Z_{cal} Neonates and second instar = 0.97; Z_{cal} Second and third instar = 0.98, Z_{cal} Neonates and third instar = 1.95

Table 3. Sensitivity of larval instars of *H. armigera* to terminal leaves of Bt cotton at 80 DAS

Instar Tested	% Mortality*			
	Bt RCH 134		N Bt RCH 134	
	2 DAT	7 DAT	2 DAT	7 DAT
Neonates	25	82	5	10
IInd	10	60	0	0
IIIrd (3)	5	50	0	0

*Mean of 30 larvae treatment^{-1}, Z_{cal} Neonates and second instar = 0.91, Z_{cal} Second and third instar= 0.43,

Z_{cal} Neonates and third instar = 1.32

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Population dynamics of fruit flies, *Bactrocera* spp. in tomato, *Lycopersicon esculentum* Mill.

T. Boopathi

Division of Entomology, ICAR Research Complex for NEH Region, Mizoram Centre, Kolasib-796 081, Mizoram, India.

ABSTRACT

Population fluctuation and seasonal incidence of fruit flies, *Bactrocera* spp. on tomato crop using methyl eugenol traps revealed that four distinct peaks was noticed during second week of April (134.3), first week of May (137.7), second week of May (167.7) and first week of June (130.0). These peaks coincided with tomato fruiting period in relation to April to June months. Among abiotic factors, the maximum temperature (r = 0.332) showed a positive correlation, while the minimum temperature, relative humidity, rainfall and rainy days had a negative correlation with trap catches of fruit flies. The abiotic factors jointly had non-significant impact on population of fruit flies. T-value of fruit flies population had a significantly positively correlated with maximum temperature (2.34), while it was significantly negatively correlated with minimum temperature (-2.22). However, the influence of relative humidity (minimum and maximum), rain fall and rainy days were found to be not significant.

Key words: Seasonal incidence, fruit flies, tomato, weather factors, methyl eugenol traps

Tomato, *Lycopersicon esculentum* Mill. carves for itself a distinct niche in the realm of the vegetable crops. Among the insect pests infesting tomato, fruit flies, *Bactrocera* spp. (Diptera : Tephritidae) is serious loss in the tomato growing areas of Mizoram. Fruit flies have high reproductive potential, wide host range, adaptability to climate and overlapping of generations, their management is rather difficult. The pest not only deteriorates its quality also due to premature droppage of infested fruits. The population build up of any insect is very intimately associated with the weather parameters prevailing during preceding and corresponding periods. The pest status does not remain static throughout the year but changes accordingly based on abiotic factors like temperature, relative humidity, rainfall, rainy days, wind speed, etc. So from the above information it becomes necessary to know the factors affecting the fly population, for effective management of the pest. Therefore the present investigations were aimed to study the seasonal abundance and population fluctuation of fruit flies infesting tomato.

MATERIALS AND METHODS

The studies on population dynamics were carried out in a tomato field at the ICAR Research farm, Kolasib, Mizoram, India. Three traps were maintained and the traps were hung in bamboo stick at about 1.5 m above ground level. These monitoring traps were loaded with vials immersed with a cotton wick containing 15 ml of methyl eugenol and malathion in the ratio of 3:1 and the solution changed once in three months. Fruit flies catches were recorded weekly and collected during March, 2009 to July, 2009. The weekly data on fruit flies incidence were subjected to correlation and regression analyses with average weekly weather data to find out the influence of abiotic factors on fruit flies infestation.

RESULTS AND DISCUSSION

The results in the table 1 showed that more numbers of fruit flies caught during April to June months and it was coincided with tomato fruiting period. The catches continued to increase from early March and reached a major peak in mid May (167.7) was followed by a sharp decline in end May. Later a minor peak was seen in early June and the catches sharply declined to reach very low numbers in mid/late July. These findings are in agreement with the reports of Sarada *et al*. (2001) and Latif and Abdullah (2005). The error variation between observed and predicted values was ranged from -65.33 to 75.56.

Population of fruit flies, *Bactrocera* spp. attracted in methyl eugenol were positively correlated with maximum temperature (r = 0.332). However, the influence of minimum temperature, relative humidity (maximum and minimum), rainfall and rainy days was found to be negative correlation. Gupta and Bhatia (2000) and Gajalakshmi *et al*. (2011) have also reported positive correlation between trap catches and maximum temperatures and hence support our findings. Rainfall found negatively correlated with population of fruit

Table 1. Population dynamics of fruit flies in tomato during March 2009 to July 2009

Date of collection	SMW	Fruit flies population*	Predicted values	Error variation between observed and predicted values	Weather parameters					
					Temperature (^{0}C)		Relative humidity (%)		Rainfall (mm)	Rainy days (2.5mm & above)
					Min.	Max.	Min.	Max.		
17.03.2009	12	24.33^{e-h}	23.67	0.66	21.8	25.9	28.6	41.3	0.0	0
24.03.2009	13	39.33^{d-h}	43.90	-4.57	21.5	27.4	33.0	77.0	0.0	0
31.03.2009	14	38.00^{d-h}	79.15	-41.15	16.1	27.4	62.4	99.0	5.0	3
07.04.2009	15	62.33^{b-e}	69.08	-6.75	18.4	27.6	50.4	90.4	9.0	3
14.04.2009	16	134.33^{ab}	98.52	35.81	21.5	30.5	47.0	55.0	0.0	2
21.04.2009	17	52.33^{d-g}	64.06	-11.73	20.7	27.3	75.0	98.0	0.0	4
28.04.2009	18	74.67^{b-e}	106.77	-32.10	23.6	32.3	57.0	86.0	0.0	0
05.05.2009	19	137.67^{ab}	124.54	13.13	21.4	32.4	52.0	64.0	0.0	1
12.05.2009	20	167.67^{a}	127.78	39.89	23.5	31.3	54.0	64.0	0.0	2
19.05.2009	21	104.33^{a-d}	76.02	28.31	19.1	30.4	60.0	85.0	50.0	5
26.05.2009	22	58.00^{c-f}	35.09	22.91	22.0	28.0	80.0	85.0	10.0	5
02.06.2009	23	130.00^{abc}	54.44	75.56	21.9	26.5	88.0	91.0	0.0	1
09.06.2009	24	58.33^{b-e}	60.06	-1.73	22.0	30.5	64.0	94.0	0.0	4
16.06.2009	25	64.00^{b-e}	54.81	9.19	22.7	29.2	58.0	75.0	16.0	4
23.06.2009	26	42.33^{d-g}	23.86	18.47	24.7	31.9	94.0	97.0	0.0	2
30.06.2009	27	18.67^{e-h}	6.31	12.36	22.8	29.3	78.0	88.0	10.0	3
07.07.2009	28	7.00^{fgh}	72.33	-65.33	23.8	30.0	60.0	95.0	0.0	3
14.07.2009	29	5.00^{h}	37.78	-32.78	23.6	27.9	88.0	95.0	27.0	3
21.07.2009	30	6.00^{gh}	67.88	-61.88	24.6	33.1	54.0	87.0	0.0	2
28.07.2009	31	3.67^{h}	1.93	1.74	23.0	26.7	84.0	93.0	27.0	5
Correlation coefficient (r)					-0.331ns	0.332ns	-0.190ns	-0.118ns	-0.288ns	-0.197ns

SMW : Standard meteorological week, ns: non-significant

* Means in column followed by a common letter are not significantly different at 5 per cent level (DMRT)

Table 2. Multiple regressions of fruit flies with weather parameters

Multiple regression	Temperature (^{0}C)		Relative humidity (%)		Rainfall (mm)	Rainy days
	Min. (X_1)	Max. (X_2)	Min. (X_3)	Max. (X_4)	(X_5)	(X_6)
Coefficient	-18.726	19.277	0.531	-0.487	-0.384	-0.514
Standard Error	8.422	8.227	0.995	1.185	0.312	8.851
T-value	-2.22*	2.34*	0.53ns	-0.41ns	-1.23ns	-0.06ns
F value	2.25 ns					
R^2	0.51ns					
Regression equation	Y= -85.61 – 19.00 (X_1) + 19.00 (X_2) + 1.00 (X_3) - 0.50 (X_4) - 0.40 (X_5) - 0.51 (X_6)					

Y = Population of fruit flies, * Significant at 5% level, ns : non significant

flies in present study had been reported by Gupta *et al*. (1990) and Gajalakshmi *et al*. (2011).

All the abiotic factors jointly had non-significant impact on population of fruit flies (Table 2). The coefficient of determination (R^2) was found to be 51 per cent. T-value of fruit flies population had a significant positively correlated with maximum temperature (2.34), while it was significant negatively correlated with minimum temperature (-2.22). However, the influence of relative humidity (minimum and maximum), rain fall and rainy days was found to be not significant. The present recovered species of fruit flies from tomato are new records for India on this host.

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Effect of phenol contents present in plant on the incidence of the pea leafminer *Chromatomyia horticola* (Goureau) (Diptera : Agromyzidae)

Yogeeta Thakur and K.C. Sharma

Department of Entomology, Dr Y.S. Parmar University of Horticulture and Forestry, Solan-173 230, Himachal Pradesh, India
Email : thakuryogeeta@gmail.com; yogeeta_3@yahoo.co.in

ABSTRACT

The pea leafminer *Chromatomyia horticola* (Goureau) is one of the highly destructive pests of pea in mid hill regions of Himachal Pradesh. The severe infestation of this pest reduces photosynthetic activities by consuming the chlorophyll content which ultimately affects the crop yield. The phenol contents have been reported to impart resistance to this pest and an inverse relation was observed between incidence of the pea leafminer and phenol contents. Three commercial varieties of pea viz. 'Arkel', 'Lincoln' and 'Azad P1' were considered for the availability of phenol contents in their foliage. It was maximum (341.33 mg g^{-1}) in Azad PI which was least infested by pea leafminer (3.14 mines $plant^{-1}$) while the minimum of 276 ppm g^{-1} was found in Lincoln variety on which the leafminer population was maximum (9.4 mines $plant^{-1}$).

Key Words: Phenol, pea, variety, *Chromatomyia horticola*

Pea (*Pisum sativum*) is one of the important cash crops grown in all the agro climatic zones of Himachal Pradesh. It is heavily attacked by the pea leafminer, *Chromatomyia horticola* (Goureau). A few chemicals have been found to be used for the control of this pest, but the appropriate knowledge of their application rate and method, as well as other management strategies are far away from poor and illiterate farming community. Therefore, frequent and non-judicial application of chemicals, poses health hazards due to their residual effect particularly in case of vegetable pea. Breeding of resistant varieties to pea leafminer is a safe and economic measure to overcome this problem.

In breeding programmes, it is imperative to season breeding material by creating artificial epiphytotics. It would, therefore, be worthwhile to assay the resistance to pea leafminer in terms of stable biochemical markers as indicator of pest infestation which are less influenced by the environment. The biological constituents present in quantities and proportions to each other in host plants have been reported to exert profound influence on the growth, development, survival and reproduction of insects in various ways (Painter, 1958). Among the biochemical markers phenolic compounds are one of the most important indicators implicated in resistance reaction (Bajaj *et al*. 1972). Information on the effect of phenolic constituents on the incidence of pea leafminer is limited. In the present investigations an effort has therefore been made to establish a possible correlation between phenolic compound of different pea varieties and pea leafminer population.

MATERIALS AND METHODS

The field trials were conducted at the farm area of the department of Entomology, Dr YS Parmar, University of Horticulture and Forestry, Solan in the form of randomized block design. Three varieties viz. 'Arkel', 'Lincoln' and 'Azad-PI' were sown separately in a bed size of 4 x 3 meters with a sowing distance of 60 x 8 cm (row to row and plant to plant). Larval population of the leaf miner was recorded at weekly interval starting from the last week of February till the crop harvest by counting the number of mines made by the larva. To determine phenol contents in the green leaves of pea, the leaf samples were collected at the time of peak pest activity i.e. during March/April. The samples, so collected were dried at 65^0C in the hot air oven for over night (till the samples got dried) and were ground separately in pestle-mortar to pass through 100 mesh size. Each sample was divided into three parts and each part was considered as replication. Total phenols in each variety were estimated as per the method of Bray and Thorpe (1954).

RESULTS AND DISCUSSION

The pea leafminers' infestation start appearing in the first week of March and its population increased with the passage of time and achieved its peak in third week of March, thereafter, pest population start decreasing (Table 1). The population of larval mines in different time intervals pea

Table 1. Effect of phenol contents on the incidence of the pea leaf miner on pea varieties

Variety	Mean larval mines plant^{-1} in the indicated week					Mean	Phenol content (ppm)
	I Week of March	II Week of March	III Week of March	IV Week of March	I Week of April		
Arkel	1.95 (1.56)	5.75	15.20	11.10	2.60	7.32 [b]	281.33 [a]
	0.05	(2.35)	(3.95)	(3.44)	(1.84)	(2.63)	
Lincoln	(1.21)	16.80	17.85	9.10	2.70	9.40 [a]	276.00 [a]
	0.85	(4.06)	(4.31)	(3.13)	(1.88)	(2.92)	
Azad-PI	(1.32)	5.20	6.85	1.90	0.90	3.14 [c]	341.33 [b]
		(2.43)	(2.74)	(1.62)	(1.34)	(1.89)	
Mean	1.12	9.25	13.30	7.37	2.07	-	
	(1.36)	(2.95)	(3.67)	(2.73)	(1.68)		

CD p = 0.05, varieties = 0.18, phenol content = 8.81

varieties in different weeks are presented in Table 1, which reveal that 'Azad P1' harbored minimum population of the pea leafminer (3.14 larval mines plant^{-1}) and was significantly different from the population observed in 'Arkel' variety (7.32 larval mines plant^{-1}). Maximum population (9.40 larval mines plant^{-1}) was found in Lincoln variety and the population on this variety was significantly different from the population observed in other two varieties.

The quantitative analysis of phenol content in these varieties revealed that there was an inverse relation between incidence of the pea leafminer population and phenol content. The phenol content was maximum in 'Azad-PI' (341.33 ppm), which was least infested by the pea leafminer and it was found minimum (276 ppm) in 'Lincoln', which was more prone to the attack of pea leafmines, therefore the pest population was recorded maximum. In case of 'Arkel', the phenol content was 281.33 ppm and was observed to be statistically at par to the phenol content estimated in 'Lincoln' (Table 1).

Similar studies were carried out by Sahoo and Patnaik (2003) in pigeon pea pod borer who observed lower incidence of the pod borer on varieties with higher phenol content. Mohan *et al*. (1987) determined that phenolics in a fairly large concentration ward off the insect pests because of their direct toxicity. Ananthakrishnana *et al*. (1990) were of the opinion that phenolic substances like resorcinol, gallic acid and phloroglucinol affected the nutritional indices, survival and growth of *Heliothis armigera*. Sithanatham *et al*. (1981) reported negative relation between phenol contents in pigeon pea pods and incidence of *Menduca obtuse*. Chhabra *et al*. (1993) also observed that high concentration of total phenol reduced the incidence of insect attack.

The present studies thus indicated that high concentration of phenol content in pea can afford resistance to the pea leafminer which can form the bases for selection of pea varieties.

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Screening of rice (*Oryza sativa* L.) germplasms against *Xanthomonas oryzae* pv. *oryzae*

A.K. Singh, B.K. Sarma, P.K. Singh and R. Nandan

Institute of Agricultural Sciences, BHU Varanasi – 221 005, India.
E-mail: anilbhu987@gmail.com

ABSTRACT

Thirty eight rice germplasm accessions were grown in Agricultural Research Farm of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during Kharif 2010 to screen their performance against *Xanthomonas oryzae* pv. *oryzae* the causal agent of bacterial leaf blight (BLB). Out of the 38 germplasm accessions, 27 accessions (HUR 36, HUR 38B, HUR 3022, HUR 4-3, HUR 105, HUBR 40, HUBR 2-1, BPT 5204, IDR 763, MTU 7029, GR 32, Swarna Sub1, Gobindbhog, Gopalbhog, Adamchini, Shivani, HUR 38, Pant 12, Jaya 3, Kalanamak, Sonachoor, Motigold, Sonam, Super Aman, Heera, Heena and Moti 360) showed moderate resistance and 11 accessions (IR 64, IR64 Sub1, Sarjoo 52, Badshahbhog, Pant 10, Pant 16, Kanchan, Rupali, Krishna, Vijay and Shakti) showed moderate susceptibility to BLB. Among these accessions, HUR 38B and Shakti showed lowest (19.95%) and highest (34.66%) disease severity, respectively. Moderately resistant germplasm accessions were recommended for general cultivation and further use in breeding programmes.

Key words: Bacterial leaf blight, rice, moderately resistant, moderately susceptible

Rice (*Oryza sativa* L.) is widely grown in tropical and subtropical regions (Ezuka and Kaku, 2000). Approximately 90 per cent of the world's rice is grown in the Asian continent and constitutes a staple food for 2.7 billion people Worldwide (Salim *et al*. 2003). Rice is placed on second position in cereal cultivation around the globe and occupies an important position in the economy of India as an export item as well as staple food.

Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922) Swings is one of the most destructive diseases throughout the world (Mew, 1987). BLB occurs at all the growth stages of rice and is manifested by either leaf blight or "*kresek*" symptoms. Disease is characterised by linear yellow to straw coloured stripes with wavy margins, generally on both edges of leaf, rarely on one edge. Some wild rice (*Oryza rufipogon, O. berthii* and *O. glaberimma*) pose resistant gene against BLB which are being deployed in *O. sativa*. Resistance to BLB is known to be widely different with rice cultivars. This is due to the fact that the presence of different pathogenic races subsequently breaks the resistance of rice cultivars. So evaluating rice cultivars for BLB resistance is a routine practice to overcome yield losses at different agro-climatic zones.

MATERIALS AND METHODS

Thirty eight rice germplasm accessions *viz.* HUR 36, HUR 38B, HUR 3022, HUR 4-3, HUR 105, HUBR 40, HUBR 2-1, IR 64, IR64 Sub1, BPT 5204, IDR 763, MTU 7029, GR 32, Sarjoo 52, Swarna Sub1, Badshahbhog, Gobindbhog, Gopalbhog, Adamchini, Shivani, HUR 38, Pant 10, Pant 12, Pant 16, Jaya 3, Kalanamak, Sonachoor, Motigold, Sonam, Super Aman, Heera, Kanchan, Rupali, Krishna, Vijay, Heena, Moti 360 and Shakti were grown to screen their performance against the bacterial leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae*. The experiment was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during the *kharif* season 2010. For the growth of rice plants in the field, the seedlings of each germplasm were transplanted in small separate plots of size (1.5 m x 0.6 m).

The culture of *Xanthomonas oryzae* pv. *oryzae* was obtained from Division of Plant Pathology, IARI, New Delhi and sub cultured on peptone sucrose agar (PSA) medium. For pathogenicity test, pinprick method (with bacterial suspension containing 10^8 cfu/ml) was used for inoculation in the rice plants with *Xanthomonas oryzae* pv. *oryzae.* The test was conducted on fully developed leaves at the age of 6-8 weeks old rice plants after transplanting. Following inoculation, the plants were observed after every 24 hours time interval to note the appearance of disease symptoms. Disease incidence was recorded at 14 and 21 days after inoculation using a disease index of 0-9. Per cent disease incidence was calculated with the help of following formula

(Gnanamanickam *et al*. 1999):

Per cent Disease incidence = (Lesion length/ Total leaf length) X 100

The score chart given in table 1 was used to evaluate the response of host plant (Anonymous, 1996).

Table 1. Scale for bacterial blight (Anonymous, 1996)

Infection (%)	Score	Host response
0	0	Highly resistant (HR)
> 1-10	1	Resistant (R)
> 10-30	3	Moderately resistant (MR)
> 30-50	5	Moderately susceptible (MS)
> 50-75	7	Susceptible (S)
> 75-100	9	Highly susceptible (HS)

The leaves of rice plants inoculated with *Xanthomonas oryzae* pv. *oryzae*, showing the symptoms of bacterial blight i.e. yellow lesions were used for isolation of the bacteria in order to confirm the bacterial blight.

RESULTS AND DISCUSSION

Thirty eight germplasm accessions of rice were evaluated to a strain of *Xanthomonas oryzae* pv. *oryzae*. The experiment was conducted in the field and found that 27 germplasm accessions (HUR 36, HUR 38B, HUR 3022, HUR 4-3, HUR 105, HUBR 40, HUBR 2-1, BPT 5204, IDR 763, MTU 7029, GR 32, Swarna Sub1, Gobindbhog, Gopalbhog, Adamchini, Shivani, HUR 38, Pant 12, Jaya 3, Kalanamak, Sonachoor, Motigold, Sonam, Super Aman, Heera, Heena and Moti 360) showed moderate resistance and 11 germsplasm accessions (IR 64, IR64 Sub1, Sarjoo 52, Badshabhog, Pant 10, Pant 16, Kanchan, Rupali, Krishna, Vijay and Shakti) showed moderate susceptibility to the pathogen (Table 2, Fig. 1). These finding were in agreement with earlier reporter Ali *et al*. (2009) and Tyagi *et al*. (2010).

Initial symptoms in the present study were observed to be linear, yellow to straw coloured stripes which appeared after 7 days in moderately susceptible accessions. This was supported by the work of Kauffman *et al*. (1973) as they reported that the disease symptoms first appear 4-5 days of inoculation in the form of leaf curling near the cut off portion when they evaluated the resistance of IR 8 and IR 20 against *Xanthomonas oryzae* pv. *oryzae* strain PXO25. The linear, yellow to straw coloured stripes symptoms were first appeared in Shakti and Vijay after 7 days from the date of inoculation, while Badshahbhog, Pant 16, Kanchan, Rupali and Krishna showed these symptoms 8 days after inoculation and then HUR 38B showed the symptoms after 10 days of inoculation. Per cent disease incidence calculated for evaluated rice plants on the basis of lesion length was used to evaluate the pathogenicity of the bacterial strains to rice germplasm accessions. Gnanamanickam *et al*. (1999) also used percentage disease in a series of experiments to evaluate the performance of *Pseudomonas putida* strain V14i as a bio-control agent to suppress bacterial blight disease in IR 24. Mew and Khush (1981) used standard evaluation system (0-9 scale) to evaluate the resistance of different rice varieties

Table 2. Comparison of per cent disease incidence on 1-38 rice germplasm accessions against *Xanthomonas oryzae* pv. *oryzae*

Germplasm accessions	(%) Disease Severity		Standard deviation	Disease score	Host response
	14 DAI	21 DAI			
HUR 38B	8.69	19.85	1.84	3	MR
HUR 3022	10.95	23.21	2.66	3	MR
HUR 4-3	9.10	20.39	2.09	3	MR
HUR 105	13.54	26.40	2.35	3	MR
HUBR 40	12.05	23.98	1.76	3	MR
HUBR 2-1	16.32	27.61	3.83	3	MR
IR 64	20.15	30.05	2.44	5	MS
IR64 Sub1	21.00	30.79	1.11	5	MS
BPT 5204	17.25	27.93	3.70	3	MR
IDR 763	13.40	25.05	4.79	3	MR
MTU 7029	13.93	25.76	2.77	3	MR
GR 32	12.33	23.72	2.10	3	MR
Sarjoo 52	21.18	30.70	1.60	5	MS
Swarna Sub1	11.87	23.89	3.97	3	MR
Badshahbhog	24.30	33.25	2.40	5	MS
Gobindbhog	9.95	21.09	1.52	3	MR
Gopalbhog	12.90	25.57	2.80	3	MR
Adamchini	10.25	21.13	1.59	3	MR
Shivani	18.20	27.66	1.43	3	MR
HUR 38	17.12	26.48	2.59	3	MR
Pant 10	20.48	30.10	3.54	5	MS
Pant 12	11.75	22.09	3.71	3	MR
Pant 16	25.33	34.47	2.70	5	MS
Jaya 3	9.70	20.25	2.54	3	MR
Kalanamak	17.05	27.93	2.13	3	MR
Sonachoor	17.35	27.37	4.93	3	MR
Motigold	14.70	26.72	2.10	3	MR
Sonam	13.65	25.45	1.79	3	MR
Super Aman	11.33	23.74	0.89	3	MR
Heera	12.98	25.47	2.07	3	MR
Kanchan	24.45	33.22	2.09	5	MS
Rupali	24.98	33.89	1.42	5	MS
Krishna	24.76	33.58	2.10	5	MS
Vijay	25.96	34.61	4.41	5	MS
Heena	13.90	26.68	2.71	3	MR
Moti 360	13.52	26.61	4.44	3	MR
Shakti	25.66	34.66	2.85	5	MS

DAI= Days after inoculation

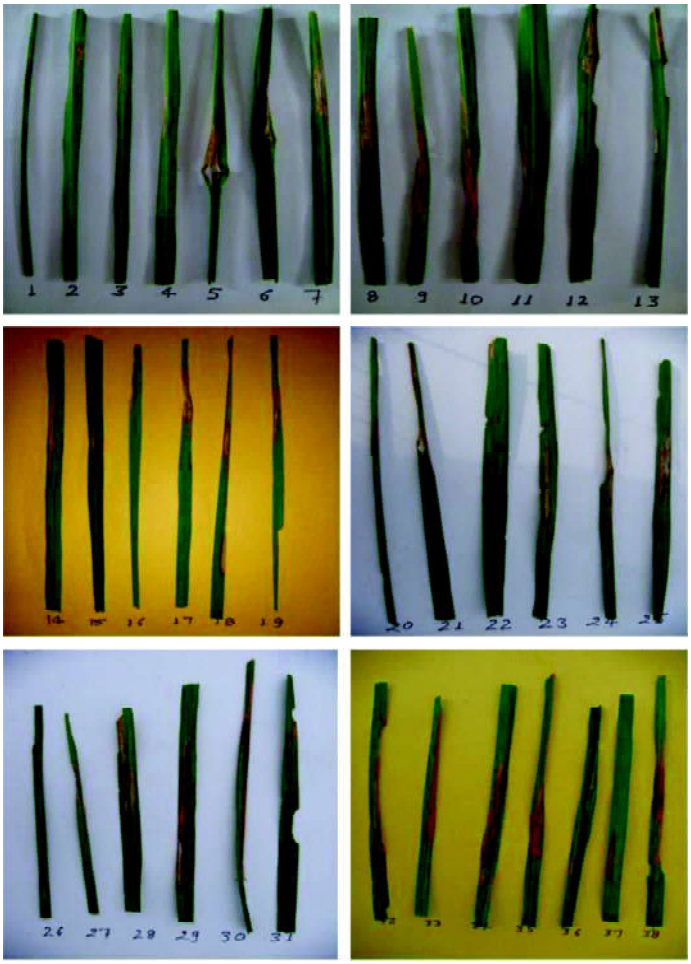

Fig 1. Reaction of bacterial blight on 1-38 germplasm accessions after 3 weeks of inoculation

to PXO61. After 14 days of inoculation Vijay showed highest (25.96%) disease severity followed by Shakti (25.66%), while HUR 38B showed lowest (8.69%) disease severity. Disease severity increased rapidly in Badshahbhog, Pant 16, Kanchan, Rupali, Krishna, Vijay and Shakti between 7-14 days from inoculation, then slowed down in 14-21 days and then became constant, while rest of germplasm accessions showed continuous increase in disease severity up to 21 days after inoculation and then became constant. Similar findings were also reported by Khan *et al.* (2009).

From our results it was obvious that 27 rice germplasm accessions were moderately resistant and 11 germplasms were moderately susceptible to the bacterial leaf blight (BLB) pathogen but none were found highly resistant to BLB. Among 38 germplasm accessions HUR 38B showed lowest (19.95%) disease severity and Shakti showed highest (34.66%) disease severity indicating future use of HUR 38B against bacterial leaf blight (BLB).

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Integrated organic management of powdery mildew disease in vegetable pea caused by *Erisyphe polygoni*

Devendra Singh Negi, J. Kumar[1], Rajan Kumar Gupta[2] and Binita Shah

Research and Development Cell, State Research and Training Centre, Majkhali (Ranikhet) Almora (Uttrakhand Organic Commodity Board), Uttarakhand – 263 652, India
[1]G.B. Pant University of Agriculture and Technology, Pantnagar
[2]Govt. Post Graduate Autonomous College, Rishikesh

ABSTRACT

The experiment was conducted to find out the efficacy of foliar spray of *Trichoderma harzianum, Pseudomonas fluorescens, Trichoderma virens* (Individual and combination), Biodynamic preparation (BD) – 501, CPP (Cow pat pit), Cow urine and Mycostat against powdery mildew disease (*Erisyphe polygoni*) in vegetable pea (*Pisum sativum* L.*)* at State Research and Training Centre for Organic Farming, Majkhali, Almora during 2007-08. Considering different parameters, Mycostat, Cow pat pit and mixture of *T. harzianum* + *P. fluorescens* showed least per cent disease incidence than other treatment and untreated control. Three foliar sprays of Mycostat indicate significant effect for disease control and disease reduction over control (PDI 6.85 in 105 days and 80.0% disease reduction over control) and produce high yield of green pods (126.6q ha^{-1}). Three foliar sprays of CPP and combination of *T. harzianum* + *P. fluorescens* also reduced disease incidence and increased yield.

Key words: Pea, biodynamic preparation, cow pat pit, Mycostat, *Trichoderma harzianum, Pseudomonas fluorescens*

Vegetable pea (*Pisum sativum* L.) covers an area of 79789 ha with a production and productivity of 488252 t and 6.12 t ha^{-1} respectively in India (Singh and Vani, 1996). In Uttrakhand hills it is cultivated in an area of 1778 ha. with a production of 1572 MT and productivity 0.77 MT ha^{-1} (Jagran, 1998) as off season vegetable. Powdery mildew appears in epidemic form almost every year when the plant are in the pod stage towards the end of September in early sown pea and February in late sown pea. Heavy reduction in pod formation in pea was occurred due to severe infection of powdery mildew.

In this experiment bioagent and biodynamic preparation were used as compost additives and field sprays. All biodynamic (BD 500 to BD 506) preparations showed large number of microbial populations (total 1,443 colonies on the plates used for counting microorganisms) from the sample.

MATERIALS AND METHODS

The experiment was conducted under natural infection condition. The study of the efficacy of foliar spray of twelve different treatments, such as bio-dynamic preparation-501, cow pat pit (CPP), bioagent combination (individual and mixture), Mycostat and cow urine were tested against powdery mildew disease of vegetable pea at State Research and Training Centre for organic farming, Majkhali, Almora during Rabi session 2007-08. For each treatment seeds of vegetable pea variety Azad were sown in randomized block design with three replications. The unit plot size was 3X2 m and seeds were sown in October last week. Recommended organic compost was applied to raise a good crop. The data were recorded from randomly selected plants for per cent disease incidence. Standard scoring scale (Anon., 1994) was used for scoring the disease i.e. 0= leaf and fruit free from infection; 1 = 1 to 5 % leaves infected; 2 = 6-20 % leaves and fruit infected 3 = 21-40 % leaves and fruit infected; 4 = 41-70 % leaves and fruit infected and 5 = up to 71 % leaves infected.

Three bioagents, *T. harzianum* (CFU 10^6) , *P. fluorescens* (10^7) obtained from the bio-control laboratory of GBPUA&T. Hill campus Ranichauri, Tehri Garhwal, Uttrakhand and *T. virens* ($2X10^9$) obtained from the NAFED Biofertilizer Rudrapur, U.S.Nagar were used as individual and combination through the foliar spray @ 10 g l^{-1}. Bio-dynamic preparation-501 obtained from Supa Biotech Pvt. Ltd. Nainital was used for foliar spray at morning time in two leaf stage in separate plots. Mycostat (Neem base formulation) were also used for foliar spray @ 5 g l^{-1}. All foliar sprays were applied on 75, 85 and 95 DAS and observations were recorded after five days of each spray.

Cow Pat Pit is known as CPP and is a specialized type of compost. It refers to 40 kg cow manure mixed with 250 g crushed egg shell and 250 g basalt dust, with the preps 502-

507 then put into pit. The dung is fermented, together for a period of 2 months. Compost water extract is made from compost suspended in a barrel of water.

Water extract of the compost material were prepared by using the procedure adopted by Steve Diver (2002). Ripe compost were soaked with water in 1:1 ratio (W/V) in a container; they were aerated periodically by tuning them with a wooden stick. The water extract were cleared by muslin cloth and fermented juice was used for seed treatment and foliar spray @ 20 %.

$$\text{Disease control percentage} = \frac{(\%\ \text{PDI in control}) - (\%\ \text{PDI in treatment})}{(\%\ \text{PDI in control})} \times 100$$

RESULT AND DISCUSSION

All the bioagent, CPP, Mycostat and BD-501 reduced the per cent disease incidence of powdery mildew in vegetable pea. Foliar sprays of Mycostat (80, 90 & 100 DAS) showed least per cent disease incidence (1.54% in 80, 11.52% in 90 and 6.85% in 100 DAS) followed by two foliar sprays of *T. harzianum* and *P. fluorescens* (1.64% in 80, 13.58% in 90 and 15.32% in 100 DAS). Individual applications of antagonist were comparatively less effective as compare to combined treatment (Table 1).

Foliar spray of CPP also showed significant result (PDI 1.95 in 90, 15.43 in 80 and 15.63 in 100 DAS). Per cent disease reduction over control was also maximum in above treatments, where as the highest per cent disease incidence of powdery mildew was observed in untreated control (34.26%).

Disease reduction over control was observed in all treatments. Disease reduction 80.0% over control was found in foliar sprays of Mycostat after final observation, while 55.28 and 54.37% in mixture of *T. harzianum* + *P. fluorescens* and CPP respectively.

On leaves, disease was scored on 0-5 scale after 5 days of final spray. 64.06% plants showed 0 rating, 21.87% showed 1 and 14.06% plants showed 2 rating and no disease rating on 3, 4 & 5 scale by the foliar spray of Mycostat followed by CPP 60.52% seedling showed 0 rating, 23.68% showed 1, 7.29% showed 2, 2.63% showed 3 and 5.92% showed 4 rating and no disease scale on 5 scale, while 26.48% seedling showed 5 rating and 27.38% seedling showed 4 rating in untreated control (Table 2).

Data recorded on green pod indicate that all bioagents, CPP and Mycostat were significantly superior over control. Highest green pod yield 126.6q ha^{-1} was recorded in plot sprayed with three foliar spray of Mycostat, followed by CPP (124.5 q ha^{-1}) and foliar spray of *T. harzianum* + *P. fluorescens* (124.1 q ha^{-1}). The same treatments indicate the maximum yield reduction over control 73.18, 70.31 and 69.76 % respectively.

Table 1. Eco-friendly management of Powdery mildew disease in vegetable pea caused by *Erisyphe polygoni*

Treatment	Dose	PDI 80 DAS	DRoC	PDI 90 DAS	DRoC	PDI 100 DAS	DRoC
3 FS by Th	10g l^{-1}	3.08	76.78	17.38	30.75	18.82	45.06
3 FS by Pf	10 g l^{-1}	2.16	83.72	16.25	35.25	17.38	49.27
3 FS by Tv.	10 g l^{-1}	9.15	31.0	23.14	7.8	29.83	12.93
3 FS by Th + Pf.	(5+5) 10 g l^{-1}	1.64	87.64	13.58	45.9	15.32	55.28
3 FS by Th + Tv.	(5 +5) 10 g l^{-1}	6.17	53.5	20.20	19.52	25.92	24.34
3 FS by Tv + Pf	(5 +5) 10 g l^{-1}	4.01	69.78	19.85	20.91	19.85	42.06
3 FS by Th + Tv + Pf	3 g each l^{-1}.	3.49	73.7	19.79	21.5	24.27	29.15
3 FS by CPP (cow pat pit)	20 %	1.95	85.3	15.43	38.52	15.63	54.37
3 FS by BD-501	1g 13l^{-1}*	4.61	65.25	21.5	14.34	24.07	29.74
3 FS by cow urine	5 %	12.13	8.59	24.17	3.7	31.78	7.23
3 FS of Mycostat	5 g l^{-1}	1.54	88.33	11.52	54.1	6.85	80.0
Untreated control	--	13.2	-	25.10	-	34.26	-
SEm	-	0.55	-	0.92	-	2.8	-
CD 5%	-	1.64	-	2.71	-	8.2	-

*As per recommendation of Biodynamic association of India.

Table 2. Disease rating (%) of powdery mildew in pea (0-5 scale)

Treatment	Infected seedling rating (%)					
	0	1	2	3	4	5
3 FS by Th	53.0	12.56	17.48	11.47	1.64	3.82
3 FS by Pf	60.95	7.10	15.97	5.32	7.1	3.65
3 FS by Tv.	17.93	12.75	24.82	11.37	9.31	23.79
3 FS by Th + Pf.	51.67	9.39	13.42	22.14	2.35	-
3 FS by Th + Tv.	33.33	21.03	16.26	7.53	7.14	14.68
3 FS by Tv + Pf	59.06	9.84	17.09	4.66	3.63	5.7
3 FS by Th + Tv + Pf	51.69	13.55	14.4	9.74	4.66	5.93
3 FS by CPP (cow pat pit)	60.52	23.68	7.29	2.63	5.92	-
3 FS by BD-501	49.57	17.94	12.82	2.56	5.98	11.11
3 FS by Cow urine	10.03	14.23	20.38	13.91	7.76	33.66
3 FS by of Mycostat	64.06	21.87	14.06	-	-	-
Untreated Control	4.16	9.82	21.13	11.01	27.38	26.48

FS- Foliar spray, Th-*Trichoderma harzianum Pf-Psedomonas fluorescens* Tv-*Trichoderma viride* PDI- Per cent disease incidence, DAS-Day after sowing, DRoC-Disease reduction over control, YRoC- Yield reduction over control

The result of the present study clearly demonstrate that the foliar spray of Mycostat for the control of *Erysiphi polygoni* of vegetable pea ranks second after the mixture of fungal and bacterial bioagents and CPP reducing the disease incidence and disease rating and increase in yield (Fig. 1).

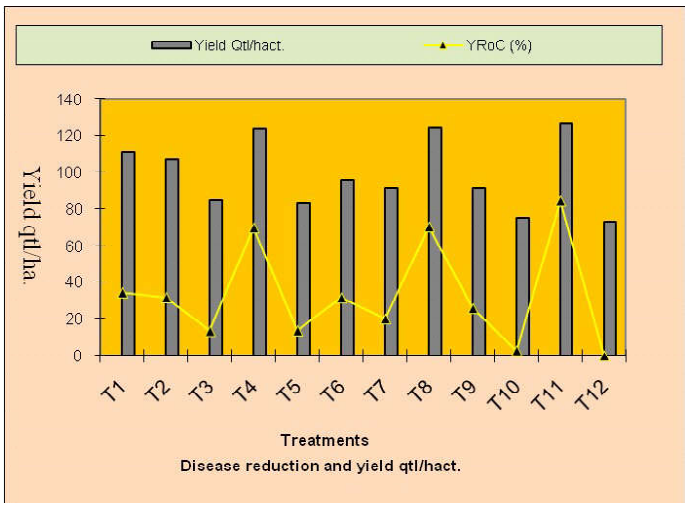

Fig 1. Yield in different treatments

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Evaluation of advance breeding lines for multiple disease resistance in rice

Jyoti Jain, J. S. Lore, M. S. Hunjan, R. Kaur and G.S. Mangat

Rice Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana – 141 004, Punjab, India

ABSTRACT

A total of 193 entries from National Screening Nursery-1 (NSN-1), 622 entries from National Screening Nursery-2 (NSN-2) and 43 entries from Donor Screening Nursery (DSN) were received from Directorate of Rice Research, Hyderabad during *kharif* 2008-09. These entries were evaluated against the prevalent pathotypes viz. PbXo-1, PbXo-5, PbXo-7 and Tar-950 of the bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*), sheath blight (*Rhizoctonia solani*) and brown spot (*Drechslera oryzae*) respectively under artificial inoculation conditions for multiple disease resistance. The promising entries selected during *kharif* 2008-09 were grown during *kharif* 2009-10 for further evaluations. Eleven entries showed resistant reaction to BB pathotype PbXo 1, Twelve entries to pathotype PbXo-5, Nine entries to both pathotypes PbXo-7 and Tar 950. Two DSN entries viz., VOHP 3102 and VL 30424 showed resistant reaction to all the four pathotypes whereas none was resistant to sheath blight and brown spot. Among NSN-2 entries, 29 entries were found resistant to all the four pathotypes of *Xanthomonas oryzae* pv. *oryzae*. Two genotypes, viz., IET 20796 (RP 4818-29-1-1-31-1-1-B) and 20861 (CRR 451-2921-1-1-1) were found moderately resistant to brown spot disease. These genotypes can either be released as new varieties or further utilized as donors in multiple disease resistance breeding programmes.

Key words: Multiple disease resistance, bacterial blight, sheath blight, brown spot, pathotypes.

Rice (*Oryza sativa* L.) is one of the most important crop and primary source of food for more than half of the world population. More than 90 per cent of world's rice is grown and consumed in the Asian region, where 60 per cent of world's people live. The current world population of 6.1 billion is expected to reach 8.0 billion by 2030 and rice production must increase by 50 per cent in order to meet the growing demand. If this goal is to be met, it is necessary to use rice varieties with higher yield potential, durable resistance to diseases and insects and tolerance to abiotic stresses (Khush and Brar, 2003). A critical aspect of enhancing production at any range of time-scale is to minimize losses to diseases (Savary *et al*,. 2000).

In India the rice crop is affected by atleast 22 diseases, out of which bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae*, sheath blight caused by *Rhizoctonia solani* and brown spot caused by *Drechslera oryzae* are of major concern. Bacterial blight (BB) can cause losses up to 50 per cent depending on stage, weather, location and variety (Mew *et al*., 1993). On the other hand it has been reported that sheath blight and brown sopt account for highest yield loss (6% for sheath blight and 5% for brown spot) across all the production situations in south and Southeast Asia (Savary *et al*., 2000a). Although, rice resistance breeding has gained large achievements in managing bacterial blight disease, but due to high variability in virulence, breeding of resistant cultivars always confronts difficulties in terms of durability of resistance. Similarly, on the other hand a large scale screening of the national rice elite material and germplasm has been done in the past against sheath blight and so far none of the material has been found to possess clear cut resistance. So keeping in view, advance breeding lines were evaluated under artificial inoculation conditions, so that multitude resistance resources can be used to manage diseases or as donors in disease resistance breeding programme.

MATERIALS AND METHODS

The present studies were conducted at PAU, Ludhiana during years 2008-09 and 2009-10 for the screening of national elite lines for multiple disease resistance in rice. A total of 193 entries from National Screening Nursery-1 (NSN-1) comprising advance varietal trials, 622 entries from National Screening Nursery-2 (NSN-2) comprising of initial varietal trials and 43 entries from Donor Screening Nursery (DSN) were received from Directorate of Rice Research, Hyderabad and transplanted in paired rows with 20 x 20 cm spacing. Agronomic practices and fertilizer application was as per PAU recommendations. These entries were evaluated against the prevalent pathotypes viz. PbXo-1, PbXo-5, PbXo-7 (Lore *et al*., 2011) and Tar-950 of the bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*), sheath blight

(*Rhizoctonia solani*) and brown spot (*Drechslera oryzae*) pathogens respectively under artificial inoculation conditions during year 2008-09 and only selected entries were grown during year 2009-10 for further evaluations (Table 1).

Bacterial blight: For bacterial blight inoculation, pathotypes (PbXo-1, PbXo-5, PbXo-7 and Tar-950) of *X. oryzae* pv. *oryzae* were isolated on Waki Moto medium. Seventy two hours old single colony virulent culture of *Xanthomonas oryzae* pv. *oryzae* was used for artificial inoculations. All the test genotypes

Table 1. Reaction of advanced breeding lines against bacterial blight, sheath blight and brown spot diseases (2008-2010).

IET No.	Designation	BLB				SHB	BS
		PbXo-1	PbXo-5	PbXo-7	Tar 950		
NSN I Entries							
NSN I	NDR 9830145						
19790	(IR 68821-355-NDR-1-1-1-1)	3	3	3	3	9	9
19795	R 1243-1224-578-1	3	3	3	3	9	9
19799	RP 4092-412-120-15	3	3	7	7	9	9
20082	OR 2315-6	5	3	3	3	7	9
20372	OR 1903-6-67	3	3	3	3	9	9
20532	UPR 2760-10-1-1	3	3	3	3	9	9
20535	UPR 2754-3-1-1	3	3	3	3	9	9
20540	R 1448-73-44-1-1	5	3	5	7	7	7
20545	GGV-05-02	3	3	5	5	7	7
20556	OR 1924-4	3	3	3	5	7	9
19972	SJR (SPR 85089-5-1-2-4)	5	5	5	5	9	7
19752	DRRII-50 (HYBRID)	7	7	7	7	5	7
20601	OR 1530-8/IR 68181-B-49	3	3	3	3	9	5
20603	CR 2499-68 (IR 72158-16-3-3)	3	5	5	3	9	9
20251	RGI 20545	3	3	3	3	7	7
DSN Entries							
DSN 11	VOHP 3102	3	3	3	3	9	9
DSN 15	VL 30424	3	3	3	3	9	9
DSN 24	VL 30917	3	5	5	7	9	9
NSN2 Entries							
NSN2							
20764	CN 1448-310-9-6	3	3	3	3	7	7
20791	R 1529-1183-3-1003-1	3	3	3	3	9	7
20792	R 1530-1196-2-1064-1	3	3	3	3	9	5
20796	RP 4818-29-1-1-31-1-1-B	9	9	9	9	7	3
19482	RP 4353-MSC-28-13-1-1-3	3	3	3	5	7	9
19483	RP 4631-146-9-1-1-1-3	3	3	3	3	7	9
19486	RP 4631-146-20-4-1-1-1	3	3	3	7	7	7
20861	CRR 451-2921-1-1-1	7	7	7	7	9	5
20827	MAUB 171	7	7	7	7	9	7
20523	NDR 2701	3	3	3	5	9	9
20883	RAU 670-5-20	5	3	3	5	7	7
20892	HKR 05-81	3	3	3	3	7	7
20893	HKR 05-20	3	3	3	3	7	7
20894	HKR 05-22	3	5	5	3	7	7
20910	PAU 3419-1-7-1	3	3	3	5	7	7
20938	CR 2495-24	3	3	3	3	9	9
20983	HKR 05-28	5	3	3	3	9	9
20984	HKR 05-33	3	3	5	3	7	7
20985	HKR 05-46	3	3	5	5	9	9

IET No.	Designation	BLB				SHB	BS
		PbXo-1	PbXo-5	PbXo-7	Tar 950		
20987	WGI 283	3	3	3	3	7	7
20991	NDR 2097	3	3	3	3	9	9
20992	NDR 2098	3	3	3	3	9	9
20994	MGD-105	3	3	3	5	9	9
20995	MGD-106	3	3	3	5	9	9
20996	RP 4092-101-74-10-2	3	3	3	7	7	7
20997	RP 4092-107-69-14-1	3	3	3	5	9	9
21006	PAU 3040-3-4-4	3	3	3	5	9	9
21007	CN 1436-111-1-5	9	7	9	7	9	9
21030	MTU 1110	3	5	5	3	7	7
20667	CRMAS 2231-36	3	3	3	3	9	9
20668	CRMAS 2231-37	3	3	3	3	9	7
20669	CRMAS 2231-48	3	3	1	3	9	9
20670	CRMAS 2232-66	3	3	1	3	9	9
20671	CRMAS 2232-71	3	3	1	3	9	9
20672	CRMAS 2232-85	3	3	3	3	9	9
21063	CRMAS 2621-7-2	3	3	3	3	9	9
21064	CRMAS 2621-97	3	3	5	3	9	9
21065	CRMAS 2621-12-9	3	3	3	5	9	9
21066	CRMAS -7-1	3	3	3	3	9	9
21067	CRMAS 2622-2-2	3	3	3	3	9	9
21068	CRMAS 2621-4-4	3	3	3	3	9	9
21069	CRMAS 2642-43-5	3	3	3	3	9	9
21070	CRMAS 2622-7-6	3	3	3	3	9	9
21071	CRMAS 2622-1-2	3	3	3	3	9	7
21149	OR 2131-1	3	3	3	7	7	9
21151	OR 2316-1	3	3	3	3	7	7
21155	OR 2316-1	3	3	5	5	9	7
21156	OR 2328-5	3	3	3	3	9	7
21200	PAU 201	3	3	3	3	9	7
21202	CR 2496-24-5	3	3	3	5	9	9
21204	CR 2597	3	3	3	5	9	9
21182	CR 2472-1-6-2-1	3	5	5	7	9	9
21188	CR 2485-7-3-45-1-1	3	3	3	3	9	7
20662	UAS ARB 8	7	5	7	7	9	9
21197	MAS ARB 109	9	7	7	7	9	7
21198	RPHR 295-5-6-5	3	7	5	7	9	7
21239	CR 2472-1-6-2-1	3	3	3	3	9	9
3602	IR 64 Sub 1	3	3	3	7	9	9
IR64		3	3	3	7	9	7
IR BB 60		3	3	3	3	9	7
TN1(Susceptible check)		9	9	9	9	7	7
IET 8585 (resistant check)		3	3	3	3	7	7

were inoculated at maximum tillering stage by clip-inoculation technique (Kauffman *et al.,* 1973) with bacterial suspension of approximately 10^{-9} cells ml^{-1}. Ten plants of each test entry were inoculated with all the aggressive pathotypes separately. TN1 and Ajay (IET 8585) were used as susceptible and resistance checks. Reaction of plants was recorded 14 days after inoculation according to Standard Evaluation System (0-9 scale) for rice (Annonymous. 1996).

Plants were characterized as resistant or susceptible based on scale 0-3 (resistant), 5 (Moderately resistant), 7-9 (Susceptible).

Sheath Blight: The pathogen was isolated from infected rice plants on PDA medium and was mass multiplied in 500 ml flasks having *Typha* bits. Ten days old growth of *R. solani* was inoculated on plants by placing 3-4 *Typha* bits hill^{-1} in the whorl of each test genotype. The disease data was recorded 15 days after inoculation based on 0-9 scale of Standard Evaluation System (SES) for rice (Annonymous. 1996).

Brown spot: The pathogen was isolated from infected rice cultivar PR116 on PDA medium, mass multiplied in 250 ml conical flasks containing 100 ml potato dextrose broth by inoculating with 8 days old small bits of actively growing culture of the pathogen and incubated at 28$\pm$ 2^{0}C. One week old culture was homogenized and the spore suspension was filtered through double-layered muslin cloth. Conidial suspension was adjusted to 1x10^6 conidia ml^{-1} with the help of a haemocytometer and sprayed on the plants with hand sprayer at the boot stage to have uniform disease. Disease was scored as per Standard Evaluation System for rice (Annonymous, 1996).

RESULTS AND DISCUSSION

Among 193 NSN-1 entries tested against bacterial blight, 6 entries showed resistant reaction all the four *Xoo* pathotypes whereas 11 entries showed resistant reaction to pathotype PbXo-1 , 12 entries to pathotype PbXo-5, 9 entries to pathotypes PbXo-7 and Tar 950. Genotype IET 20082 (OR 2315-6) was resistant to pathotypes PbXo-5, PbXo-7 and Tar 950 but was moderately resistant to pathotype PbXo-1. Similarly genotype IET 20540 (R 1448-73-44-1-1) showed moderately resistant reaction to pathotype I. On the other hand, one genotype IET 19799 (RP 4092-412-120-5) was resistant to pathotypes PbXo-1 and PbXo-5 but moderately susceptible to pathotypes PbXo-7 and Tar 950. Only one genotype namely IET 19752 (DRR 11-50, Hybrid) was found moderately susceptible to all the four *Xoo* pathotypes whereas it showed moderately resistant reaction to sheath blight.

Further, among these NSN-1 entries, none showed resistance to sheath blight. Two DSN entries namely VOHP 3102 and VL 30424 showed resistant reaction to all the four *Xoo* pathotypes whereas none was resistant/moderately resistant to sheath blight and brown spot respectively.

Among 622 NSN-2 entries, 29 showed resistant reaction all the four *Xoo* pathotypes. Three entries IET 20996 (RP 4092-107-69-14-1), IET 3602 (IR 64 Sub 1) and IR 64 were resistant to three pathotypes (PbXo-1, PbXo-5 and PbXo-7) but were susceptible to pathotype Tar 950. None of entries showed resistant reaction to sheath blight. However, 2 genotypes, viz., IET 20796 (RP 4818-29-1-1-31-1-1-B) and 20861 (CRR 451-2921-1-1-1) were found moderately resistant to brown spot disease.

Bacterial blight is a major disease of rice in Punjab and 7 pathotypes of the *Xanthomonas oryzae* pv. *oryzae* have been reported in Punjab (Lore *et al.* 2011). Pathotypes PbXo-7 have been reported as more dominant in Punjab state and virulent to known *Xa* genes namely *Xa* 1, *Xa* 3, *Xa* 4, *Xa* 25, *Xa* 7, *Xa* 8, *Xa* 10 and *Xa* 11 and mega varieties PR 114, PR 116 and PR 118 having genetic resistance to four pathotypes namely PbXo-1, PbXo-2, PbXo-3 and PbXo-4 (Lore *et al.* 2011). Another newly identified pathotype Tar 950 highly virulent on newly identified gene *Xa* 38 and cultivar PR 120 grown in Punjab. In the present study a total of 58 entries showed resistance to PbXo-7 and 46 entries showed resistance to newly identified pathotype Tar 950 and a total of 36 entries showed resistance to all the four pathotypes of *Xanthomonas oryzae* pv. *oryzae*.

Sheath blight and brown spot are emerging diseases of rice in Punjab and till date, no complete resistance has been reported against these diseases (Hunjan *et al.* 2010). In the present study only one entry namely, IET 19752 (DRR 11-50, Hybrid) was found moderately resistant to sheath blight and two entries viz., IET 20796 (RP 4818-29-1-1-31-1-1-B) and 20861 (CRR 451-2921-1-1-1) were found moderately resistant to brown spot disease. Similarly, Hunjan *et al.* (2010) evaluated advanced stage breeding lines of rice comprising of 127 entries (non basmati) and 43 entries (basmati) for multiple disease resistance. Of these, 41 entries (non basmati) and 5 (Basmati) were found to possess genetic resistance to all the seven pathotypes of bacterial blight pathogen, while eight and three entries from non basmati and basmati rice respectively, exhibited tolerance to brown spot. However, only one entry from non basmati rice showed tolerance to sheath blight.

These sources of resistance from advanced breeding lines of rice can be exploited in breeding programmes for the development of resistant commercial cultivars after determining their genetics and if they are found to possess other desirable agronomic characters.

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Epidemiology and management of Alternaria blight of Ber

Jyoti Jain and K. S. Verma

Rice Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana - 141 004, Punjab, India

ABSTRACT

The intensity of Alternaria blight of Ber caused by *Alternaria alternata* (Fr.) Keissl. was found to vary from 27.25 to 59.75 per cent on different cultivars at Ludhiana, while at Patiala, Sangrur and Nawanshahar, the disease intensity was recorded to be 28.25, 23.20 and 20.75 per cent respectively. The disease was more severe on un-pruned trees as compare to pruned trees. The older trees were more affected by the disease as compared to younger trees. The development of the disease under field conditions showed positive correlation with average maximum and minimum temperature, maximum relative humidity and total rainfall, whereas it was negatively correlated with minimum relative humidity. The pathogen successfully survived on infected Ber leaves under both *in vivo* and *in vitro* conditions. Out of the seven test fungicides, Indofil M-45 (0.3%), Bordeaux mixture (1.0%) and Antracol (0.3%) proved most effective against Alternaria blight providing 78.79, 75.17 and 65.55 per cent disease control repectively.

Key word : Ber, disease intensity, disease mangement, epidemiology

Ber (*Zizyphus mauritiana* Lamk.) is an important fruit crop of north India and is extensively grown in arid and semi arid regions of Punjab and adjoining states. Commercial Ber cultivars are susceptible to a number of pathogenic fungi among which *Alternaria alternata* (Fr.) Keissl inciting blight is serious pathogen particularly on grown up trees. The disease was reported by Jeyarajan and Cheema (1972) from Punjab and subsequently by Gupta and Madaan (1977) from Haryana. The disease appear in the form of small irregular brown spots on the upper surface of the leaves and gray to brown spots on the lower surface which generally starts from the tip and margins of the leaves and spreads gradually downwards and inwards. Many spots coalesce to form large patches involving most of the leaf lamina, resulting into premature defoliation of diseased leaves (Gupta and Madaan, 1977). Detailed investigations have not been done on this disease in India. The present investigation was carried out to study the prevalence and severity of the disease, perpetuation of the pathogen, correlation of some meteorological parameters with disease under field conditions and management of disease through fungicides.

MATERIALS AND METHODS

Periodical surveys of Ber plantations at Ludhiana, Patiala, Sangrur and Nawanshahar were conducted to study the prevalence of the disease. To study the correlation of some pre-disposing meteorological factors, viz. average atmospheric temperature, relative humidity and total rainfall with the development of the Alternaria blight disease under field conditions, the data on per cent disease index (PDI) were recorded at 10 days intervals starting from the January till mid of April during 2002-03 and correlated with the averages of meteorological factors prevailing during those periods obtained from the observatory of the Department of Agronomy and Agrometeorology, PAU, Ludhiana. PDI was calculated on the basis of 0-4 scale using the formula given by Mc Kinney (1923).

To study the perpetuation of the pathogen naturally infected Ber leaves were collected from the New Orchard, PAU, Ludhiana in the mid April 2002. Infected leaves were kept in wire gauge baskets under field conditions and also in polythene bags in the laboratory at room temperature. Periodical isolations were made from theses leaves were done on potato dextrose agar (PDA) slants after every 30 days to ascertain the longevity and viability of the spores upto mid of December 2002. Observations in terms of per cent recovery of pathogen on PDA slants were recorded.

Poisoned food technique (Nene and Thapliyal, 1979) was employed to study the relative efficacy of different fungitoxicants viz. Indofil M-45 (Mancozeb), Blitox-50 (Copper oxychloride), Antracol (Propineb) (each at 0.1% and 0.2%); Bayleton (Triademefon) (at 0.05% and 0.1%); Bordeaux mixture (at 0.6% and 0.8%); Neem oil (at 0.5% and 1.0%); Neem extract (at 1.0% and 2.0%) concentrations on a.i. basis under *in vitro* conditions against *Alternaria alternata* isolated from blight infected Ber leaves. Potato dextrose agar (PDA) medium was prepared in the 250ml Erlenmeyer conical flasks and was sterilized by autoclaving. To this medium was added requisite quantity of the fungicide so as to get the final concentration. A series of concentrations were prepared. The fungicide was thoroughly mixed by stirring. The

poisoned medium was then poured into sterilized Petri plates under aseptic conditions in a laminar air flow cabinet and allowed to solidify. Petri plates were inoculated with 8mm discs of the inoculums cut with a sterilized cork borer from 15 days old culture of *A. alternata* raised on PDA. The fungal discs were placed in an inverted position so that the fungus may come in direct contact with the medium. Checks were kept where the culture discs were grown under similar conditions on PDA without fungicide. Each treatment was replicated thrice in Completely Randomised Design. The fungus colony diameter (mm) was measured after seven days of inoculations. The per cent inhibition in radial growth was calculated using the formula devised by Vincent (1947) :

$$\text{Per cent radial growth inhibition} = \frac{\text{Radial growth in control - radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

For the management of the disease under field conditions fungicidal chemicals belonging to different groups, viz. Indofil M-45 (Mancozeb), Blitox-50 (Copper oxychloride), Antracol (Propineb) each (at 0.2% and 0.3%); Bayleton (Triademefon) at (0.05% and 0.1%); Bordeaux mixture at (0.8% and 1.0%); Neem oil (at 0.5% and 1.0%); Neem extract (at 1.0% and 2.0%) concentrations were sprayed under field conditions on *ber* trees at New Orchard, PAU, Ludhiana. Three sprays were given starting from 3rd week of January after light showers at 15 day intervals. Each treatment was replicated thrice. The observations on efficacy of different concentrations were recorded in terms of per cent disease index (PDI) as well as per cent disease control (PDC).

$$\text{Per cent disease control} = \frac{\text{PDI in control - PDI in treatment}}{\text{PDI in control}} \times 100$$

Data were recorded one month after the last spray. Randomized block design was used to evaluate the data.

RESULTS AND DISCUSSION

The incidence of Alternaria blight of Ber was recorded on cultivar 'Umran' in four different Ber growing districts of Punjab in the first week of March during the year 2003 (Table 1). Periodical surveys of Ber orchards conducted at 10-day intervals at Punjab Agricultural University, Ludhiana and no symptoms of the disease were observed uptil the last week of December when the average maximum and minimum atmospheric temperatures were 24.39^{0}C and 10.29^{0}C respectively though average maximum and minimum relative humidity prevailed to the extent of 99.2 and 59.8 per cent respectively with 0.39 mm rainfall. Gradual decline in

Table 1. Per cent disease index (PDI) of Alternaria blight of Ber trees (cultivar Umran) in different Ber growing areas of Punjab during 2003

District	Location	Per cent Disease Index (PDI)*
Ludhiana	New Orchard, PAU, Ludhiana	29.75
Patiala	Fruit Research Station, Bahadurgarh	28.25
Sangrur	Village Changal	23.20
Nawan Shahar	Ballowal Saunkhri	20.75

*Observations recorded in first week of March.

atmospheric mean maximum temperature to 16.14^{0}C with increase in mean maximum and mean minimum relative humidity and with total rainfall of 0.30 mm during the first week of January favored the initiation of the disease on the nursery plants as well as on the grown up trees of the Ber. The disease started as faded green areas on the margins of the leaves which later enlarge gradually to form irregular brown necrotic lesions on the upper surface of the leaves and grey to brown lesions on the corresponding lower surface with black colored sporulation. Many lesions coalesce to form large blightened patches leading to pre mature defoliation. Further increase in the mean maximum relative humidity accompanied by total rainfall showed a sharp upward trend in the index (52.89%) of the disease up to last week of March. Beyond this, the per cent disease index started declining gradually due to pre-mature fall of severely blightened leaves. However, no symptoms were observed on any other part of the plant. Similar type of symptoms and pattern of disease development have been described by Gupta and Madaan (1977) from Haryana.

Further, interpretation of the correlation of the individual factors with development and progress of the disease lead to the conclusion that the mean maximum atmospheric and mean minimum atmospheric temperature were positively correlated with the disease progression having r values of 0.572 and 0.578 respectively, whereas it bears a positive correlation with mean maximum relative humidity with r values of 0.116 and -0.586 respectively. Rainfall was positively correlated (r=0.269) with the progression of the disease. The disease development followed a linear equation of y = 1.265x + 0.241 and y = 0.162x + 0.334 with respect to average maximum and minimum temperature respectively. The linear equation between disease development and average maximum and minimum relative humidity found to be y = 0.083x + 85.961 and y + -0.641x + 72.472 respectively and between disease development and

total rainfall it was calculated as y = 0.049x – 0.078. Information on this aspect of study is scanty for comparision, however, preliminary observations recorded by Madaan and Chand (1984) that temperature of 20-30^{0}C with 100 per cent relative humidity favours maximum disease development on Ber leaves are in complete agreement with the present findings (Table 2).

As evident from Table 3, periodical isolations revealed that pathogen could survive on infected leaves kept protected under field conditions as well as at room temperature. There was cent per cent recovery of the pathogen up to 60 days in case of leaves kept protected under field conditions, whereas cent per cent recovery up to 180 days was observed in case of infected Ber leaves kept at room temperature. After 270 days i.e. up to mid December, there was recovery from the infected leaves protected under field conditions and those kept at room temperature was 38.30 and 90.60 per cent respectively. It was inferred from the observations recorded above that the pathogen survived on the infected leaves and which helps in its perennation. This observation is in agreement with that of Madaan and Chand (1986).

The data presented in the Table 4 revealed that Indofil M-45 ranked first by providing 82.08 per cent inhibition of vegetative growth at 0.2 per cent concentration whereas, the same fungitoxicant at 0.1 per cent concentration inhibited the vegetative growth to the extent of 69.44 per cent. Bordeaux mixture provided 79.22 and 73.93 per cent inhibition of growth at 0.8 and 0.6 per cent concentration whereas Antracol at 0.2 and 0.1 per cent induced 72.71 and 59.40 per cent radial growth inhibition, respectively. Blitox-50, Neem oil, and Bayleton also exhibited significant inhibition of radial growth. Neem oil at a concentration of 1.0 per cent was found to be least effective (26.68%) among all the test fungicides.

The analysis of the data presented in Table 5 clearly indicated that the fungicides that proved more promising

Table 2. Effect of some meteorological factors on the development of Alternaria blight of ber during 2002-03

Date of observation	Mean atmospheric temperature (^{0}C)		Mean relative humidity (%)		Total rainfall	Per cent disease index (X_i)	RPD* ($X_i - X_{i-I}$)
	Max.	Min.	Max.	Min.			
25/12/02	24.39	10.29	99.20	59.80	0.39	0.00	0.00
04/01/02	16.14	5.94	97.40	69.00	0.30	1.70	1.70
14/01/02	11.28	4.36	87.90	84.50	0.00	4.12	2.42
24/01/03	14.88	3.79	100.0	77.40	0.00	8.30	4.18
03/02/03	19.12	9.04	98.30	65.90	0.00	21.83	13.53
13/02/03	20.52	6.41	97.60	49.30	0.00	33.90	12.07
23/02/03	20.36	10.10	93.30	60.70	11.43	39.61	5.71
05/03/03	22.88	11.19	89.50	53.90	3.29	47.07	7.46
15/03/03	25.45	11.10	91.20	42.60	0.00	52.89	5.82
25/03/03	29.35	14.08	88.60	44.70	0.00	48.98	-3.91
04/04/03	29.77	15.96	85.30	45.90	1.50	43.24	-5.74
14/04/03	35.71	16.83	80.90	24.00	0.00	33.4	-9.77
24/04/03	36.72	18.94	51.70	20.80	0.50	24.82	-8.65

*Relative Progression of the Disease

Table 3. Mode of perpetuation of *Alternaria alternata* causing blight of Ber.

Mode of perpetuation	*per cent recovery of pathogen after (days) starting from mid April								
	30	60	90	120	150	180	210	240	270
A. Infected leaves kept in wire gauge baskets under field conditions	100.0	100.0	93.3	86.6	66.6	60.0	46.6	41.6	38.3
B. Infected leaves kept at room temperature	100.0	100.0	100.0	100.0	100.0	100.0	96.6	93.3	86.6

*mean of 15 observations

Table 4. Comparative efficacy of different fungitoxicants against *Alternaria alternata* under *in vitro* conditions

Fungicide	Concentration (%)	Radial growth (mm)*	Radial growth inhibition (%)
Indofil M-45	0.3	10.33	87.37
	0.2	17.00	79.22
Bordeaux mixture	1.0	14.66	82.08
	0.8	21.33	73.93
Antracol	0.3	18.66	77.19
	0.2	22.33	72.71
Blitox-50	0.3	27.00	67.00
	0.2	32.00	60.89
Neem Oil	1.0	42.66	47.86
	0.5	46.33	43.17
Bayleton	0.1	49.00	40.12
	0.05	55.00	32.78
Neem extract	2.0	55.00	32.78
	1.0	60.00	26.68
Control	-	81.83	-

*Based on three replications

CD (P=0.05), Chemicals = 1.154, Concentration = 0.617, Chemicals ´ Concentration = NS

Table 5. Management of Alternaria blight of ber through fungicidal sprays under field conditions

Fungicide	Concentration (%)	Per cent Disease Index (PDI)*	Per cent Disease Control (PDC)
Indofil M-45	0.3	12.66	78.79
	0.2	21.25	64.44
Bordeaux mixture	1.0	14.83	75.17
	0.8	25.33	57.04
Antracol	0.3	20.60	65.55
	0.2	33.10	44.60
Blitox-50	0.3	38.25	35.98
	0.2	43.58	27.06
Neem Oil	1.0	38.95	34.81
	0.5	43.91	26.51
Bayleton	0.1	41.21	31.04
	0.05	47.91	19.78
Neem extract	2.0	46.31	22.48
	1.0	50.93	15.31
Control	Plain water sprayed	59.75	-

*Based on three replications;

CD (P=0.05), Chemicals = 4.024, Concentration = 2.15, Chemicals ´ Concentration = NS

under *in vitro* conditions were also found more effective under *in vivo* conditions. Indofil M-45 (at 0.3%) concentration provided maximum disease control of 78.79 per cent followed by Bordeaux mixture (at 1.0%) and Antracol (at 0.3%) giving 75.17 and 68.55 per cent disease control. Blitox-50 (0.3%), Neem oil (1.0%) and Bayleton (0.1%) provided 35.98, 34.81 and 31.04 per cent control over the disease.

These findings are similar to those of Prasad *et al.* (2002) that Indofil M-45 was most effective agaist Alternaria species and Verma and Jain (2002) that Bordeaux mixture, Indofil M-45 and Neem oil were most effective, while Blitox-50 was found to be least effective against *Alternaria alternata* causing blight of pear.

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Assessment of genetic diversity of *Fusarium solani* from different agro-ecological regions of India

Rupesh Kumar Mishra, B. K. Pandey, Muthukumar M., A. K. Misra, Vijay Singh, Amita J. Mathew, Neelam Pathak and Mohd. Zeeshan

Central Institute for Subtropical Horticulture, Lucknow- 226101, U.P., India
E-mail: rupeshkmishra@rediffmail.com

ABSTRACT

Fusarium solani is one of the causal agent of wilt disease of guava (*Psidium guajava* L.) in subtropical regions. Identification of the pathogenic isolates by microscopic and cultural characteristics is not sufficient and reliable for characterization of pathogenic isolates from guava as they are mostly influenced by environmental factors. PCR assays used such as RAPD and internal transcribed spacer (ITS) region for discrimination and also sequenced the ITS region and generated the phylogenetic relationship among *F. solani*. A phylogenetic tree based on RAPD data has been generated showing the three major clades. Additionally, specific primer used for detection of *F. solani* and all tested isolates showed positive result in PCR assay. However, three clades were obtained using the sequences of ITS region. It can be used to develop new molecular marker for identification of *F. solani*. These finding provide a new insight for molecular level discrimination of *F. solani* form different agro-ecological regions of India.

Key words: *Fusarium solani*, RAPD, wilt disease, ITS region, phylogenetic tree, molecular marker.

The soil borne fungus *Fusarium solani* contains many host-plants that cause vascular wilt disease. *Fusarium* wilt is a threatening disease in guava (*Psidium guajava* L.). It is most destructive disease of guava and causes a 5–60 per cent loss (Misra, 2006). Although numbers of pathogens are reported associated with guava wilt, two species *F. oxysporum* f. sp. *psidii* and *Fusarium solani* are more frequently reported (Prasad *et al*., 1952; Edward, 1960; Chattopadhyaya and Bhattachariya, 1968a, Mishra *et al*., 2012). However, reports revealed wide variations in cultural and morphological characteristic of different isolates of *F. solani* (Gupta *et al*., 2010). The fungus first colonizes the surface of roots and then enters through epidermal cells. Thereafter, intercellular mycelium establishes first in epidermal cells and then spreads into cortical cells which get considerably damaged and filled up with the mycelium. *Fusarium solani* enters the xylem vessels, grows inside and blocks them. Previous studies on progressive natural wilting of guava plants during different months have been reported (Misra and Pandey, 1999a,d) with the maximum wilting during September-October (Misra and Pandey, 2000b). Conventional methods for identification and discrimination of fungal pathogen are time consuming and difficult. Thus, PCR assay for rapid and correct identification is useful. Random amplification of polymorphic DNA (RAPD) method has been applied (Williams *et al*., 1990) for detection and genetic characterization. Some recent reports are available for discrimination of phytopathogenic fungi including *Fusarium* species from different crops (Kim *et al*., 1993; Miller 1996; Brown 1998; Gulino *et al*., 2003). Cultural and genetic diversity have been recorded in different isolates of *F. solani*, when tested *in vitro* against different cultural and physiological parameters (Gupta *et al*., 2010, Chattopadhyay and Sengupta, 1955, Dwivedi and Dwivedi, 1999). In recent years, the identification of members of the genus *Fusarium* is based on the characteristic colony morphology and the microscopic characters (multiseptated sickle-shaped conidia called macro-conidia) however, identification may be difficult when the macro-conidia are not produced in culture or resemble with another species. This usually occurs with soil borne pathogen in unfavourable conditions. In this case, the isolates can be confused with other genera or species. Furthermore, the specific determination of *Fusarium* species remains a problem.

In recent years, several techniques have been employed in development of molecular tools to identify fungi, such as single sequence repeat (SSR), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and sequence based specific detection. PCR-RFLP technique that enables easy and rapid identification of the fungi residing in the three phylogenetic clades of *F. oxysporum* defined by O'Donnell *et al*. (1998). Identification of *F. solani* isolates causing the vascular wilt disease in the guava plants is important for efficient management. The objective of the

present investigation was to detect and characterize *Fusarium solani* isolates using molecular techniques.

MATERIALS AND METHODS

Survey and sample collection

Soil and infected root samples from different locations were collected before onset of rains to find out the presence of *Fusarium* present in the soil of guava orchards. *Fusarium solani* were isolated from eight different agro-climatic regions of India (Table 1). The reference culture of *Fusarium solani* (NAIMCC-F-2010) was collected from NAIMCC, NBIAM, Mau, Uttar Pradesh for this study.

Table 1. List of *Fusarium solani* isolates indicating geographic location and colony colour used in this study

Culture No.	Geographic origin	Colony colour	Wilt symptom (%)	Source of isolation
F. solani-1	Chandigarh	Light brown	100	Soil
F. solani-2	Rewa	Dark yellow	70-80	Root
F. solani-3	Lucknow	Light brown	100	Soil
F. solani-4	Punjab	Creamy	50	Soil
F. solani-5	West Bengal	Light pink	100	Soil
F. solani-6	Allahabad	No colour	100	Root
F. solani-7	Faizabad	Pale yellow	50	Root
F. solani-8	Kanpur	Pale yellow	50-70	Soil
F. solani-9	Varanasi	Cream colour	100	Soil
NAIMCC-F-2010	Kanpur	Brown	50	Root

Isolation and characterization of F. solani

Infected root samples were surface sterilized and were laid on each (90mm) sterilized Petri dish containing PDA. Serial dilution was done to isolate the fungus from soil. All the cultured petri dishes were incubated at 25 ± 2^{0}C for 5-7 days. Each fungus isolated and cultured on PDA; and kept in a refrigerator at 8^{0}C. The causal pathogen of wilt was identified based on the morphological and cultural characteristics on PDA, and microscopic observation following the fungi identification key of Barnett and Hunter (1972).

Extraction of genomic DNA

All *F. solani* isolates were maintained at 4^{0}C on potato dextrose agar (PDA) (HiMedia). Mycelium for DNA extraction was grown on potato dextrose agar (PDA) for 5 days at 28^{0}C. The mycelium grown over the media was harvested and ground to a fine powder in a sterile mortar with liquid nitrogen. DNA was extracted by using Fast DNA isolation kit (MP-Biomedical) according to the manufacturer's instructions.

Screening of a RAPD marker

Fifteen arbitrary 10-mer primers (Table 2) (Eurofins Genomics India Pvt. Ltd.) were used for RAPD marker studies. RAPD PCR was performed in 25 µl reaction volume containing 25 ng genomic DNA, 10 pmole primer, 0.5 mM of each dNTPs, 2.5 µl of 10x PCR buffer with $MgCl_2$ (15 mM), 1.25 µl (5 U/µl) of *Taq* DNA polymerase (Fermentas). DNA was amplified by Master Cycler (Eppendorf) programme to provide first denaturation for 5 min at 94^{0}C followed by 36 cycles of 1 min at 94, annealing at 36^{0}C for 1 min followed by 2 min at 72^{0}C and final extension for 5 min at same temperature. PCR products were resolved by electrophoresis using agarose gel (2%) with 1X TBE buffer with ethidium bromide.

PCR amplification using species-specific primer

The ITS region (ITS1, ITS2) was amplified with primers ITS-Fu2f (5'-CCAGAGGACCCCCTAACTCT-3') and ITS-Fu2r (5'-CTCTCCAGTTGCGAGGTGTT-3') (Arif *et al.*, 2012). The PCR amplification was carried out in 25 ìl reaction mixture containing 10x PCR buffer, 1.5 mM $MgCl_2$, 0.5 mM of each dNTPs, 0.5 µM of each primers, 1.25 U *Taq* polymerase and 10 ng genomic DNA. The following cycling conditions were used for PCR amplification 95^{0}C, 4 min. (denaturation), 56^{0}C, 1 min (annealing), 72^{0}C, 1 min (extension) for 30 cycles on a Master Cycler (Eppendorf). The PCR products were checked on 1.2% agarose gel with ethidium bromide (EtBr) and visualized under a UV transilluminator.

Cluster Analysis

The comparison of genetic similarity was assessed based on DNA banding patterns of RAPD and PCR-RFLP was carried out on the basis of presence (1) or absence (0) of amplified PCR products of the same length by using Jaccard's coefficient (Jaccard, 1908). A dendrogram was derived from the distance matrix by the Unweighted Pair-Group Method Arithmetic Average (UPGMA) obtained using NTSYS-pc 2.02e (Numerical Taxonomy and Multivariate Analysis System) (Rohlf, 1998).

Table 2. Sequences of reliable RAPD primers and the number of scorable polymorphic bands of each primer

S. No.	Primer	Sequence detail	No. of amplified bands	No. of polymorphic bands	Polymorphic ratio (%)
1.	OPA 01	CAGGCCCTTC	11	5	45.45
2.	OPA 02	CTGGGGACTT	9	5	55.55
3.	OPA 03	CCCAAGGTCC	10	5	50
4.	OPA 04	TCACCACGGT	10	7	70
5.	OPA 05	GGTCTAGAGG	8	4	50
6.	OPA 06	AAGCGGCCTC	9	5	55.55
7.	OPA 07	GAAACGGGTG	13	6	46.15
8.	OPA 08	GTGACGTAGG	7	5	71.42
9.	OPA 09	GGGTAACGCC	9	4	44.44
10.	OPA 10	GTGATCGCAG	9	8	88.89
11.	OPA 13	CAGCACCCAC	19	17	89.48
12.	OPB 08	GTCCACACGG	8	5	62.5
13.	OPD 18	GAGAGCCAAC	7	4	57.14
14.	OPD 19	CTGGGGACTT	12	10	83.33
15	OPX 01	CTGGGCACGA	No amplification	0	0
Average		-	9.4	6	63.82
Total		-	141	90	_

RESULTS AND DISCUSSION

In present study, genetic diversity of 10 isolates of *Fusarium solani*, collected from different geographical regions of India is determined by RAPD and sequencing result of ITS region. Out of fifteen RAPD primers (Table 2) used for the study OPA-10, OPA-13 and OPD-19 revealed maximum polymorphism in the isolates. The primer OPA-13 (5'-CAGCACCCAC-3') generated 89.48 per cent polymorphic ratio (%) in isolates (Fig. 1). Amplified fragments were ranged from 250-2400 bp. The number of polymorphic DNA fragments, amplified and scored per isolate for individual primer, ranged from 4 to 17. These data show that RAPD is a convenient method for distinguishing the isolates of *F. solani* and also reveal a high degree of genetic variation among the isolates. A total of 141 reproducible fragments are scored with wide range of diversity within the pathogenic isolates. Out of 141 amplified bands 90 fragments were scored polymorphic bands and it shows 63.82 per cent average polymorphic ratio. Similarly, RAPD markers have been previously used to study inter- and intra-specific variation of twelve *Fusarium* species isolated from cotton-growing areas in Egypt (Abd-Elsalam *et al*., 2003). In a RAPD analysis of *F. oxysporum* Assigbetse *et al*. (1994) reported a correlation between genetic similarity and geographic origin. RAPD provides more comprehensive information regarding the genetic variability among the pathogen populations as it is based on the entire genome of an organism (Achenback *et al*., 1997). RAPD-PCR polymorphisms found in *Fusarium* potentially provide a method for identifying the fungi both at isolate and species level (Gupta *et al*., 2009). There are several research groups (Hyun *et al*., 1998; Migheli *et al*., 1998; Ibrahim and Nirenberg, 2000; Jana *et al*., 2003) study the *Fusarium* spp. population from different plant host by using RAPD analysis which have been suggested that RAPD markers can be a quick and reliable alternative method for differentiating isolates of *Fusarium* species.

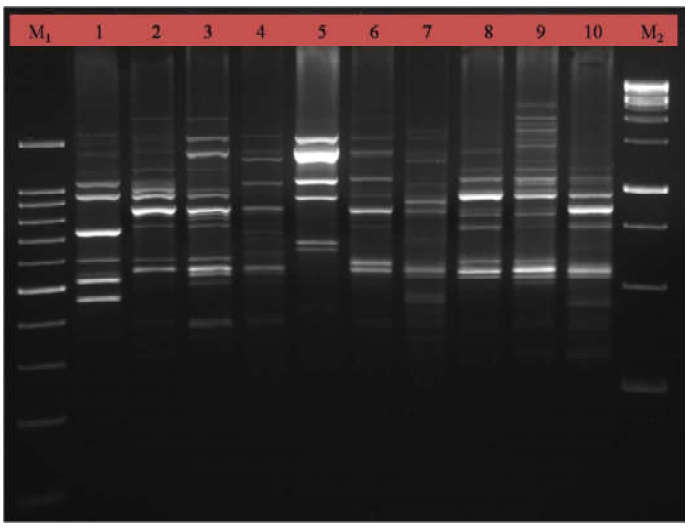

Fig.1. RAPD patterns on 2% agarose gel of amplified fragment generated from 10 isolates of *Fusarium solani* with random primer OPA-13. Lane M_1- 100 bp DNA ladder, Lane M_2- 1kb DNA ladder, lane 1-10 *Fusarium solani* isolates.

Table 3. Genetic similarity coefficient matrix among 10 isolates in the *Fusarium solani* was calculated from RAPD profiles by OPA-13 primer

	F.solani-1	*F.solani-2*	*F.solani-3*	*F.solani-4*	*F.solani-5*	*F.solani-6*	*F.solani-7*	*F.solani-8*	*F.solani-9*	*NAIMCC-F-2010*
F.solani-1	1.0000000									
F.solani-2	0.6250000	1.0000000								
F.solani-3	0.3750000	0.5555556	1.0000000							
F.solani-4	0.8333333	0.7500000	0.5000000	1.0000000						
F.solani-5	0.1428571	0.2222222	0.2857143	0.2857143	1.0000000					
F.solani-6	0.4285714	0.6250000	0.5714286	0.5714286	0.3333333	1.0000000				
F.solani-7	0.4285714	0.4444444	0.8333333	0.5714286	0.3333333	0.4285714	1.0000000			
F.solani-8	0.2500000	0.4444444	0.5714286	0.3750000	0.1428571	0.6666667	0.4285714	1.0000000		
F.solani-9	0.3333333	0.6666667	0.6250000	0.4444444	0.2500000	0.7142857	0.5000000	0.7142857	1.0000000	
NAIMCC-F-2010	0.5000000	0.6666667	0.8571429	0.6250000	0.2500000	0.7142857	0.7142857	0.7142857	0.7500000	1.0000000

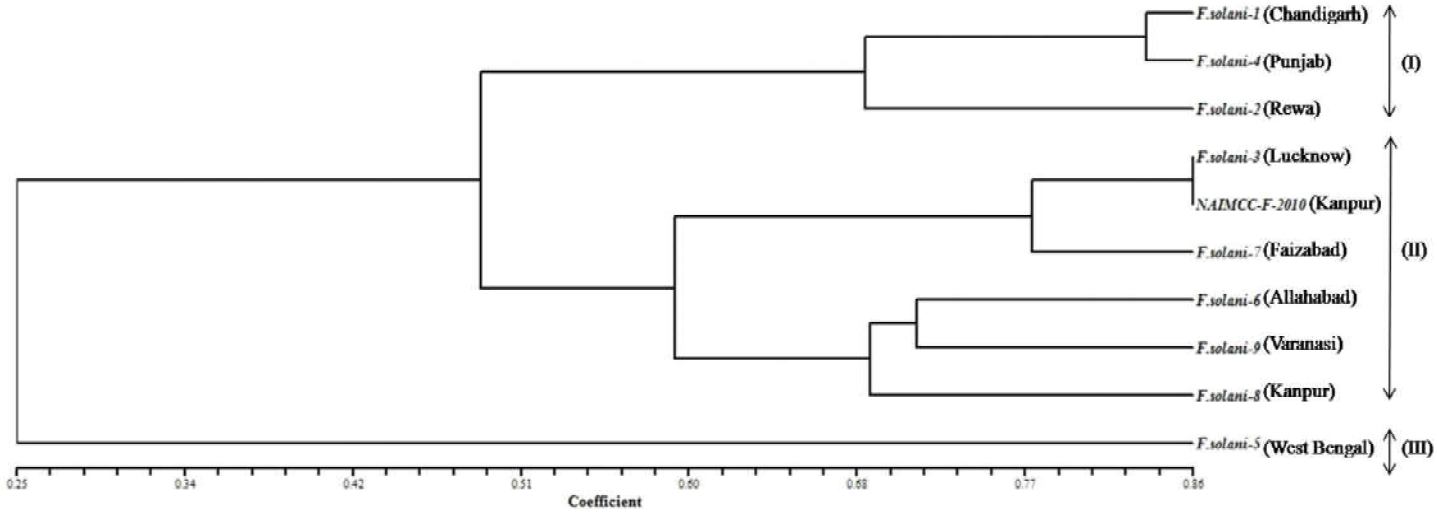

Fig. 2. Dendrogram of 10 *Fusarium solani* isolates derived from data of RAPD fingerprints generated by UPGMA based on Genetic distance calculated from Presence (1) or absence (0) of polymorphic bands with OPA-13

Relatedness among the isolates was estimated by means of scorable polymorphic DNA bands and the genetic similarity of isolates was assessed based on RAPD by using Jaccard's coefficient and the data was used to construct a dendrogram (Fig. 2, Table 3) using the NTSYS 2.02e software package (Rohlf, 1998). The cluster analysis of data separated the 10 isolates into three major groups. *F. solani*-1 (Chandigarh) and *F. solani*-4 (Punjab) are 83.33 per cent similar formed a group with *F. solani*-2 (Rewa) of 68 per cent genetic similarity. Major group of isolates *F. solani*-3 (Lucknow), *F. solani*-8 (Kanpur), *F. solani*-6 (Allahabad), *F. solani*-7 (Faizabad) and *F. solani*-9 (Varanasi) are clustered with reference isolate *NAIMCC-F-2010* (Kanpur) and form two sub groups containing *F. solani*-3 and *NAIMCC-F-2010* of 86 per cent similarity with *F. solani*-7 (78%) and in another subgroup *F. solani*-6, *F. solani*-8 and *F. solani*-9, while *F. solani*-5 (West Bengal) lies in separate branch and found only 25 per cent genetic similar with other isolates. On the basis of RAPD cluster analysis region specific grouping was observed in isolates.

The rapid and sensitive detection of pathogenic fungi is important in formulating strategies for disease management in plant nurseries. Using the *Fusarium solani* specific primers developed by Arif *et al.* (2012), PCR amplicon of 590 bp were generated from all of the isolates. As shown in Figure 3 the DNA banding patterns obtained for ITS region amplified from a selection of different *Fusarium solani* isolates. The amplicon size clearly shows that no such variation in amplified region. The optimized PCR parameters for the specific amplification using ITS-Fu2f and ITS-Fu2r were 56^{0}C for annealing temperature, 1.5 mM for $MgCl_2$ concentration,

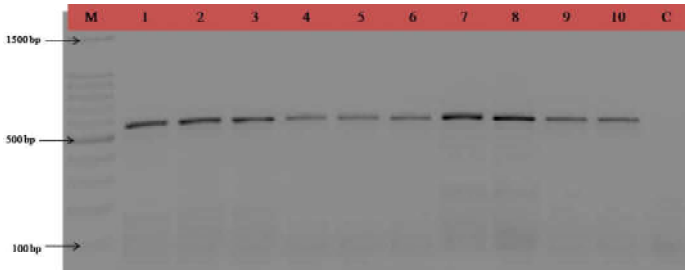

Fig. 3. *Fusarium solani* species-specific PCR amplification of 590 bp amplicon size using primer pair ITS-Fu2f and ITS-Fu2r, Lane M contains 100 bp DNA marker

and 0.5 ìM for each primer concentration. The primer sets developed by Arif *et al*. (2012) could be particularly useful in identification of *Fusarium solani* causing infection on a variety of plants (Arif *et al*., 2008a, b; Romberg and Davids, 2007) and also implicated in mycotic ocular keratitis (Godoy *et al*., 2004). The developed primers have been used for discrimination of population's biology of *F. solani* using sequencing of amplified PCR products (Arif *et al*., 2012).

To confirm the genetic profile of each isolate, the partial region of ITS with 5.8s rDNA using ITS1 primer was sequenced. Amplified product on agarose gel was cut and purified with gel purification kit (QIAquick gel extraction kit (Qiagen, Hilden, Germany) DNA elution kit and the amplified products were sequenced in single directions (Forward) using the above primer was carried out by Xcelris Bio-lab Hyderabad, India. Data obtained by sequencing (570 bp of ITS+ 5.8s rDNA of each *F. solani* isolates) was used for homology search analysis and result shows 99-100 per cent sequences are similar with isolates reported from other host plant. Molecular evolutionary analyses were carried out using MEGA5 and a phylogenetic tree (Fig. 4) was constructed using these sequences, showed three major clades. The distribution of isolates was found as RAPD analysis. The Sequence Data Explorer (Fig. 5) shows the aligned sequence data and provides a number of useful functionalities for exploring the statistical attributes of the data and also for selecting data subsets. The data obtained by analysis revealed some variation in their ITS regions. The dendrogram derived have also three main clusters like RAPD analysis. PCR assays have been implemented successfully for identification and detection of economically important

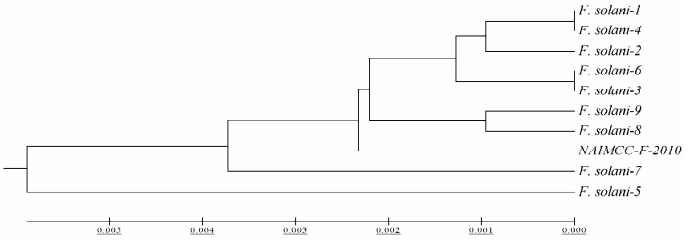

Fig. 4. The phylogenetic tree based on the nucleotide sequence of the ITS1 region in the *Fusarium solani*

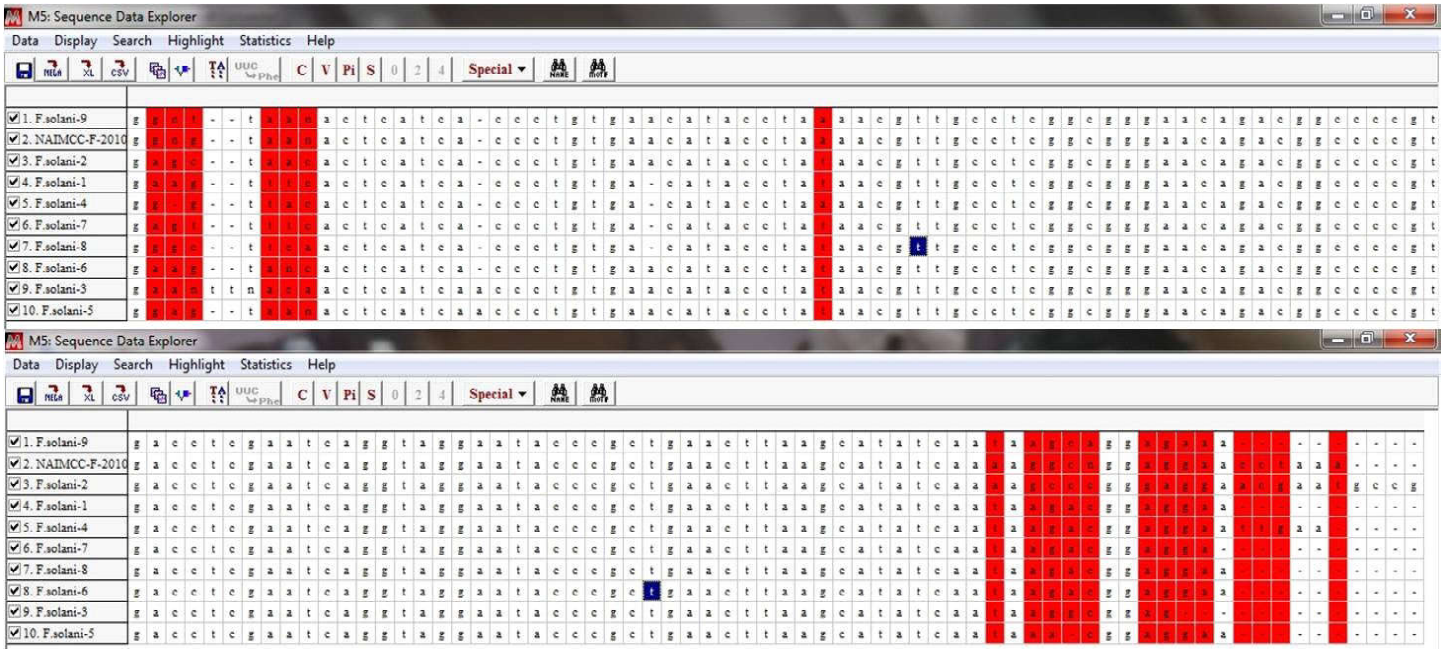

Fig. 5. Nucleotide sequence alignment of the partial ITS1 and 5.8S rDNA gene and colour rows shows variation in the sequences

Fusarium species such as *Fusarium avenaceum* (Schilling *et al*., 1996; Turner *et al*., 1998), *Fusarium culmorum* (Klemsdal and Elen, 2006; Nicholson *et al*., 1998; Schilling *et al*., 1996), *Fusarium graminearum* (Nicholson *et al*., 1998; Schilling *et al*., 1996; Yoder and Christianson, 1998), *Fusarium langsethiae* (Wilson *et al*., 2004), *Fusarium moniliforme* (the official name is now *F. verticillioides*; Moller *et al*., 1999), *Fusarium subglutinans* (Moller *et al*., 1999), *Fusarium poae* (Parry and Nicholson, 1996), *Fusarium sambucinum* (Yoder and Christianson, 1998), *Fusarium sporotrichioides* (Wilson *et al*., 2004) and *Fusarium venenatum* (Yoder and Christianson, 1998). Most of these molecular assay are based on the development of species-specific primers. The results were in accordance with Lee *et al*. (2000) in which they suggested that the ITS with 5.8S is more useful in differentiation of *Fusarium* species.

Our investigations confirm the high level of genetic variability in *Fusarium solani*, pointing out the dissimilarity between these isolates obtained from different agroclimatic region of India. This study demonstrates the reliability of the RAPD as tools for addressing questions on geographical distribution of population of this pathogen. In this preliminary study, the *F. solani* isolates were found to be closely related regardless of the location and guava cultivars. *F. solani* isolates were different from each other and have existing high level of genetic variation. These observations could be helpful for further studies of guava cultivars resistant *F.solani* pathogen.

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Utilization of banana agro-horti waste for production of carboxymethyl cellulase

Khan Mohd. Sarim, Neelima Garg, Devendra Kumar and Preeti Yadav, U.P., India

Central Institute for Subtropical Horticulture, Rehmankhera, P.O.Kakori, Lucknow – 226 101
E-mail : neelimagargg@rediffmail.com

ABSTRACT

Banana is a major cash crop of India and is cultivated all over across the country, generating large amount of agro waste including pseudostem and leaves after fruit harvest. Microbial production of enzymes using low valued agro industrial waste is gaining importance globally. Since banana is rich in cellulose, it was utilized for cellulase production. Out of the ten fungi screened for cellulase production PF4 identified as *Fusarium* sp. exhibited maximum carboxymethyl cellulase activity. Maximum production of carboxymethyl cellulase was achieved on the fifth day of fermentation ((3.9 U ml^{-1}). pH 5 was found optimum for maximum carboxymethyl cellulase production (4.75 U ml^{-1}). Statistical analysis showed that fermentation temperature had significant effect on enzyme production ($p > 0.01$) and maximum carboxymethyl cellulase production (6.90458 U ml^{-1}) was recorded at 30^0C. The K_M and Vmax values for carboxymethyl cellulase were observed to be 0.108 µg ml^{-1} and 0.0452mM ml^{-1} min^{-1}.

Key words : Banana, enzyme, cellulose, *Fusarium* sp.

Enzymes from microbial sources are preferred compared to plant or animal sources because enzyme production from former is relatively cheap and controllable. The enzyme production cost can further be reduced if negative value or cheap value substrates such as fruit processing industry wastes are used (Abdul Hamid *et al*., 1999). This might solve waste disposal problem of fruit industries to some extent and also help in reduction of the pollution problem. India ranks first in the production of banana with 16.8 million metric tons followed by Brazil with 6.7 million metric tons. It is a major cash crop of India. It is cultivated across all over the country, chiefly in Maharashtra, Uttar Pradesh, Andhra Pradesh, West Bengal, Tamilnadu, Karnataka, Gujarat and Bihar, generating large amount of agro waste including pseudostem and leaves after the harvest. Since banana is rich in cellulose, potential exists for its use as substrate for cellulase production (Baig *et al*., 2004). The enzyme produced might be utilized by the processing industries and reduce the production cost of processed products besides reducing environmental pollution. In the present study potential of banana waste for cellulase production was explored.

MATERIALS AND METHODS

Substrate: Banana waste (dried and powdered) was used for cellulase production.

Organisms: The microorganism were isolated from cellulose rich substrates and plated on carboxymethyl cellulose (CMC) agar (Speck, 1985). The microorganisms showing growth were screened for cellulase production.

Isolation of cellulolytic microorganisms

One gram orchard soil and degrading leaves were added in to CMC broth and incubated at 30^0C for 4 days. One ml of fermenting suspension was added to carbohydrate utilization medium containing 1 per cent CMC (Speck, 1985). After 2 days of growth, 1 ml was plated over CMC agar. Repeated purification of microorganism was performed to get pure colonies of microorganisms.

Enzyme assay for carboxymethyl cellulase

Enzyme was incubated with 1 per cent CMC in 0.1M sodium acetate buffer pH 4.0 at 30^0C for 30 min. The amount of glucose was estimated by measuring the amount of reducing sugar released in the medium. One unit of enzyme is the amount of the enzyme that produced 1 ìmol of glucose per minute under assay condition (Miller, 1959).

Screening of micro-organisms for carboxymethyl cellulase activity

The micro-organisms were subjected to plate assay method as described by Carder, (1986). The results were confirmed by growing the fungal isolate in banana waste medium at 1 per cent substrate concentration and determining the reducing sugar released in the medium.

Standardization of pH for optimum carboxymethyl cellulase production

pH of the cellulose utilizing medium was adjusted to 4, 6 and 8. The flasks were incubated at 30±2^{0}C for 5 days with selected fungal strain, showing maximum enzyme activity. The pH at which maximum enzyme activity was observed was selected for further studies (Wood and Bhat, 1988).

Standardization of temperature for optimum carboxymethyl cellulase production

The flasks (cellulose utilizing medium) were incubated at 30, 40 and 50^{0}C (in triplicate) for 5 days with selected fungal strain; showing maximum enzyme activity. The temperature at which maximum enzyme activity was observed was selected for further studies.

Determination of pH for maximum carboxymethyl cellulase activity

Sodium acetate buffer of different pH viz. 3, 4, 5, 6 and 7 was prepared. Then 2 % of CMC solution at different pH was prepared. 0.5 ml of substrate was mixed with 0.5 ml of the extracted enzyme from solid state fermentation by banana waste. These were incubated 30^{0}C at for 60 minutes. After that enzyme activity was determined in the mixture. The pH of buffer at which maximum enzyme activity was observed, was determined as the optimum pH.

Protein quantification

The protein concentration of crude as well as partially purified enzyme was determined by the method of Lowry *et al*. (1951) using bovine serum albumin (BSA) as standard.

Enzyme characterization

Optimization of pH and temperature for maximum enzyme activities

The effect of pH (3.0-7.0 in the increments of 1 unit) on cellulase activity was studued using citrate/phosphate (0.02 M) was studied as described by Ali and El-Dein (2008). For temperature optimization studies, the enzyme extract was incubated at incremental temperatures of 10^{o}C from 20-60^{o}C for 30 min and then assayed for carboxymethyl cellulase activity.

Determination of Kinetic constant

Kinetics of the enzyme was analyzed using Lineweaver-Burke and Michaelis-Menten plots (Sanghi *et al*., 2010). The enzyme kinetics experiment was performed by measuring the initial enzyme reaction velocity at different substrate concentration of carboxymethyl cellulose in 50mM acetate buffer. The Lineweaver-Burke plot was used to establish the Michaelis constant (K_M) and maximum velocity (V_{max}) of the enzyme reaction. In all experiments 3 replication were taken and mean values have been reported.

Statistical analysis

Values are presented as mean, standard deviation (n=3), the significance of all terms in the polynomial functions including substrate concentration, nutrient addition, day of incubation, temperature and pH were assessed statistically using F-value at a probability (p) of 0.001, 0.01 or 0.05.

RESULTS AND DISCUSSION

Out of the ten fungi screened for carboxymethyl cellulase production microbial isolate no. PF4 identified as *Fusarium* sp. exhibited maximum cellulase activity (Table 1). *Fusarium* sp. is known to have high cellulase activity. Wood (1969) has described the cellulase enzyme complex of *Fusarium solani*. Murali *et al*. (1994) have compared FPase (filter-paper cellulase ml^{-1}) activity of *Fusarium* sp. with that of *Trichoderma reesei*. Higher activities were observed with former fungus (0.33 units ml^{-1}), than later (0.8 U ml^{-1}) under aerated conditions.

Table 1. Secondary screening of microorganisms for cellulase production

Microbial isolate	μM min^{-1}	protein (mg ml^{-1})	sp. activity (U mg^{-1})
T. viride	1.051	0.11	9.39
C1W	0	0.12	0
T. harzianum	1.57	0.17	9.22
C6	0.61	0.16	3.72
PF4	1.57	0.09	16.11
PF3	0.96	0.23	4.19
WF1	0.79	0.17	4.78
A. fumigatus	0.88	0.13	6.79
WF3	1.23	0.25	4.94
WF4	0.17	0.16	1.13

Trivedi and Rao, 1981 have reported that *Fusarium* produced significantly higher cellulase within a short period of time as compared with *Trichoderma reesei*. Our results indicated that maximum production of carboxymethyl cellulase was achieved on the fifth day of fermentation (3.9 U ml^{-1}). Temperature and pH are the most important factors, which markedly influence enzyme activity. Enzymes are active over a limited pH range only. The pH value of

maximum activity is known as optimum pH and this is the characteristics of enzyme. The variation of activity with pH is due to the change in the state of ionization of the enzyme protein and other components of the reaction mixture. In synthetic medium containing cellulose is the carbon source. Highest cellulase production (4.77496 U ml^{-1}) was observed at pH 5 (Fig 1). Statistical analysis showed that fermentation temperature had significant effect on enzyme production ($p > 0.01$). Maximum cellulase production (6.90458 U ml^{-1}) was recorded at 30^0C. Further increase in temperature resulted into decrease in the activity of the enzyme (Fig 2). Most carboxymethyl cellulose degrading enzymes are known to exhibit maximum activity in the temperature range of 30-60^0C (Ariffin *et al*., 2006; Ali *et al*., 2010).

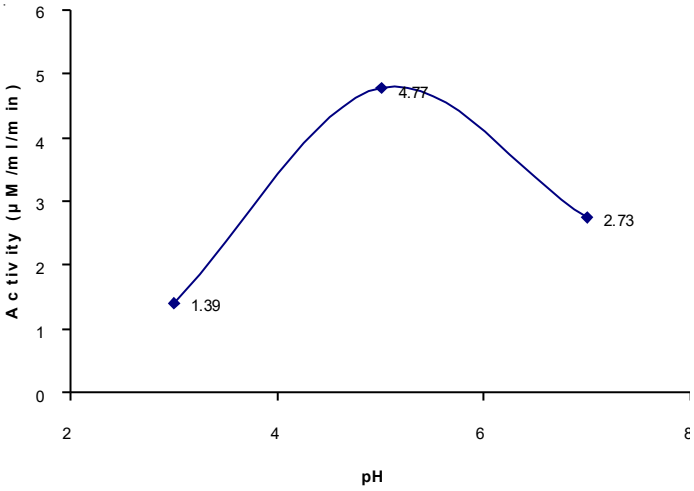

Fig. 1. Effect of pH on cellulase production

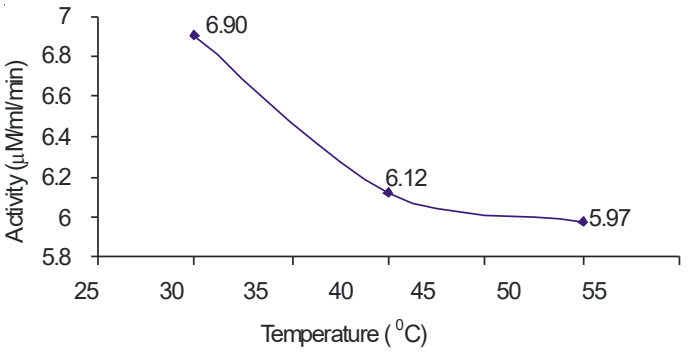

Fig. 2. Effect of temperature on cellulase production

Enzyme kinetics: The pH 5 and temperature 40^0C were found optimum for carboxymethyl cellulase activity (6.069 U mg^{-1}). The effect of the substrate concentration on the enzyme activity indicated that increase in carboxymethyl

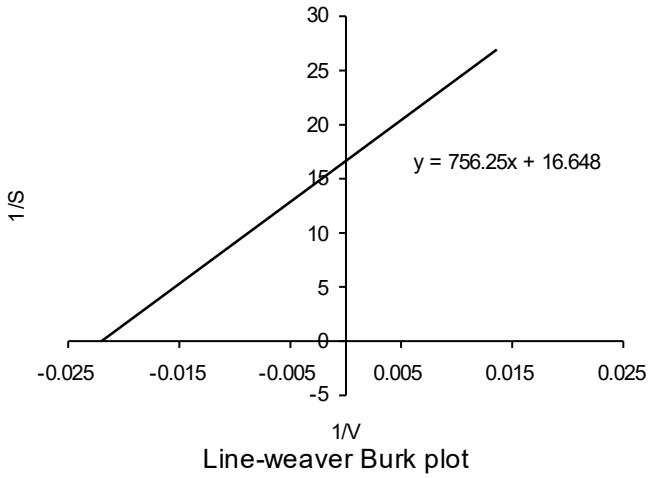

Fig. 3. Lineweaver-Burke plot showing the Michaelis–Menten-type kinetics of CM cellulase on the carboxylmethyl cellulose under the standard assay condition specified, indicating the K_M value under maximum velocity (Vmax)

cellulose concentration up to 24 mg ml^{-1} and decreased thereafter. The Michaelis constant (K_M) and V_{max} values were found to be 0.108 µg ml^{-1} and 0.0452 mM ml^{-1} min^{-1} glucose released sec^{-1} mg^{-1} protein, respectively. Using Line weaver-Burke plot (**3**) the R^2 value was worked out to be higher than 0.9, which depicted Michaelis–Menten-type of kinetics for endoglucanase on the carboxymethyl cellulose under the standard assay conditions. De Castro *et al.* (2010) reported K_M (19.39 mg ml^{-1}) and V_{max} (0.0948 m.mole L^{-1}) for endoglucanase production using *Trichoderma harzianum* on pure carboxymethyl cellulose. The lower K_M values observed in our study might be due to aonla pomace as substrate.

The study indicates that banana waste could serve as potential substrates for production of cellulases. Recently more and more emphasis is being laid on utilization of fruit processing waste for value added products, in this context the present research may find useful application.

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