RNI No. UPENG/2006/22736
ISSN 2229-628X
Registration No. 131380

Vol. 14, No. 2, July, 2019

Journal of Eco-Friendly Agriculture

Doctor's Krishi Evam Bagwani Vikas Sanstha
(Doctor's Agricultural and Horticultural Development Society)
Registered under Society Registration Act 21, 1860
Lucknow - 226 016, India

JOURNAL OF ECO-FRIENDLY AGRICULURE

(A Bi-annual Scientific Research Journal)
Doctor's Krishi Evam Bagawni Vikas Sanstha
(Doctor's Agricultural and Horticultural Development Society)
Registration No. 131380, ISSN 2229-628X

ANNUAL MEMBERSHIP FEE EFFECTIVE FROM JANUARY, 2019

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Reprint	250.00 per page	250.00 per page	20.00 per printed page	20.00 per printed page
Life Membership	8000.00	15000.00	1000.00	2500.00

Owner : Doctor's Krishi Evam Bagwani Vikas Sanstha, Publisher : Dr. Ram Prakash Srivastava, 108, Lakhraj Khazana, Faizabad Road, Lucknow, Printed at : Army Printing Press, 33 Nehru Road, Sadar Cantt. Lucknow.

Journal of Eco-Friendly Agriculture

Vol. 14 **No. 2** **July, 2019**

Acknowledgment : DKEBVS acknowledges the financial support from ICAR, New Delhi
Journal is approved by UGC (Journal No. 48500) and ICAR (Journal ID-154)

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Registered under Society Registration Act 21, 1860
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Journal of Eco-friendly Agriculture **14**(2): 1-10. 2019

Ecological chemistry of insect-plant interactions

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ABSTRACT

A clear scientific understanding of the behavioural/chemical ecology of the interactions with its hosts, conspecifics and natural enemies at different trophic levels is key for the development of reliable and sustainable push-pull strategies. Insects have mastered the art of using semiochemicals as communication signals and rely on them to find mates, hosts or habitats. The potential semiochemicals could be rapidly screened by Computational Reverse Chemical Ecology (CRCE) approach. The literature on phytosemiochemicals has shown that even common and structurally simple compounds can act as important chemical signals and exhibit biological activity on many different species. Future applications on semiochemicals depend on the availability of the potential cues that enable efficient manipulation of mate-and host-finding behaviour in horticultural pests.

Key words: Semiochemicals, CRCE, behavioural/chemical ecology

"Certainly insects cannot think, but they can react"- Chemical cues (= semiochemicals/infochemicals)are used by insects to interact with their environment for survival and reproduction.This reliance of insects on chemical cues offers a number of opportunities for their control (Kamala Jayanthi *et al*., 2015a; Bruce, 2010).

At this juncture, when we are celebrating ~60 years of ***'Pheromone research'*** (way back in 1959, when bombycol, the first pheromone from silkworm was identified, thanks to **Butenandt**; and simultaneously when two scientists**, Peter Karlson** and **Martin Lüscher** coined the word *"pheromone"* to describe the chemicals used for communication between members of the same species), the exploitation of these powerful infochemicals used by insects in horticulture pest management programs is still in its infancy.

In recent past, push-pull strategies or Stimulo-Deterrent Diversionary Strategies (SDDS) are behavioural manipulation methods that use repellent/deterrent (push) and attractive/stimulant (pull) stimuli to direct the movement of pest or beneficial insects for pest management. However, their potential is under exploited particularly in horticultural crop pests. This may be mainly because of lack of thorough understanding of chemical mediated processes to manipulate trophic interactions to manage pests. Thus, development of reliable, robust and sustainable push-pull strategies requires a clear scientific understanding of the behavioural/chemical ecology of the interactions with its hosts, conspecifics and natural enemies at different trophic levels to underpin key processes that can be exploited as weak links (Kamala Jayanthi *et al.,* 2015a). Further, in order to understand/manipulate the various phyto/semio-chemicals (pheromones, allelochemicals, kairomones, allomones, synomones) and to maximize their usefulness in integrated pest management, collaborations between biology, chemical ecology,physiology, analytical chemistry and molecular biology are paramount for India (Kamala Jayanthi *et al.,* 2015a).

Conventional way of doing chemical ecology-A time honoured practice

Insects have mastered the art of using semiochemicals as communication signals and rely on them to find mates, host or habitat. However, discovering semiochemicals is a laborious process that involves a plethora of behavioural and analytical techniques, making it expansively time consuming (Verghese *et al*., 2013, Kamala Jayanthi *et al.,* 2014a). Chemical ecology in nutshell elucidates the chemical signals involved in trophic interactions through series of bioassay guided approaches that are not only labour intensive but also time consuming. For the isolation and identification of active fractions, the researchers usually start their investigation with preliminary bioassays augmented with electrophysiological approaches, such as gas chromatography-electroantennographic detection (GC-EAD). Although GC-EAD may provide a "shortcut" to identify the active fractions, a solid and consistent bioassay is still needed to avoid false-positives (a compound may generate an electrical signal and be behaviourally inactive) (Young-Moo Choo *et al*., 2018; Leal, 2017).

Computational Reverse Chemical Ecology (CRCE)

Recent research upsurge has uncovered the role of distinct proteins involved in insect olfaction enhancing our

understanding of molecular basis of odour perception in insects and in the last two decades this approach has opened the door for reverse chemical ecology, a term coined for using olfactory proteins as another "bioassay tools" to screen active semiochemicals (Young-Moo Choo *et al.,* 2018; Leal, 2017). Therefore, reverse chemical ecology approach using odorant binding proteins (OBPs) as target for elucidating behaviourally active compounds is gaining eminence. This methodology often described as "Computational Reverse Chemical Ecology" approach (CRCE) helps in rapid screening of potential semiochemicals (Leal, 2005; Leal *et al*., 2008; Kamala Jayanthi *et al.,* 2014a). Though different schools of thought exists regarding the exact mechanism of olfaction that is being taken place in insects, the most widely accepted one is achieved through two low-molecular weight (10-20 kDa) proteins, *viz.,* odourant binding proteins (OBPs), odorant receptors (ORs) (Sun *et al*., 2012; Pophof, 2002). OBPs are the first proteins to recognize and bind to odour molecules in the long cascade of olfactory signal transduction (Yin *et al.,* 2012; Leite *et al.,* 2009; Jiang *et al.,* 2009). OBPs interact with odors that enter through tiny pores present on the insect's antenna forming an OBP-Odor complex. The complex transports odor molecules to ORs thereby starting the signal transduction cascade leading to behavioural outputs. This methodology was tried as pilot study in Oriental fruit fly, *Bactrocera dorsalis* (Hendel), a frugivorustephritid that causes huge losses in several cultivated fruits and vegetables. We generated *in-silico* data for estimating kairomone efficiency of possible insect attractants that were previously identified (Kamala Jayanthi *et al.,* 2012). A total of twenty four host cues were selected randomly representing a varying range of high, medium and low attraction categories.

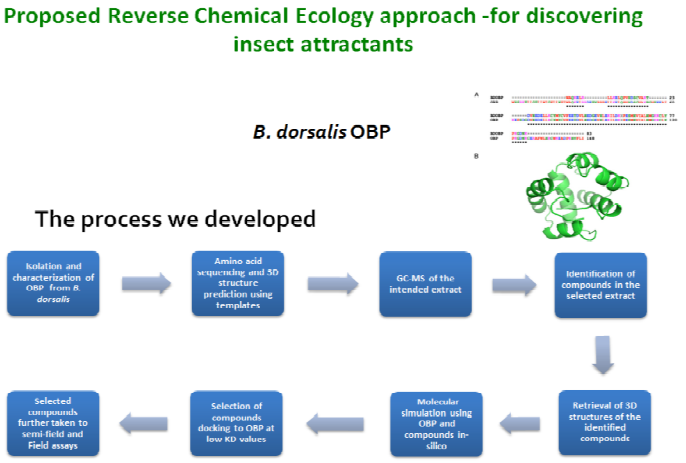
A major, female-enriched odourant binding protein (OBP) ~14 kDa from *B. dorsalis* was isolated from the antenna of females and sequenced through MALDI-TOF (Kamala Jayanthi *et al.,* 2014a). The partial sequence was blasted with submitted OBPs of *B. dorsalis* that showed 100 per cent sequence match with a previously isolated and characterized OBP of *B. dorsalis* (GenBank ID: ACB56577.1) and the later was used for 3D model prediction. The profile 3D score of the selected model was 48.34 and exceeded the minimum requirement value of 26.85. The predicted model consists of 6 α-helices that are located between 47-65 (α1), 72-85 (α2), 96-102 (α3), 105-118 (α4), 126-140 (α5) and 142-146 (α6). There also existed 3 pair of disulphide bridges that may play a role in stabilizing the structure.

Molecular docking, prediction and bioassay of behaviorally active compounds

A 3D structure of the isolated OBP with the highest score was selected and used in docking studies. We used an online molecular docking tool "Docking Server". The 3D structures of protein and selected semiochemicals were loaded to the server. The results were processed and arranged for prediction. Thermodynamically, a ligand binds tightly to the active site of the protein when the free binding energy is low. Therefore, compounds with lower free binding energy were predicted to be behaviourally active. To aid in our prediction process compounds showing free binding energy less than -4.00 were considered behaviourally active and compounds with free binding energy more than -4.00 were used for comparison. First, we conducted a tryptophan quenching assay to find the binding potential of the selected compounds. Second, we conducted a behavioural assay to validate if the predicted compounds were behaviourally active or not. Tryptophan quenching was carried out with the isolated OBP and predicted compounds at concentrations ranging from 0–5000 nM. Kd value was estimated by fitting the fluorescence quenching data to an equation describing a single binding site present as a default in Prism Graph Pad version 5.01 for OS X. Per cent quenching was determined. The predicted compounds showed high quenching as evident by the Kd values. Kd values ranged from 600–6000 nM. The results regarding the Trp fluorescence quenching spectrums of OBP with test compounds are interesting because the compounds we predicted behaviourally active had tighter binding as evident by quenching and lower Kd values. Computer simulations or *in-vitro* binding assay of OBPs may not be an exact measure of the behavioural activity of an insect, however, it may be relevant to the functional characterization of an OBP. Therefore, behavioural assays are needed to ascertain the nature (attractant or repellent) of the predicted compounds.

Accordingly, behavioural assays were conducted to find the activity of predicted compounds. Using the behavioural assay data, a unified estimator, Attraction Index (AI), was calculated. From the data we found that methyl eugenol that was predicted as highly behaviourally active by its free binding energy showed the highest attraction 74.4 per cent (Free binding energy = -5.63; AI = 0.50) and the lowest attraction was exhibited by ethanol with 6.67 per cent (Free binding energy = -2.43; AI = -0.84) of flies attracted towards them.

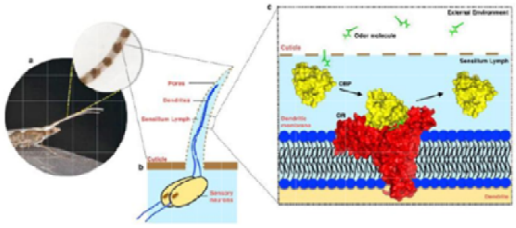
Statistical validation of computational and behavioural assays is crucial in these types of studies. Further, as *Kd* and free binding energy are dependent, considering both for validation of the method is not sensible. Thus, analysis carried out to standardize the dependable scoring functions [free binding energy (ΔGbind), vdW + Hbond + desolv energy, electrostatic energy, total intermolecular energy,



interaction surface are considered along with *Kd*]for estimating the semiochemical efficiency showed significant correlation for both *in silico Kd* (Pearson r = -0.7974; P < 0.0001) and free binding energy (Pearson r = -0.9728; P < 0.0001) to AI. Regression analysis showed that the scoring function 'free binding energy' (F = 90.41; P < 0.001; r^2 = 0.9464) to be the best variable to predict behaviourally active compounds. Therefore, free binding energy was used as a dependable and robust scoring function. Then, correlation between *in silicoKd* and experimental *Kd* was significantly positive with r^2 = 0.9408 (P < 0.0001) and demonstrated that *in silico* data could be used for predicting behaviourally active compounds.

In this study we described and validated a simple and efficient *in silico* method for estimating the kairomone efficiency of host cues for chemical ecology studies. Instead of using lengthy and costly procedures for identification of kairomones, our method comes in handy to cut short time and cost.

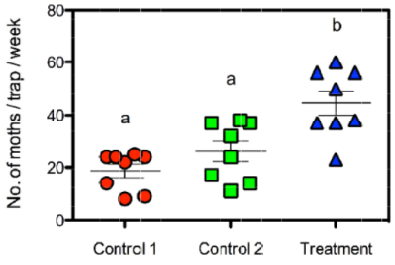
Later we revised the above CRCE approach by simulating the interactions of OBP-odour complex with ORs to mimic the working environment of olfactory proteins (OBPs and ORs) in live insect antenna using volatile cues from cabbage and diamondback moth, *Plutellaxylostella* as test insect (Kamala Jayanthi *et al.*, 2016a). We screened 17 volatile compounds from cabbage, *Brassica oleracea*, in order to discover attractants for the *P. xylostella*. Three-dimensional structure of OBP1 and Odourant receptors OR2 from *P. xylostella*were modelled and used as molecular targets. The interaction of OBP-odourant complex with OR was used in identifying behaviourally active compounds. From the docking studies, four compounds from the headspace volatiles of cabbage namely allylisothiocyanate, E-2-hexen-1-ol and Z-3-hexen-1-ol were predicted as behaviourally active. Semiochemicals ascertained active were further



Schematic representation of the molecular mechanism of olfaction inside a moth's antenna. a) Image of *P. xylostella* antenna. b) Schematic diagram represents a sensillum present in moths. c) The odor molecules (green molecules) from external environment enter the sensillum through pores on the cuticle. The OBPs (yellow proteins) present in the sensillum lymph capture the odor molecules and form an OBP-odor complex. The complex then moves towards the OR (red protein) and starts signal transduction leading to behavioral outputs.

subjected to interaction studies in the laboratory and in field studies. It was observed that the predicted compounds were attractive to *P. xylostella* adults both in bioassays and in the field.

Of several insect pests that ravage fruits and vegetables in general, fruit flies belonging to Genus *Bactrocera* are particularly important in horticulture not only because of their polyphagy but the quarantine restrictions they carry across the globe adds to the management complexity. The current IPM program for fruit flies mainly revolves around MAT (Male Annihilation Technique). The Male Annihilation Technique involves lure and kill of males using bait stations that consist of attractants such as methyl eugenol lures combined with an approved insecticide as a killing agent. It is recommended for area wide management of fruit flies (Mumford, 2006). However, the existing methyl eugenol baited traps are effective only in intercepting male *B. dorsalis,* and because oriental fruit flies are highly polygamous a few surviving males can fertilize a substantial number of females (Cunningham, 1989). Therefore, more successful control of oriental fruit fly would be achieved by targeting female B. dorsalis because they are responsible for oviposition and subsequent damage to fruits. Crude attractants based on fermenting sugars, hydrolysed protein, and yeast are available but are limited by lack of potency, short shelf life, and lack of specificity (Siderhurst and Jang, 2006). Identification of kairomones would facilitate the development of lures based on synthetic female attractant.



Attraction of *P. xylostella* to natural blend of predicted compounds. Sticky traps containing natural blend of predicted compounds were erected in cabbage fields. The treatment traps (natural blend of predicted compounds) attracted significantly (P< 0.005) more moths than control 1 traps (empty traps) and control 2 traps (pheromone traps) for a period of 8 weeks. Each point represented one-week of trap catch. Data was subjected to one-way ANOVA followed by Tukey's multiple comparison test. Error bars represent SE (Standard error).

As a first step in this direction, we attempted isolation and identification of host cues to attract gravid female fruit fly, *B.dorsalis* (Kamala Jayanthi *et al*., 2012). We identified new attractants for *B. dorsalis* from overripe mango fruits. Headspace samples of volatiles were collected from two cultivars of mango, 'Alphonso' and 'Chausa', and a strong positive behavioural response was observed when female *B. dorsalis* were exposed to these volatiles in olfactometer bioassays. Coupled GC-EAG with female *B. dorsalis* revealed 7 compounds from 'Alphonso' headspace and 15 compounds from 'Chausa' headspace that elicited an EAG response. The EAG-active compounds, from 'Alphonso', were identified, using GC-MS, as heptane, myrcene, (*Z*)-ocimene, (*E*)-ocimene, allo-ocimene, (*Z*)-myroxide, and ã-octalactone, with the two ocimene isomers being the dominant compounds. The EAG-active compounds from 'Chausa' were 3-hydroxy-2-butanone, 3-methyl-1-butanol, ethyl butanoate, ethyl methacrylate, ethyl crotonate, ethyl tiglate, 1-octen-3-ol, ethyl hexanoate, 3-carene, p-cymene, ethyl sorbate, α-terpinolene, phenyl ethyl alcohol, ethyl octanoate, and benzothiazole. Individual compounds were significantly attractive when a standard dose (1 µg on filter paper) was tested in the olfactometer. Furthermore, synthetic blends with the same concentration and ratio of compounds as in the natural headspace samples were highly attractive ($P< 0.001$), and in a choice test, fruit flies did not show any preference for the natural samples over the synthetic blends.

Further studies were carried out to identify specific cues that can instigate oviposition behaviour in gravid female *B. dorsalis* (Kamala Jayanthi *et al*., 2014b). We screened all the 21 EAD-active volatiles identified from mango in the previous study for their oviposition stimulant activity. 1-Octen-3-ol, ethyl tiglate, ã-octalactone and benzothiazole instigated oviposition in gravid *B. dorsalis* females. Flies deposited most of their eggs into pulp discs with oviposition-stimulants, and only a small fraction of eggs were laid into control discs. In a binary choice oviposition assay, 95.1, 93.7, and 65.6 per cent of eggs were laid in discs treated with 1-octen-3-ol, ethyl tiglate, andbenzothiazole, respectively. Single plate two-choice assays proved that oviposition-stimulants were crucial in oviposition site selection by gravid female *B. dorsalis*. In simulated semi-natural assays, gravid *B. dorsalis* females accurately differentiated between fruits with and without 1-octen-3-ol, ethyl tiglate, and ã-octalactone by laying more eggs on the treated fruit. However, benzothiazole did not elicit an increase in oviposition when presented in this context. Our results suggest that the identified oviposition-stimulants are 'key' compounds, which the flies associate with suitable oviposition sites. Elucidation of these kairomones will improve our perception of the chemical basis of ovipositionbehavior in *B. dorsalis*, and the identified cues will likely enhance control operations against this highly polyphagous, invasive quarantine pest of invincible economic importance.These studies helped us further in developing a lure for female *B. dorsalis* to bait traps with. A female attractant lure *ArkaDorsolure F* was standardized and patented for commercialization.

Further, these studies also helped us in enhancing the existing mass rearing protocols for *B. dorsalis* particularly for sterile insect technique programs (Kamala Jayanthi *et al*., 2017). The sterile insect technique (SIT) can be an effective, target-specific and economically feasible control method for fruit fly management elsewhere. However, implementation of SIT depends on mass production of high-quality insects. Production of superior quality eggs is of foremost importance in insect mass production. The present protocols make use of fruit juices or fruit domes that attract saprophytic insects or microorganisms, reducing the quality of eggs. Furthermore, fermentation of juices is known to decrease oviposition efficiency, and daily sanitation of oviposition devices is required. We evaluated the effectiveness of four synthetic oviposition stimulants (OS) of *B. dorsalis* for egg production in dual choice tests, using oviposition devices similar to those used in mass rearing. Results indicated that ã-octalactone, benzothiazole, and octen-3-ol and ethyl tiglate significantly increased egg laying compared to controls (water). Of these, ã-octalactone was particularly effective and elicited a 263-fold increase in oviposition on treated oviposition devices compared to control. Our findings demonstrate the potential of using OS to improve the efficiency and cost effectiveness of mass production of *B. dorsalis*.

Exploring the scope of chemical elicitors as IPM components

Studies have demonstrated that insects use precise ratios of volatiles for host location (Bruce *et al*., 2005; Kamala Jayanthi *et al.,* 2012). Even subtle changes in volatile ratios of host plants confuse insects and alter their perception and orientation (Beck *et al*., 2012). However, *plants* have evolved with their insect pests and have developed an array of strategies for defense. One such strategy is the utilization of elicitors in priming and/or increasing or decreasing the production of certain volatile compounds upon insect attack (Schmelz *et al*., 2006). Thus, exogenous applications of elicitors may impact insect-plant interaction through modified host plant volatile emissions.

Chemical elicitors, *viz*., salicylic acid (SA), jasmonic acid (JA), ethylene, abscisic acid (ABA), gibberellic acid (GA_3) are well studied and known to induce both direct and indirect defenses against insect pests (Erb *et al*., 2009). Such

induced responses in plants are important components of pest management and can be triggered by external application of elicitors (Zhao *et al*., 2009). Among the elicitors listed above, SA is well studied in non-woody plants for its role in regulating plant defense and in triggering 'systemic acquired resistance' (SAR) (Bruinsma *et al*., 2007).

In our constant endeavor to identify synergistic components for the integrated management of fruit flies in mango, we explored a strategy that has received negligible attention is the induction of 'natural plant defenses' by phytohormones (Kamala Jayanthi *et al.,* 2015b). In this study, we investigated the effect of salicylic acid (SA) treatment of mango fruit (cv. Totapuri) on oviposition and larval development of *B. dorsalis*. In oviposition choice assays, gravid females laid significantly less eggs in SA treated compared to untreated fruit. Our olfactometer results showed that headspace volatiles collected from untreated fruit attracted *B. dorsalis* whereas volatiles from SA treated fruit did not attract the flies. This result indicated that the SA treated fruit volatiles were less attractive to gravid flies. GC-MS analysis of the headspace volatiles from SA treated and untreated fruit showed noticeable changes in their chemical compositions. As we know volatile chemical cues from the host plant play a major role in the orientation of gravid females to their hosts from a distance. Thus, perception of right mix of these volatile blends plays a pivotal role in host recognition and determines the probability of phytophagous insect alighting on a given host (Bruce and Pickett, 2011). GC/MS analysis confirmed the complete absence of volatiles, *viz.,* cis-ocimene and 3-carene in SA treated fruit. These volatiles are reported to elicit significant EAG response as well as positive behavioural responses in gravid female *B. dorsalis (*Kamala Jayanthi *et al*., 2012). Therefore, these two chemical cues, cis-ocimene and 3-carene are important attractants and involved in host location of *B. dorsalis* (Kamala Jayanthi *et al.* 2012). The subsequent inhibition of cis-ocimene, 3-carene after the exogenous application of SA would have led to the observed altered behavior of *B. dorsalis* as herbivorus insects are known to use plant volatiles as key for host location and as indication of suitable oviposition site (Bruce *et al*., 2005; Kamala Jayanthi *et al*., 2014b).

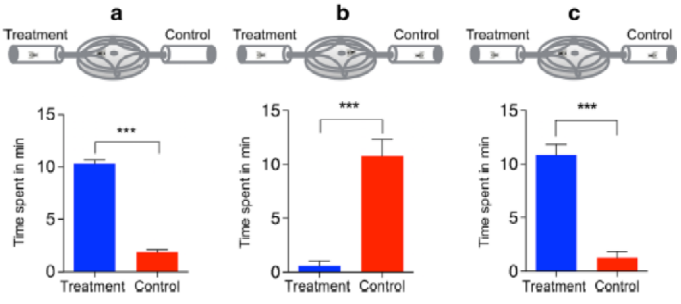
Further, reduced pupae formation and adult emergence was observed in treated fruit compared to control. Increased phenol and flavonoid content was recorded in treated fruit. We also observed differential expression of anti-oxidative enzymes namely catalase (CAT), polyphenoloxidase (PPO) and peroxidase (POD). These results indicate that SA treatment reduced oviposition, larval development and adult emergence of *B. dorsalis* and suggest a role of SA in enhancing mango tolerance to *B. dorsalis*. As fruit flies infest mango fruits usually at the fag end of the harvest, application of SA as pre-harvest spray to curb fruit fly infestation would fit well in to the sustainable eco-friendly management module (Kamala Jayanthi *et al*., 2015b).

Commensal bacteria aid mate-selection in the fruit fly, *Bactrocera dorsalis*

Commensal bacteria influence many aspects of an organism's behaviour. However, studies on the influence of commensal bacteria in insect mate-selection are scarce. We generated empirical evidence that commensal bacteria mediate mate-selection in the Oriental fruit fly, *B. dorsalis*. Male flies were attracted to female flies, but this attraction was abolished when female flies were fed with antibiotics, suggesting the role of the fly's microbiota in mediating mate-selection. In this study, the removal or reinfection of commensal bacteria, in female flies clearly affected mate selection behaviour in *B. dorsalis* males.

Schematic representation of the olfactometer experiment setup and graphical representation where males were significantly more attracted to the olfactometer arm containing virgin female (Treatment) than control. b Antibiotic treatment abolishes male attraction to female. Virgin female flies fed on BD medium containing antibiotics (treatment) and female flies fed on BD medium without antibiotics (control) are transferred into the two arms of the olfactometer. A virgin male in the center is allowed to make a choice between treatment and control females. Time spent by the male in each arm is recorded for a period of 15 min. Male fruit flies spent significantly more time in the control then the treatment arm, confirming the involvement of commensal bacteria in attraction of male to female flies.c Schematic and graphical representation of re-infection experiments. Antibiotic-treated virgin females are introduced into vials containing sterile BD medium inoculated with the female flies' microbiota for reinfection. Re-infected female flies served as treatment and antibiotictreated female flies serves as control (Kamala Jayanthi *et al.,* 2016b).

We showed that male flies were attracted to and ejaculated more sperm into females harbouring the



microbiota. Using culturing and 16S rDNA sequencing, we isolated and identified different commensal bacteria, with *Klebsiellaoxytoca* being the most abundant bacterial species. This study will enhance our understanding of the influence of commensal bacteria on mate-selection behaviour of *B. dorsalis* and may find use in devising control operations against this devastating pest (Kamala Jayanthi *et al.,* 2016b).

Oviposition-site selection in insects is mediated through Innate Recognition Tunes (IRTs) tuned to specific volatile cues

Insects make vital decisions about selection of food, mates or oviposition sites through pre-constructed recognition templates (Del Campo *et al.,* 2001; Sherman *et al.,* 1997). These templates can be innate or acquired through learning and experience. Innate recognition templates (IRTs) are embedded into the genome of insects and are transferred genetically to offspring so that when they are exposed to a fixed cue, or a fixed set of cues, a particular behavior is elicited without having to be learnt. Such recognition templates in insects are thought to be numerous; however, there is a paucity of information about the cues that trigger them. IRTs are triggered by external stimuli that occur within the context of the insect's ecology.

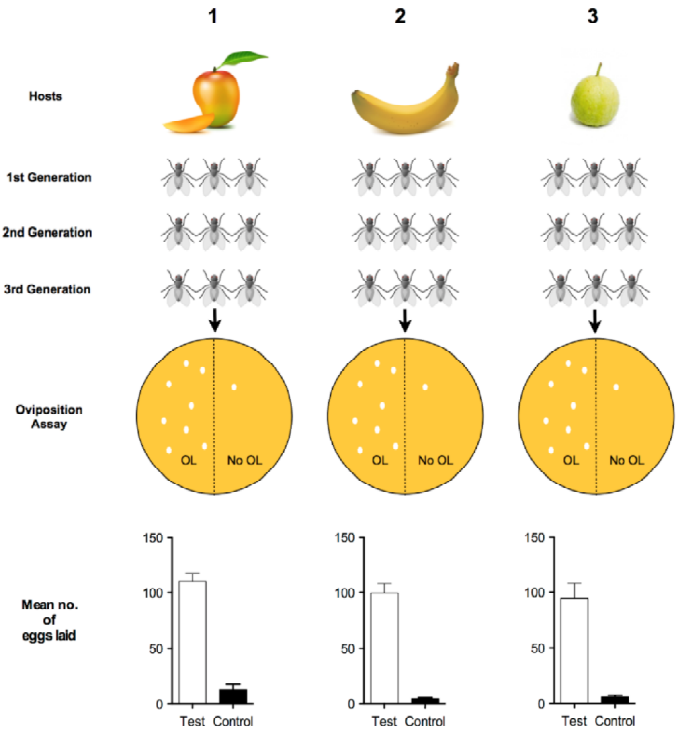
An example of a behavior that may be directed through an IRT is oviposition site-selection by insects (Schwartz *et al.,* 2012). A predilection for favorable oviposition sites by adult insects is essential for successful development and fitness of their progeny (Ryan *et al.,* 2009). Owing to strong competition for oviposition sites and processing time constraints, a female insect has to rapidly evaluate and direct her eggs into suitable oviposition sites. This process of evaluation and egg-laying is instigated only while the insect is gravid. Oviposition site-selection in insects is assisted by a plethora of site-specific cues, but insects have to choose the most reliable and specific cues to override noise and channel appropriate sensory information efficiently during this crucial process. In insects, learning of certain crucial behaviors is impossible due to their short life span and cost incurred during learning. Therefore, crucial behaviors become innate, are embedded into the genome, and passed on by the parents to offspring as IRTs. Through co-evolution with their hosts, insects construct recognition templates to crucial cues that aid in faster processing of information in their brain. However, the oviposition-stimulants to which insects have developed IRTs remain elusive (Kamala Jayanthi *et al.,* 2014c).

Insect olfactory driven behaviors can have an "innate bias" towards certain stimuli or be learned by association. When innate bias occurs, the insects respond to the critical stimulus, rather than the other environmental stimuli; it is possible that the insect can be tricked by these cues even if they are outside their usual context.We studied the oviposition behavior of the oriental fruit fly, *B. dorsalis*, in relation to exposure to volatile semiochemicals from ripe mango (cv. Alphonso) that are known to be electrophysiologically active for this insect (Kamala Jayanthi *et al.,* 2012). We found that oviposition site-selection depends almost entirely on one of these volatile compounds, γ-octalactone. Upon careful experimentation, involving insects with different rearing histories, it was observed that oviposition behavior in *B. dorsalis* is mediated through an IRT tuned to γ-octalactone (Kamala Jayanthi *et al.,* 2014c).

During experimentation γ-octalactone, triggered oviposition behavior where the flies extended their ovipositors and probed the filter paper. Although filter papers are not appropriate oviposition sites, the females showed probing action, which is commonly observed only during egg laying. This observation indicated the involvement of a cue that activated IRT specific for oviposition and suggested that this particular CRT is conserved in the mango fruit flies and passed on through succeeding generations even after being reared on non-specific fruits like banana and guava that are devoid of the cue. Thus, proving γ-octalactone activates a dedicated IRT for oviposition that overrides other exploratory behaviors in *B. dorsalis*. Discovering oviposition-stimulants triggering an IRT will help us 'trick' gravid *B. dorsalis* females, a devastating invasive pest of horticultural importance, into traps (Kamala Jayanthi *et al.,* 2014c).

Schematic representation of the response of flies with different rearing histories towards γ-octalactone. *B. dorsalis* was reared on 3 different hosts. The final (third) generation gravid females were used in oviposition bioassays. A one-tailed *t* test revealed a significant difference ($P<0.0001$) between Test (OL, γ-octalactone present) and Control (No OL, γ-octalactone not present). One-way ANOVA between the tests of group 1 (mango), 2 (banana) and 3 (guava) showed no significant difference in the mean no. of eggs laid.

Another interesting insect-plant interaction, we chanced upon is about the fidelity of gravid female *Bombyx mori* oviposition site choice after ~5000 years of domestication by humans (Kamala Jayanthi *et al.*, 2015c). In insects, selecting an appropriate oviposition site is a complex process that involves multiple modalities and is crucial to the fitness of their offspring (Ponnusamy *et al.,* 2008). As insect eggs are easy targets for predators and egg parasitoids, gravid females must make decisions on the suitability of an



oviposition site in order to keep their offspring safe (Dweck *et al.*, 2013). During egg-laying, insects assess a plethora of factors such as color, nutrition, temperature and microbial composition (Ponnusamy *et al.*, 2008). Furthermore, recent studies have shown that insects tend to select oviposition sites that are repulsive to parasitoids and other threats that may harm eggs or neonate larvae (Dweck *et al.*, 2013). However, it was unclear whether such oviposition site-selection function is still preserved in the highly domesticated insect, *B.mori* and the current study was designed to test if it was maintained after a long history of domestication. We hypothesised that certain innate behaviours allowing location of suitable oviposition sites may be preserved. A recent study has shown that domestication has impaired the olfactory system of *B. mori* so that it is less sensitive to environmental cues (Bisch-Knaden *et al.*, 2014). However, oviposition site selection is an 'innate behavior' and usually passed on to offspring from parents over generations (Kamala Jayanthi *et al.*, 2014c).

We demonstrated that centuries of domestication of silkmoth have not impaired its oviposition site-selection function. We first assessed the egg-laying preference of *B. mori* towards mulberry leaves or its traditional oviposition site, paper, using a binary choice assay. The moths had no restrictions and were allowed to move around freely in the choice arena. In this assay, moths consistently preferred and laid most of the eggs on mulberry leaves. Moths (n = 1 per trial, 10 trials, each lasting 24 h) deposited 93.97 ± 3.7 per cent (mean ± s.e.m) of eggs on mulberry leaves, compared to 6.03 ± 0.14% on filter paper. Silkmoths clearly showed preference for mulberry leaves as oviposition sites (Paired *t*-test; $t = 11.82$; $df = 9$; $P < 0.0001$; n = 10). Presumably, moths may have used volatile cues to navigate towards and oviposit on mulberry leaves. Therefore, we concluded that silkmoth, although domesticated for centuries without access to mulberry plants as oviposition sites; still retain their oviposition site preference. Since the tested moths had no prior experience with leaves, except in their larval stages, we further concluded that the preference for mulberry leaves is innate.Further oviposition assays with filter paper, filter paper treated with leaf volatiles and leaf alone proved that surface texture was not a significant criterion for oviposition site-selection, but volatile cues were.

The moths laid a similar number of eggs on filter paper treated with leaf volatiles (662.4 ± 46.96 eggs; mean ± s.e.m) and mulberry leaf (721.7 ± 49.99 eggs) compared to filter paper alone (226.3 ± 33.64 eggs). One-way ANOVA followed by Tukey's multiple comparison test between the number of eggs in different treatments showed that eggs laid on filter paper treated with leaf volatiles and mulberry leaf were significantly not different (n = 10; $q = 1.345$; $P = 0.38$). This demonstrates that volatiles from mulberry leaves were sufficient for the oviposition site selection process. We presumed that due to many decades of domestication where gravid moths are given only paper as an oviposition substrate, the silkmoth may have lost the ability of detecting physical cues of mulberry leaves. This also proves that insects retain IRTs (Innate Recognition Templates) of olfactory cues (host volatiles) that remain unaltered for generations.

Electrophysiology studies revealed that the silkmoth's antenna responded to specific volatile compounds of mulberry leaves. Therefore, we asked which volatile compounds from mulberry might be mediating the oviposition preference in the silkmoth, *B. mori*. Although intact leaves are virtually odorless to humans, they release volatile plumes that can be detected by insects. GC-EAD results revealed that silkmoths can detect 7 volatile compounds from mulberry leaves. Individual electrophysiologically active volatiles were further subjected to oviposition assays. Interestingly, the moths showed oviposition preference for certain EAD-active volatiles by laying most of the eggs onto filter papers treated with valencene (OI = 0.57 ± 0.06; mean ± s.e.m) and á-humulene (OI = 0.62 ± 0.01) compared to other cues. ANOVA followed by Tukey's multiple comparison test showed that there was

no significant difference between eggs laid on valencene and á-humulene treated filter papers (n = 10; $q = 0.8649$; $P = 0.45$). We inferred that the presence of valencene and á-humulene is necessary for the increased rate of oviposition as seen on mulberry leaves. Oviposition assays with electrophysiologically active compounds from mulberry revealed that two of the volatiles, valencene and á-humulene, aided moths in choosing suitable oviposition sites and enhanced egg-laying significantly. Moreover, we show that generalist egg-parasitoids like *Trichogramma chilonis* are strongly repelled by valencene and α-humulene. Our results demonstrate that IRTs tuned to cues that aid crucial functions like oviposition site-selection are less likely to be impaired even after centuries of domestication (Kamala Jayanthi *et al*., 2015c).

Herbivore-Induced Plant Volatiles as aggregation cues for sap feeders

Herbivore-induced plant volatiles (HIPVs) have been opined as 'indirect or direct defenses'of plants, are extensively studied. In contrast, HIPVs may also indicate that plant defenses have been overcome by herbivores infesting the plant; however, studies on this aspect have so far received little attention. Using the interaction of *Capsicum annum* (Bell pepper) with its pest *Scirtothrips dorsalis* (Chilli thrips) as a model system, we studied the role of HIPVs in this selected insect-plant interaction. Multiple-choice olfactometer assays with headspace volatiles collected from different growth stages of un-infested *C. annum* plants represented by pre-flowering (PF), flowering (FL) and fruiting stages (FR) proved FR volatiles to be highly attractive to *S. dorsalis*. Further, FR plants were infested with *S. dorsalis* adults and HIPVs released by infested plants were collected and subjected to multiple-choice olfactometer bioassays. Thrips were significantly attracted to HIPVs than to headspace volatiles of un-infested FR plants or thrips body odour. Coupled GC-EAG with *S. dorsalis* and HIPVs or FR plant volatile revealed specific compounds, *viz*., n-dodecane, n-docosane, o-cymene, â-elemene, n-dodecyl iodide (in FR volatiles); octadecane, n-docosane, tricosane, ä-3-carene, dodecyl iodide (in HIPVs) that elicited an EAG response. Individual EAG-active compounds were less attractive to thrips, however, synthetic blends of EAG-active compounds at the ratio similar to headspace samples were found to be highly attractive. However, when given a choice between synthetic blends of HIPVs and FR, thrips were significantly attracted to synthetic blend of HIPVs. Our study provides empirical data on signals HIPVs may provide to conspecific herbivores and suggests that the role of HIPVs, mostly generalized as defense, may vary based on the interaction and must be studied closely to understand their ecological functions (Subhash *et al*., 2017).

Similarly in case of onion also, an attempt was made to study behaviour of onion thrips*Thrips tabaci* towards HIPVs and healthy plant volatiles. Olfactometer bioassays revealed that *T. tabaci* significantly preferred HIPVs from conspecific infested onion to volatiles from healthy onion plants. Gas chromatography-Mass spectrometry (GC-MS) analysis of HIPVs and volatiles from healthy onion plants revealed substantial changes in their volatile profiles. This study provides empirical data on signals HIPVs may provide herbivores and suggests that the role of HIPVs, mostly generalized as defense, may vary based on the interaction and must be studied closely to understand their ecological roles. It also provides basis for the development of kairomone based management strategies against this devastating pest (Prasanna Kumar *et al*., 2017).

Scope

Future applications of semiochemicals depend on the availability of the potential cues that enableefficient manipulation of mate- and host-finding behaviour in horticultural pests. Detailed studies (crop wise and pest wise) would throw light on the ecological chemistry involved in insect-insect and insect-plant interactions that will help us to understand the trophic interactions in toto. Such studies will aid to pin-point the weak links that can be exploited for IPM. Further, it is now within our reach to facilitate the discovery of relevantchemical cues with emerging molecular/sensitive biochemical-behavioural equipment. Nevertheless, detection and identification of potential semiochemicals for several Indian horticultural crop pests are still rudimentary and particularly needed (Kamala Jayanthi *et al*., 2015a).

Recent advances in analytical chemistry coupled with more definitive behavioral analyses have allowed more rigorous identification of many insect pheromones. Literature on phyto-semiochemicals has already proved that even rather common and structurally simple compounds can act as important chemical signals and exhibit biological activity on many different species and that one compound can have different functions in different species. Therefore, systematic, goal oriented multidisciplinary research work in the proposed area of ecological chemistry will definitely yield powerful cues for integrated pest management of targeted horticultural crop pests enabling better appreciation of the frontier technologies and sensitive analytical instruments, we have today.

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Manuscript recived on 25.12.2018

Manuscript accepted for publication 3.2.2019

Journal of Eco-friendly Agriculture **14**(2): 11-17. 2019

Development of decision support tools for effective pest management

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ABSTRACT

Simulation models have been used for several applications in the area of pest management which helped to increase the efficiency of field research greatly. These have become more relevant in emerging research areas such as climate change impacts on pest dynamics and crop-pest interaction and pest forewarning. The application of geo-spatial techniques holds promise for efficient pest surveillance and risk analysis on wide-area basis. The appropriate pest management decisions require a holistic crop loss assessment and estimation of multi-pest EILs. The natural enemy populations need to be considered in decision making to prevent unwarranted pesticide applications.

Key words: Pest management, simulation models, pest dynamics, multi-pest EILS, decision making.

Pest management is a complex system involving many interrelated components such as crop, pests, natural enemies, beneficial organisms and non-target organisms subjected to man's production oriented interventions under variable weather (Teng and Savary, 1992). Development of an IPM scheme for a sustainable farming system demands thorough analysis of the agro-ecosystem, which may be useful in identifying new control techniques by indicating intervention points (Rabbinge *et al*., 1989; Boote *et al*., 1983; Pinnschmidt *et al*., 1994). Models help to understand and analyze inter-relationships among various components of a system. A system is a limited part of real world having inter-related components. The components of a system interact in such a way that a change in any component has repercussions for the whole system. A model is a simplified representation of a system. Basically models can be qualitative or quantitative. Quantitative models can be further classified as empirical or descriptive and mechanistic or explanatory models. The use of simulation models in pest management commenced with the development of single species population dynamics models in late 60s. Crop growth simulation models were coupled with pest damage mechanisms in mid 80s (Rabbinge, 1983; Boote *et al*., 1983; Rouse, 1988). However, it was described as one–way approach of analyzing crop-pest interactions because these models accounted for pest effects on crop growth without reverse being true. Two-way approach for crop-pest interactions came into being with interlinking of pest population dynamics models with crop growth simulation models in 1990s, which increased our understanding of the complex pest-crop systems.

Pest management involves intensive decision making that requires effective decision support tools for timely action. Pest management research thus is required to derive such practical tools for developing tactics and strategies for pest management. Decision support tools such as economic injury levels (EILs) play an important role in need-based application of pesticides. Systems approach provides such tools in the form of simulation and decision models (Teng and Savary, 1992). Besides, geo-spatial techniques such as remote sensing and geographic information system (GIS) can play an important role in pest surveillance and pest risk analysis.

Simulation of crop losses due to pests

There are mainly two types of decisions in pest management, tactical and strategic decisions. Assessment and extrapolation of yield losses are mandatory at both the strategic and tactical decision levels. These are required at the strategic level for research prioritization and at tactical level for taking action or improving pest management.

(i) Empirical approach: Empirical approach has been used for establishing damage functions between pest damage and crop yield. Empirical models are the regression relationships among various variables, e.g., simple regression between yield of the crop and pest incidence (y = a-bx). These models do not explain the physiological mechanism of yield loss due to pests and are thus location and time specific and cannot be extrapolated without the risk of error. This is a weakness common to all empirical regression models (James and Teng, 1979). Empirical damage functions were established for the rice leaf folder and the EILs based on them were computed to be 4 and 8 per cent leaf damage, respectively, that depicted temporal variability of this approach (Chander and Singh, 2001).

(ii) Mechanistic approach: Assessment of crop losses due to pests with mechanistic approach, i.e., with simulation models is based on the concept of pest damage mechanisms. Damage mechanisms of the pest can be defined as plant physiological process affected by the pest injury. Crop simulation model coupled with pest effects is called crop-pest simulation model. Output of simulation models have been observed to be more reliable and can be extrapolated as it is based on physiological basis of pest damage. Simulation models are also capable of addressing injury to the crop due to multiple pests which is very difficult to achieve in field experiments. These models enhance efficiency of field experiments greatly and have great potential for applications in the field of pest management.

A generic crop growth model, INFOCROP (Aggarwal *et al.,* 2004; Aggrawal *et al.,* 2005a; Aggarwal *et al.,* 2005b) developed at ICAR-Indian Agricultural Research Institute, New Delhi has been coupled with different pest damage mechanisms. These coupled models further have been calibrated and validated through field experiments.

(iii) Pest damage mechanisms: The plant physiological processes affected by pests are called damage mechanisms (Table 1). Different categories of damage mechanisms encompass reduction in germination, plant killing, competition for resources (light, water and nutrients), reduction of assimilation rate, assimilate consumption, tissue consumption and turgor reduction by hampering water and nutrient uptake (Rabbinge *et al.*, 1994). Accordingly, different pests can be broadly categorized as germination reducers, stand removers, light (resource stealers), assimilation rate reducers, assimilate sappers, tissue consumers and turgor reducers, respectively.

The crop capture light for photosynthesis. Defoliating beetles, grasshoppers, leaf folders, leaf miners, node borers, grain feeders and foliar diseases like leaf blights, blast and sheath blight can be grouped in this category. Their effect can be simulated by reducing rates of leaf area, leaf weight, stem area, stem weight or storage organ weight depending upon their nature of damage.

These damage mechanisms categories have to be seen as a broad framework. Defining the category for a pest is difficult because some pests affect the crop in several ways like brown plant hopper in rice acts as assimilate sapper as well as light stealer. Likewise, foliar diseases act as light stealer, assimilation rate reducer as well as tissue consumers. Ear cutting caterpillar functions as tissue consumer as well as assimilation reducer. For quantifying damage mechanisms, all the possible damage mechanisms of a pest are hypothesized and prioritized. Most important damage mechanisms are then coupled to crop growth model at appropriate plant growth processes level. These are then validated through field experiments. After quantification and validation of damage mechanisms, crop-pest simulation models can be used for various applications in pest management.

Geo-spatial techniques

Remote sensing and GIS can play increasingly vital role in pest surveillance, pest risk analysis and predictive pest zoning.

(i) Remote sensing

Remote sensing is the acquisition of knowledge or information about objects from a distance without coming into direct contact with them. Remote sensing in entomology was first used in the field of forestry, wherein slow moving ships were found useful for visually assessing the extent of damage by spruce budworm, *Choristoneura fumiferana* in United States and Canada (Craighead, 1921). In remote sensing, the information about objects is gathered based upon reflected radiation from their surface. Remote sensing of crop canopies involves measurement of electromagnetic radiation reflected or emitted from plant parts. Human eye along with

Table 1. Different pest damage mechanisms coupled to InfoCrop model

Damage mechanism	Plant processes affected	Pests
Germination reducer	Germination	Mole cricket, diseases
Stand reducer	Leaf area, plant organ weights	Stem borer, damping off
Light stealer	Photosynthetic active radiation (PARINT)	Sucking insects, weeds
Assimilation rate reducer	Radiation use efficiency	Powdery mildew, sucking insects
Assimilate sapper	Increase maintenance cost of plants	Sucking insects
Tissue consumer	Leaf area, stem weight, panicle weight	Defoliators, blights
Turgor reducer	Nutrient and water uptake	Nematodes

a highly developed brain forms a natural remote sensing system, which can perceive only reflected radiation of visible band of the electro-magnetic (em) spectrum. Therefore, man tries to imitate already existing natural remote sensing system and improve upon it, using both the visible and Infrared (IR) bands of electo-magnetic spectrum.

Spectral signatures: Spectral signatures of any object comprise a set of values for its reflectance or emittance in different spectral bands of em spectrum. The amount and quality of light reflected from crop canopies are strongly dependent on both the crop species and condition of the crop. Plant pigments, leaf structure and total water content are three important factors affecting spectral reflectance of vegetation. External factors, which influence spectral reflectances of vegetation are: moisture stress, soil nutrients/ salinity, pests, seasonal variation and climatic factors. The spectral reflectance of healthy vegetation/crop is characterized by:

i) High absorption, *i.e.,* low reflectance in blue and red regions of em spectrum; ii) high reflectance in near IR due to internal cell structure and iii) water absorption bands, *i.e.,* low reflectance in the mid IR. Any deviation in reflectance from the above pattern indicates some sort of stress on the crop. High value of reflectance in visible blue and red regions, low reflectance in near IR and high reflectance in mid IR would show that there is some stress on the crop.

Ground truth: Stress on vegetation or damage symptoms may be caused by any of the biotic and abiotic factors, *viz.,* moisture, nutrients, pests and diseases. Simply by observing the reflectance the cause of stress cannot be known. The cause of the stress has to be ascertained or confirmed from the field. This is called the ground truth. The damage symptoms due to various factors need to be thoroughly differentiated and standardized for future comparisons.

Sensors for measuring spectral signatures: In field studies, spectroradiometer, infrared thermometer, scatterometer and photographic cameras are used, while in air and space, multispectral scanner, imaging spectometer, microwave radiometer and synthetic aperture RADAR are utilized.

(ii) Geographic information systems: It can be used for pest risk analysis and predictive pest zoning. Pest population dynamic model can be run with requisite weather data and probability of pest outbreak for a site can be determined. Site predictions can then be extrapolated through GIS to carve out the zones of equal epidemic potential for a pest. Yadav *et al*. (2010) undertook agro-ecological zoning of rice BPH, *Nilaparvata lugens,* where in Andhra Pradesh was divided into zones of differential pest epidemic potential. Knowledge of pest epidemic potential in different zones allows strategic decisions with respect to selection of crop cultivars and appropriate management options.

Climate change impact on pests and pest management tactics

Climate change is likely to impact insect pest populations as well as pest management tactics.

(i) Impact on insects: The global climate change may affect the crop yields, incidence of pests and economic costs of agricultural production. Climate change is expected to have significant impacts on pest distribution and abundance. The rising CO_2 concentration may not only have a variety of direct effects on plants, but may also have indirect effects on herbivores and their natural enemies (Stiling *et al.,* 2002). The climate has profound effects on populations of invertebrate pests like insects, mites and others affecting their development, reproduction and dispersal (Sutherst, 1991).

Climate change will have both direct and indirect effects on insect population. Indirect effects will be through their host plants. Direct impacts of climate change on insects may include shifts in species distribution with shift in geographic ranges to higher latitudes and elevations; changes in phenology with life cycles beginning earlier in spring and continuing later in autumn; increase in population growth rates and number of generations; change in migratory behavior; alteration in crop-pest synchrony and natural enemy-pest interaction and changes in interspecific interactions (Sutherst, 1991; Root *et al*., 2003). Plant-herbivore interactions are of particular significance because of their agricultural importance as well as their potential to affect ecosystem nutrient and carbon fluxes (Frost and Hunter, 2004). Extreme weather events such as intense rainstorms, wind or high temperatures also affect survival of insect populations. For species, to survive changing climates, they must either adapt *in situ* to new conditions or shift their distributions in pursuit of more favourable ones. Many insects have large population sizes and short generation times, and their phenology, fecundity, survival, selection and habitat use can respond rapidly to climate change. These changes to insect life history may in turn produce rapid changes in abundance and distribution. Changes in rainfall pattern also have implications for insect survival. More intense rainfall as projected under climate change may thus reduce incidence of small pests on crops. Aphid population on barley was found to have negative relation with January mean minimum temperature and February total rainfall (Chander, 1998; Chander *et al*., 2003).

(ii) Assessment of impact of climate change on insects

Impact of global climate change on crop productivity and pest population can be assessed through experiments as well as through crop growth and insect population models.

(a) Experimental approach

Direct impact of temperature: Probable impact of temperature rise on insect populations can be known by comparing current and projected temperature conditions at a location with a species' favourable temperature range. Data on temperature dependent development period and survival can be used to determine favorable temperature range and computing thermal constant and development thresholds for the species. Based on development at a range of constant temperatures, thermal constants and development thresholds were computed for various development stages of BPH and pink borer (Sujithra and Chander, 2013; Selvaraj and Chander, 2015).

Indirect impact of CO_2: Impact of CO_2 on insect population *via* host plants can be studied through open top chambers (OTCs) and free air carbon dioxide enrichment (FACE) facilities. Effect of higher carbon dioxide on insect multiplication and damage can be studied by releasing a known number of insects on plants in these structures. The OTCs are essentially plastic enclosures, consisting simply of an aluminum frame covered by panels of polyvinyl chloride plastic film. Prasannakumar *et al*. (2012) studied effect of elevated CO_2 on BPH population in OTCs and observed its positive effect on the pest multiplication that resulted in more than a doubling of its population. Ainsworth and Long (2005) reviewed and summarized the research in various FACE facilities world over.

(b) Modeling approach: Simulation models can be used to simulate climate change impact on pest dynamics as well as crop-pest interactions. This aspect has been elaborated further under simulation of climate change impact.

(iii) Climate change impact on pest management tactics

Host-plant resistance, biological control, cultural control and chemical control are the major pillars of IPM. These components are likely to be affected by climatic change and thus would need appropriate modifications for sustaining their effectiveness. Breakdown of temperature-sensitive resistance under increased temperature regimes may lead to more rapid evolution of pest biotypes (Sharma *et al*., 1999). This calls for exploration of new sources of plant resistance against insects for their efficient management. It is also important to understand the effect of climate change on the efficacy of transgenic plants, which are also important resource in pest management. Environmental factors such as soil moisture, soil fertility and temperature have strong influence on the expression of *Bacillus thuringiensis* (*Bt*) toxin proteins deployed in transgenic plants (Sachs *et al*., 1998). Cultural practices are expected to play greater role in pest management in changing climate. Global climate change would cause alteration in sowing dates of crops, which may alter host-pest synchrony. There is thus a need to explore changes in pest-host plant interaction due to sowing date in other crops. Climate change can have diverse effects on natural enemies of pests. Natural enemy and host insect populations may respond differently to climate change. There is a need to breed temperature-tolerant natural enemies of pests. Fungi such as *Metarhizium anisopliae, Beauveria bassiana*, *Baculovirus,* nuclear polyhedrosis virus (NPV), cytoplasmic virus and bacteria like *Bt* have great potential for development as microbial control agents.

Climate change could affect efficacy of crop protection chemicals through changes in temperature and rainfall pattern, and through morphological and physiological changes in crop plants (Coakley *et al*., 1999). An increase in probability of intense rainfall could result in increased pesticide wash-off and reduced pest control. In contrast, increased metabolic rate at higher temperature could result in faster uptake by plants and higher toxicity to pests. Likewise, increased thickness of epicuticular wax layer under high CO_2 could result in slower or reduced uptake by host plant, while increased canopy size may hinder proper spray coverage and lead to a dilution of the active ingredient in the host tissue. Among different spray volumes, *viz*., 400-700 l ha^{-1} evaluated for efficacy of Imidacloprid (0.006%) against BPH under elevated CO_2 (570±25ppm), Imidacloprid was found to be most effective @ 700 l ha^{-1}. Higher spray volume may thus be required for effective pest suppression under climate change (unpublished data) and pesticide application thus have to be modified according to new situations.

Development of decision support tools for pest management

Crop-pest models are being used for various applications like rationalizing pesticide use, pest risk analysis, predictive pest zonation and assessing impact of climate change and for pest forewarning.

(i) Pest surveillance

Surveillance is the backbone of the IPM. Geo-spatial techniques can be utilized for pest monitoring in large areas. Besides, natural enemy based monitoring plans will help to

reduce dependence on pesticides. Sequential sampling plans formulated for rice planthoppers with incorporation of predator effect suggested need for management measures at higher planthopper population, thereby avoiding unwarranted pesticide application and ensuring natural enemy conservation and favourable benefit- cost to farmers (Rajna and Chander, 2013). Hyperspectral remote sensing used to detect BPH stress on potted rice plants revealed that the pest damage influenced reflectance of rice plants compared to uninfested plants in the visible and near-infrared regions of the electromagnetic spectrum. Based on plant reflectance corresponding to the sensitive wavelengths, a multiple-linear regression model was developed and validated, which would facilitate assessment of BPH damage based on rice plant reflectance, thereby ensuring prompt forewarning to stakeholders (Prasannakumar *et al*., 2013).

(ii) Determination of location-specific economic injury levels (EILs): Traditionally, the EILs for pests are based on empirical yield-infestation relationships, which are site specific and have little scope for extrapolation. It would however be very expensive and time consuming to use field experiments to establish such yield-infestation relationships for different pest species, crops and locations. Crop-pest simulation models can be used to establish location and weather-specific EILs thereby helping to increase the efficiency of field experiments substantially. Coupled models have been used for simulating EILs for bean leaf beetle infesting soybean (Nordh *et al*., 1988), Russian wheat aphid damage in winter wheat (Chander *et al*., 2005) and leaf folder damage in rice (Satish *et al*., 2007).

(iii) Pest risk analysis: Simulation models can also be utilized for analyzing the risk associated with the introduction of exotic pests into new ecosystem. The foreign pests under quarantine cannot be brought deliberately into new countries to test their damage potential. Therefore, models are very useful to evaluate situations where actual fieldwork on the pest is not possible (Teng, 1991). Yang *et al*. (1991) showed the possibility of the approach with a soybean rust model, SOYRUST, which when run with continental USA weather data predicted potential areas for epidemics. When model disease estimates were further linked to soybean crop model, the potential losses attributable to rust epidemics were determined.

(iv) Predictive pest zoning: Pest zoning is a concept that is particularly applicable for large area pest management. In this approach pest dynamic model is run with requisite weather data and probability of various pest infestation levels for a site is determined (Yuen and Teng, 1990). Site predictions are then extrapolated through GIS to carve out the zones of equal outbreak potential for a pest. Predictive pest zoning has been carried out for rice BPH, leaf folder and stem borer, wherein the states could be divided into zones of different epidemic potential for the pests (Chander *et al*., 2004; Yadav and Chander, 2010; Reji *et al*., 2003). Knowledge of pest epidemic potential in different zones would allow strategic decisions with respect to selection of crop cultivars and appropriate management options.

(v) Pest forewarning: Conventionally, pest forewarning is done following empirical approach. The empirical pest-weather models have been developed for BPH (Prasannakumar and Chander, 2014) and leaf folder (Singh *et al*., 2015). However, such models are location-specific and cannot be extrapolated and also do not account for the processes behind the pest population change. Insect population simulation models, based on various bio-ecological processes *viz*., fecundity, sex ratio, migration, abiotic and biotic mortality factors, development thresholds and thermal constants can also be used for pest forewarning (Reji and Chander, 2008; Sujithra and Chander, 2013; Selvaraj and Chander, 2015) . These models are mechanistic model and thus explain the causes underlying population change. These can be easily adapted for different agro-ecological situations thereby addressing location-specificity. Such models thus prove better than the empirical models that need to be developed for each situation. These models improved the understanding of the pest as an element of crop ecosystem and further detect the crucial knowledge gaps in view of a holistic assessment of pest status (Graf *et al*., 1992).

(vi) Simulation of climate change impact: Pest population simulation model can be coupled to crop growth model at relevant plant processes depending on pest damage mechanisms. Crop-pest model can then be used to analyze impact of climate change on insect dynamics as well as crop-pest interactions (Kaukoranta, 1996). A thermal constant-based mechanistic hemimetabolous-population model was adapted for BPH and linked with InfoCrop crop simulation model and used to simulate climate change impact on BPH population and crop yield under New Delhi and Aduthurai conditions (Sujithra and Chander, 2013). Likewise, a mechanistic holometabolous population simulation model for *S. inferens* was developed and coupled to InfoCrop-rice model and used to simulate climatic change impact on *S. inferens* population and rice crop in accordance with four 'standard special report on emissions scenarios' (Selvaraj and Chander, 2015).

The coupled crop-pest model can be easily adapted to diverse agro-environments and applied to simulate the pest

dynamics and crop losses under location-specific situations. The population dynamics models linked to crop growth model could predict pest population peaks and thereby spray schedules based on economic thresholds can be developed (Benigno *et al*., 1988).

Simulation models have been used for several applications in the area of pest management which helped to increase the efficiency of field research greatly. These will be of even greater relevance in emerging research areas, such as climate change impacts on pest dynamics and crop-pest interactions and pest forewarning. In view of fast changing pest scenario and invasive species, the application of geo-spatial techniques holds promise for efficient pest surveillance and risk analysis on 'Wide-Area' basis. The situation also demands a holistic crop loss assessment and estimation of multi-pest EILs for appropriate pest management decisions. The natural enemy populations need to be considered in decision making to prevent unwarranted pesticide applications, thereby ensuring better income to growers and avoiding adverse environmental effects. Besides, climate change will also impinge upon effectiveness of various pest management components. It thus becomes very important to assess climate change impact on insects and pest management components and adopt appropriate mitigation and adaptation measures to sustain agricultural productivity.

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Manuscript recived on 16.12.2018
Manuscript accepted for publication 7.2.2019

Journal of Eco-friendly Agriculture **14**(2): 18-22. 2019

Isolation and characterization of PGPRs from organic preparations

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ABSTRACT

Rhizobacteria have been described as plant growth promoting as they offer their plant hosts an additional fortification against pathogen by expression of multiple activities that directly and indirectly inhibit pathogens. Most microbial diversity of the soil ecosystem is confined to the rhizosphere. These rhizospheric microbes have the potential to stimulate plant growth and manage soil and plant health. Nowadays, management of the rhizosphere bacterial population has advanced toward the concept of plant growth promoting rhizobacteria (PGPR) owing to emergence of fungicide-resistant pathogens and health concerns for the producers and consumers. Using PGPR as bio-fertilizer is an efficient approach to replace chemical fertilizers and pesticides for sustainable cultivation. In the present study bacteria isolated from *Amritpani* and *Panchagavya* were analysed for their biochemical characters and molecular confirmation using molecular tool. The substrate utilization pattern showed that the characteristics of the majority of the tested strains share a similar pattern. However, few isolates displayed varied patterns. Identification of these isolates showed that most of them belong to genus *Bacillus* and *Alcaligenes* spp. This was further confirmed with sequencing of the 16s rDNA gene of the bacteria. The present study has resulted in the isolation of PGPRs *Bacillus pumilus* (CISH-PGPR96), *Bacillus subtilis* (CISH-PGPR102) and *Alcaligenes faecalis* (CISH-PGPR104) isolates that can be utilised for sustainable agriculture production.

Key words: Microbial population, *Panchagavya, Amritpani, Bacillus pumilus, Alcaligenes faecalis*

Use of organic liquid preparations is an age old practice in India (Ram *et al*., 2019). Preparation of *Kunapajala* which involves boiling of flesh, fat and marrow of animals such as deer, pig, fish, sheep, goat in water, placing it in earthen pot, and adding milk, powders of sesame oil cake, black gram boiled in honey, decoction of pulses, ghee and hot water used to be the common booster of plant vigour (Nene, 2007). These liquid preparations are organic preparations, obtained by active fermentation of animal and plant residues over specific duration, e.g., *Panchagavya, Amritpani, Jeevamrit,* etc. These are rich source of microbial consortia, macro- and micro-nutrients and plant growth promoting substances. They are used for seeds/seedlings treatment and fast decomposition of organic wastes in composting. *Panchagavya* is the most effective bio-enhancer demonstrated by many researchers (Sangeetha and Thevanatham, 2010). In India, availability of quality organic inputs, *viz*., organic manures, bio-enhancers, bio-pesticides, etc., for organic farming is challenging. That's why organic inputs must be produced at the farm itself. Analysis of organic inputs for their quality is very essential aspect for organic production.

Biodiversity is an important ingredient of environmental conservation and is central to agriculture production. Most microbial diversity of the soil ecosystem is confined to the rhizosphere. These rhizospheric microbes have the potential to stimulate plant growth and manage soil and plant health (Sturz, 1986; Israr *et al*., 2012). Plant growth-promoting bacteria (PGPB) are associated with many plant species and are commonly present in many environments. The most widely studied group of PGPB are plant growth-promoting rhizobacteria (PGPR) colonizing the root surfaces and the closely adhering soil interface (Israr *et al*., 2014). Rhizobacteria have been investigated as possible replacements for chemical fertilizers due to the severe deterioration of the chemical, physical and biological health of the cultivated land (Swaminathan and Jana, 1997). Nowadays, management of the rhizosphere bacterial population has advanced toward the concept of plant growth promoting rhizobacteria (PGPR) owing to emergence of fungicide-resistant pathogens and health concerns for the producers and consumers. Using PGPR as biofertilizer is an efficient approach to replace chemical fertilizers and pesticides for sustainable cultivation (Ross, 2000). Considering the multiple applications of rhizobacteria, it is essential to study their diversity, which could be useful in designing strategies to use these native strains as bioinoculants for sustainable and organic agriculture without causing harm to the environment and farmers. With the currently available tools, the microbial community structure can be examined at several levels. The simplest analysis is based on DNA profiles, generated by PCR followed by restriction digestion of PCR product, to identify differences

in the community composition. Furthermore, sequencing (16s rRNA gene) provides greater discrimination over functional diversity studies and better characterization of an isolate. The objective of the present study was to isolate and characterise PGPRs and their community structure associated with *Amritpani* and *Panchagavya* by *in vitro* assays and gene amplification techniques.

MATERIALS AND METHODS

Preparation of *Panchagavya*

Panchagavya was prepared by mixing five products of cow, *i.e.*, dung (5 kg), urine (3 l), milk (2 l), curd (2 l) and ghee (1 l). To this, sugar cane juice (3 l), jaggery (0.5 kg), tender coconut water (3 l), toddy (2 l) and ripe bananas (12 nos.) were mixed in a wide mouthed mud container. Preparation was ready for use in 18 days (Ram *et al*., 2017).

Preparation of *Amritpani*

Amritpani was prepared by incubating 10 kg cow dung along with 250 g cow ghee, 500g honey and 150 litres of water in a plastic container. Preparation was ready for use in 7-10 days (Ram *et al*., 2017).

Enumeration of microbial population

Enumeration of different beneficial microbial populations, *viz.,* bacteria, fungi, actinomycetes, *Pseudomonas*, gram positive bacteria, gram negative bacteria, p-solubilizing bacteria, Rhizobium, *Azotobacter and Azospirillum* were carried out by using dilution plate count method using selective media, *viz*., Nutrient agar, Rose Bengal Chloramphenicol Agar (RBCA), actinomycetes isolation agar, King's B (King *et al.*, 1954), methyl red agar (Hagedorn and Holt, 1975), crystal violate agar (Goud *et al*., 1985), Pikovskaya's agar (Pikovskaya, 1948), Yeast extract mannitol agar with congo red (CRYEMA, Fred *et al*., 1932), modified Jensen's agar (Jensen, 1954; Norris and Chapman, 1968) and N-free malate medium (Okon *et al.*, 1977), respectively.

Petri dishes were made by pouring each specific solid medium. Then 10 ml of each bio-enhancers sample were diluted to 90 ml sterile water and that was considered being 10^{-1} dilution factor. Transferring of 1 ml of 10^{-1} dilution to 9 ml sterilized water with the help of a sterilized pipette yielded 10^{-2} dilution. In this way, a series of up to 10^{-8} dilutions were prepared under aseptic conditions. Point one ml (0.1 ml) of the suspension from required dilution (e.g., 10^{-8}) was taken and poured into the respective agar media on petri dish and spread with L-spreader with the help of Plate Master (Hi Media). Then plates were incubated at 28 ± 2°C for 3-5 days. The numbers of visible colonies were counted. The total count was obtained by multiplying number of visible colonies on the plate by the dilution factor.

Morphological and biochemical characterization

All the isolates were morphologically and phenotypically characterized on the basis of Gram's staining, motility test and differential utilization of 10 different substrates (all from Hi Media). The reactions tested were indole formation, phosphate solubilization, protease production, citrate and oxidase, acid from glucose, sucrose, lactose, starch hydrolysis and H_2S production. The results were then interpreted after addition of the respective reagents where necessary with the help of the colour change.

DNA extraction and nucleotide sequence accession numbers

All cultures were maintained on Nutrient Agar (NA, Hi Media) at 25^{0}C. For DNA extraction pure culture of 16 isolates were grown in liquid culture of Nutrient broth (Hi Media) at 25^{o}C for 24 to 48 hours. Pellets were collected by centrifugation at 10,000 rpm, 4^{0}C. Total genomic DNA was extracted by using the method described earlier. Pure DNA was dissolved in 60µl 1x TAE buffer. Purity of DNA was quantified by UV spectrophotometry and ethidium bromide fluorescence. Primer PA ($^{5'}$AGAGTTTGATCCTGGCTCAG$^{3'}$) and PH ($^{5'}$AAGGAGGTGATCCAGCCGCA$^{3'}$) were used to amplify a fragment of 16s rDNA. PCR amplification were performed in a total volume of 100µl by mixing 100 ng of genomic DNA, 0.2 µm conc. of each primer, 2.5 mM dNTPs and 1 unit of Taq DNA polymerase in 1x PCR buffer. The reaction were subjected to initial denaturation of 94^{0}C for 5.0 min. followed by 35 cycle of 94^{0}C for 40 sec., 48^0 C for 30 sec, 72^0 C for 1.0 min. with a final extension of 72^0 C for 5 min. in a applied biosystem thermal cycler. PCR amplified products were analysed by running in 1 per cent Agarose gel prepared in 1xTAE buffer and containing 0.5 µg ethidium bromide and photographed over a transilluminator. 1.5 kb PCR products (Fig. 1) were sent to commercial gene sequencing laboratory of Xcelrise, India for sequencing. Sequence analysis of these isolates was performed using BLAST (blastn) 2.0 search tool (http://www.ncbi.nlm.nih.gov) available on the NCBI homepage. The nucleotide sequences of 16s rRNA were deposited in Gene Bank. The accession numbers of the 16S rRNA nucleotide sequences of some of the isolates are presented in Table 3.

RESULTS AND DISCUSSION

Microbial population dynamics in *Panchagavya*

Bacterial population was gradually increased during the fermentation period and highest number was recorded on 18th day (62.50 x 10^7cfu ml^{-1}) (Table 1). Population of fungi

was initially increased up to 6th day (4.0 x 10^5 cfu ml^{-1}), but then gradually decreased at maturity stage. Interestingly, actinomycetes population was increased from 0 day (0.19 x 10^6 cfu ml^{-1}) to 20th day (8.0 x 10^6 cfu ml^{-1}). Gram positive bacterial population was decreased towards maturity stage of *Panchagavya*. Gram negative bacteria increased over time and reached at a very high level (35.8 x 10^6 cfu ml^{-1}) on 20th day. Similar phenomenon was also observed with *Pseudomonas*. Population of *Rhizobium* was almost stable at the initial period but, slightly increased and highest was recorded on 20th day (4.14 x 10^6 cfu ml^{-1}). The p-solubilizing microbes were also increased gradually from initial number of 0.29 x 10^6 (0 day) to 2.42 x 10^6 (20th day). Cow dung is an active ingredient of *Panchagavya* and it is a rich source of beneficial microbes as reported earlier (Girija *et al*., 2013). Cow milk also contains beneficial microbes (Crielly *et al*., 1994). Cow curd is a rich source of *Lactobacillus* spp. The highest number of bacteria in *Panchagavya* might be due to nutrient richness of the mixture obtained from ingredients of cow origin, *viz*., cow milk, curd and milk, etc. Amalraj *et al*. (2013) have reported highest population of total bacteria (22 x 10^9 cfu ml^{-1}), actinomycetes (60 x 10^4 cfu ml^{-1}), p-solubilizers (103 x 10^6 cfu ml^{-1}) and fluorescent pseudomonas (151 x 10^5 cfu ml^{-1}). Chadha *et al*. (2012) have also reported highest load of viable bacterial populations, *Azotobacter* spp., actinomycetes as well as p-solubilizers in *Panchagavya*. Microbial and biochemical analysis of *Panchagavya* were also worked out by Radha and Rao (2014) and Ram *et al*. (2018).

Microbial population dynamics in *Amritpani*

Bacterial population was increased in the preparation from an initial value of 2.40 x 10^8 cfu ml^{-1} at 0 day to 5.49 x10^8 cfu ml^{-1} at 9th day but then gradually decreased (Table 2). Fungi population was decreased gradually from 0.12 x 10^6 cfu ml^{-1} at 0 day to 0.046 x 10^6 cfu ml^{-1} at 9th day. Similarly, actinomycetes population was decreased up to 6th day but increased on 9th day then again decreased. Population of gram positive bacteria decreased with fermentation, while gram negative bacteria increased at 9th day. Interestingly, the *Pseudomonas* population was gradually increased from an initial value of 0.71 x 10^7 cfu ml^{-1} at 0 day to 4.80 x 10^7 cfu ml^{-1} at 14th day.

The search for PGPRs and their mode of action are increasing at a rapid rate in order to use the best PGPR strain as commercial biofertilizer. Investigations into the mechanisms of plant growth promotion by PGPR strains indicated that the effective PGPRs increased plant growth basically by changing the whole microbial community structure in rhizosphere. The general mechanisms of plant growth promotion by PGPR includes associative nitrogen fixation, lowering of ethylene levels, production of siderophore and phytohormones, induction of pathogen resistance, solubilization of nutrients, promotion of mycorrhizal functioning, decreasing pollutant toxicity, etc. (Castro *et al*., 2009; Van Loon, 2007; Israr *et al*., 2014). PGPR can alter root architecture and promote plant development with the production of different phytohormones. PGPR seemed as successful rhizobacteria in getting established in soil ecosystem due to their high adaptability in a wide variety of environments, faster growth rate and biochemical versatility to metabolise a wide range of natural and xenobiotic compounds. PGPR can promote growth and yield of crop plants by direct and indirect mechanisms. In some PGPR species, plant growth promotion dominates with nitrogen fixation, phosphate solubilization and production of phytohormones like auxin and cytokinin and volatile growth stimulants. Potential role of PGPRs in conferring resistance to water stress in different crops has been investigated (Mayak *et al*., 2004; Israr *et al*., 2014). Fluorescent *Pseudomonas* and *Bacillus* species were reported with very high efficiency in host root colonization and production of growth metabolites resulting in improved strategic crop yield (Khalid *et al*., 2004). The various modes of action of *Bacillus subtilis* against phytopathogens, suggested the role of the bacterium in plant vitality.

In the present study, bacteria isolated from *Amritpani* and *Panchagavya* were analysed for their biochemical characters and molecular confirmation using molecular tool. The substrate utilization pattern showed that the characteristics of the majority of the tested strains share a similar pattern. However, few isolates displayed varied patterns. Identification of these isolates showed that most of them belong to genus *Bacillus* and *Alcaligenes* spp. Sequence analysis of these isolates were performed using BLAST (blastn) 2.0 search tool (http://www.ncbi.nlm.nih.gov) available on the NCBI homepage. The nucleotide sequences of 16s rRNA were deposited in Gene Bank (Table 3).

Phosphorus is one of the most essential nutrient requirements in plants. Ironically, soils may have large reservoir of total phosphorous (P) but the amounts available to plants are usually a tiny proportion of this total. Several phosphate solubilizing microorganisms (PSMs) are now recorded to convert the insoluble form of phosphorus to soluble form through acidification, secretion of organic acids or protons (Richardson *et al*., 2009) and chelation and exchange reactions (Hameeda *et al*., 2008). The ability of PGPRs to solubilize mineral phosphate, therefore, has been of immense interest to agricultural microbiologists since it can enhance the availability of phosphorus for effective plant

growth. PGPRs have been recorded to solubilize precipitated phosphates to plants, representing a possible mechanism of plant growth promotion under field conditions (Verma *et al*., 2001).

The present study has resulted in the isolation of PGPRs *Bacillus subtilis* CISH-PGPG102, *Alcaligenes faecalis* CISH-PGPR104 and *Bacillus pumilus* CISH-PGPR96 that can be utilised in microbial consortia preparation and bio-formulation of organic preparations.

Table 1: Different microbial population in *Panchagavya*

Type of microbe	Multiplication factor	Microbial population (cfu ml^{-1}) after days of preparation (Mean ± sd)	CD (at 5%)
Bacteria	10^8	5.49 ± 0.21	0.86
Fungi	10^6	0.046 ± 0.001	0.08
Actinomycetes	10^7	1.31 ± 0.19	0.40
Gram positive bacteria	10^8	0.30 ± 0.12	0.53
Gram negative bacteria	10^8	1.35 ± 0.50	0.55
Pseudomonas	10^7	1.53 ± 1.45	1.07
Rhizobium	10^6	3.03 ± 0.01	0.36
P-solubilizing microbes	10^6	4.80 ± 0.001	0.20

Table 2: Different microbial population in *Amritpani*

Type of microbe	Multiplic-ation factor	Microbial population (cfu ml^{-1}) after days of preparation (Mean ± sd)	CD (at 5%)
Bacteria	10^8	5.49 ± 0.21	0.86
Fungi	10^6	0.046 ± 0.001	0.08
Actinomycetes	10^7	1.31 ± 0.19	0.40
Gram positive bacteria	10^8	0.30 ± 0.12	0.53
Gram negative bacteria	10^8	1.35 ± 0.50	0.55
Pseudomonas	10^7	1.53 ± 1.45	1.07
Rhizobium	10^6	3.03 ± 0.01	0.36
P-solubilizing microbes	10^6	4.80 ± 0.001	0.20

Table 3: Identification of bacteria based on maximum similarity with 16sRNA sequences

Organism Name	Isolate code	NCBI Acc. No.
Bacillus subtilis	CISH-PGPR 102	KY427114
Alcaligenes faecalis	CISH-PGPR 104	KY427117
Bacillus pumilus	CISH-PGPR 96	KY523254

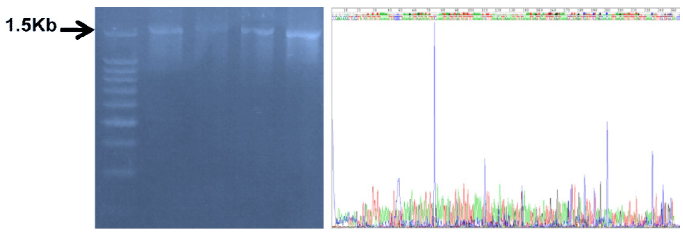

Figure 1: Amplification of 1.5kb PCR product of 16sRNA gene in all the isolates and its sequencing

The study concluded that isolation of PGPRs *Bacillus pumilus* (CISH-PGPR96), *Bacillus subtilis* (CISH-PGPR102), *Alcaligenes faecalis* (CISH-PGPR104) from *Panchagavya* and *Amritpani* can be utilised for soil health management and sustainable production in various crop production.

ACKNOWLEDGEMENTS

The present study was under taken under the Network project on organic horticulture, funded by ICAR. Authors are thankful to the Director, ICAR-Central Institute for Subtropical Horticulture, Lucknow for providing necessary facilities to complete the study.

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Manuscript recived on 16.1.2018
Manuscript accepted for publication 26.3.2019

Journal of Eco-friendly Agriculture **14**(2): 23-27. 2019

Synchronizing nitrogen and potassium supply with crop demand to enhance nutrient use efficiency and water use efficiency on Bt cotton hybrid (NSPL-999)

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ABSTRACT

A field experiment was conducted to assess N and NK split application to synchronize nutrient application with crop demand in Bt cotton hybrid (NSPL-99). Eight timings of application and different dose of N and NK were taken as different treatments. Timings were planned to supply the fertilizer at different crop growth stages, i.e., at 10, 30, 45, 60 and 75 DAS. Split application of N and NK did not have any significant effect on plant height (cm), monopodia per plant, sympodia per plant, boll per plant, boll weight, and G.O.T. (Ginning percentage). Bolls per plant was affected significantly with the different mode of fertilizers application as well as timing of split application. The seed cotton yield did not deviate significantly due to split-cum-basal fertilizer application; however, timings of split application of fertilizers resulted in significant changes in the productivity parameter. The extra net income from F_2 was only Rs. 2884 ha^{-1} over F_1. Amongst the timing of split application, S_4 and S_5 having three splits up to 60 DAS proved significantly superior to some of the remaining treatments. Seed cotton yield ranged from 21.15 to 21.19 q ha^{-1} with extra net income from Rs. 6109 to Rs. 6205 ha^{-1} over S_1. Other treatments except S_7, showed monetary loss from Rs. 900 to Rs. 2250 ha^{-1}. The treatment interactions were found to be significant GMR, and NMR the characters under study.

Key words: Bt cotton, nitrogen, potassium, split application, water use efficiency and nutrient uptake

Cotton as a prime cash crop plays vital role in Indian economy. In India, it is cultivated in about 9 million hectares area, the largest of any nations in the world, which is about 25 per cent of the total acreage in the world and 5 per cent of India's cultivated area. Nonetheless, productivity levels are very low with 502 kg ha^{-1} as against world acreage of 731 kg ha^{-1}. In northern cotton growing states, viz., Punjab, Haryana and Rajasthan, six Bt cotton hybrids were approved for commercial cultivation. Bt cotton hybrids, two each have been developed by Mahyco (MRC 6301 Bt and MRC 6304 Bt), Rasi seeds (RCH 134 Bt and RCH 317 Bt) and Ankur seeds (Ankur 651 Bt and Ankur 2534 Bt) (Singh and Kaushik, 2007). Split application of both nitrogen and potassium is recommended in many states. Nitrogen being the most essential nutrient for plant growth needs to be supplied in proper time and quantities. A positive correlation between vegetative growth and the number of fruiting points produced by cotton is well known. N supplement, therefore, by split application becomes important as it is supplied ideally in a time when crop critically requires. Bt cotton differs in its requirement either by total of it in the different stages of crop. Split applications of nitrogen fertilizer can play an important role in a nutrient management strategy that is productive, profitable and environmentally responsible.

Application of nitrogen in two or more than two split doses can help growers enhance nutrient efficiency, promote optimum yield and mitigate the loss of nutrient. Potassium (K) is the third major essential plant nutrient along with N and P. It plays a specific role in most plant species in opening and closing of stomata which cannot be done by other cation (Saxena, 1985). It increases root growth and improves drought resistance, activates many enzymes systems, reduce water loss and wilting, prevent energy losses and aids in photosynthesis, respiration and food formation (Tiwari, 2001). As the requirement of plants to potassium differ from stage to stage (Brady, 1996) and there might be better response of plants to potassium, if potassium is applied in splits at different stages. Hence, the present study was carried out using Bt cotton as test crop to assess the nutrient uptake pattern and fertility balance.

MATERIALS AND METHODS

The field experiment was conducted in medium black clay – loam soil at the JNKVV, Regional Agricultural Research Station, Khandwa (M.P.) during 2010-11 and 2011-12. The soil was slightly alkaline with low in organic carbon (0.30-0.20%), available N (191-198 kg ha^{-1}), medium in P_2O_5 (13.0-19.2 kg ha^{-1}) and K_2O. (282-288 kg ha^{-1}) The treatments consist of 02 : Mode of fertilizers application- (F_1-N_{120} as split & P_{60}, K_{40} as basal and F_2-N_{120}, K_{40} as split & P_{60} as basal)

and 08 : Timing of split application- (S_1-2 splits (10 & 45 DAS), S_2- 3 splits (10, 45 & 75 DAS), S_3- 3 splits (10, 30 & 45 DAS), S_4- 3 splits (10, 30 & 60 DAS), S_5- 3 splits (10, 45 & 60 DAS), S_6-3 splits (10, 30 & 75 DAS), S_7-4 splits (10, 30 45 & 60 DAS) and S_8-4 splits (10, 30, 45 & 75 DAS).

The experiment was laid out in split – plot design with three replications. The hybrid cotton, Bt NSPL-999 was sown on 26.05.2010 and 28.05.2011 keeping 90 x 90 cm planting geometry. The fertilizers were applied @ 120 kg N, 60 kg P_2O_5 and 40 Kg K_2O ha^{-1} through Urea, SSP and MOP, respectively, as per the treatments. In NK split dose plots, only P was applied as recommended dose, while N and K were applied at the same rates in splits as per treatments. The crop was grown as per recommended package of practices. The crop was harvested in three pickings ending by 25 and 28 February in 2011 and 2012, respectively. Samples were analyzed for N by colorimetric method (Snell and Snell, 1939), P content was determined by Vanadomolybdo phosphoric acid yellow method (Jackson, 1973) and K by flame chloral meter photometer. The soil fertility balance was estimated by using standard formula.

RESULTS AND DISCUSSION

Seed cotton yield (SCY) kg^{-1} fertilizer applied

The data on FUE with respect to SCY kg^{-1} N, P and K applied are highlighted in Tables 1 and 2. In this case, the applied mode of fertilizer application (F_1 and F_2) indicates that there was no significant impact due to these treatments with respect to quantitative fertilizer use efficiency. Nitrogen and K in splits might have supplied adequate quantity of nutrients to coincide with peak demand for nutrients to cotton. Besides, the external application of K might have increased the quantity of readily available K to cotton thus resulting in higher K uptake (Srinivasan, 2003). The range in seed cotton yield (SCY) was 13.57 to 14.86 kg kg^{-1} N applied, 25.35 to 27.86 kg kg^{-1} P applied and 36.10 to 39.74 kg kg^{-1} K applied under F_1 and F_2 having $N_{120}K_{40}$ as split performed little better than F_1 having only N_{120} as split. Thus, the split application of K_{40} proved better than K_{40} applied as basal application. This is related to the increased availability of potassium to the growing plants. In fact, potassium is universally required by all the living organism. Munson (1985) reported that absence of potassium application in fields often decreases efficiency of fertilizers. The deficiency of potassium directly or indirectly affects photosynthetic activity (Huber, 1985), disturbs carbohydrate metabolism (Beringer *et al.*, 1986), inhibits chlorophyll development, induces accumulation of certain elements and increases soluble nitrogenous compound contents (Kock and Mengel, 1977). Potassium is known to play a vital role in osmoregulation (Hsiao and Lauchli, 1986) and it is the most efficient cation for activation of several enzymes (Suelter, 1970). Quality of plant produces is also influenced by changes in potassium concentration as it is the quality nutrient. In the deficiency of potassium size, shape, colour, taste and shelflife of fruits are disturbed (Hughes and Evans, 1969). The varied changes in cotton metabolism due to potassium stress have been reported by Gopal *et al.* (2010).

As regards the timings of split application of N and K fertilizers, S_4 and S_5 having three splits of these nutrients (at 10, 30, 60 DAS or 10, 45, 60 DAS) performed the best, maximum FUE was 16.50 to 16.52 kg SCY kg^{-1} N applied, 31.10 to 31.19 kg SCY kg^{-1} P applied and 44.72 to 44.86 kg SCY kg^{-1} K applied. This was closely followed by S_6 having

Table 1: Yield, NUE (kg SCY kg^{-1} nutrient applied) and N uptake by *Bt* cotton hybrid NSPL-999 as influenced by N and K supply at different timings of split application (2010-11 and 2011-12)

Treatments	Seed cotton yield (q ha^{-1})			NUE (kg SCY kg^{-1} N nutrient applied)			N, uptake (kg ha^{-1})			Nitrogen use efficiency (%)		
	2010-11	2011-12	Mean	2010-11	2011-12	Mean	2010-11	2011-12	Mean	2010-11	2011-12	Mean
(A) Mode of fertilizer application												
F_1 N_{120} (P_{60} K_{40} basal)	18.15	18.92	18.53	13.33	13.80	13.57	169.86	183.79	176.82	21.52	23.42	22.48
F_2 N_{120} K_{40} (P_{60} basal)	19.02	19.79	19.40	14.65	15.07	14.86	167.86	181.77	174.82	25.93	27.83	26.89
CD (P = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	3.02	3.39	3.22
(B) Timings of split applications												
S_1 2 splits (10 & 45 DAS)	18.50	19.28	18.88	13.53	14.17	13.85	156.88	170.85	163.83	15.58	17.48	16.54
S_2 3 splits (10, 45 & 75 DAS)	16.97	17.73	17.35	13.24	13.66	13.45	146.79	160.70	153.74	22.08	23.98	23.04
S_3 3 splits (10, 30 & 45 DAS)	17.74	18.50	18.12	12.82	13.24	13.03	154.34	168.24	161.29	24.94	26.84	25.90
S_4 3 splits (10, 30 & 60 DAS)	20.81	21.58	21.19	16.31	16.73	16.52	203.06	216.97	210.02	23.24	25.14	24.20
S_5 3 splits (10, 45 & 60 DAS)	20.77	21.54	21.15	16.29	16.71	16.50	177.94	191.85	185.06	26.97	28.87	27.93
S_6 3 splits (10, 30 & 75 DAS)	16.70	17.46	17.08	15.19	15.61	15.40	167.58	181.48	174.53	28.40	30.30	29.36
S_7 4 splits (10, 30 45 & 60 DAS)	19.77	20.53	20.15	11.87	12.29	12.08	184.14	198.04	191.09	28.39	30.29	29.35
S_8 4 splits (10, 30, 45 & 75 DAS)	17.43	18.19	17.81	12.66	13.08	12.87	160.18	174.08	167.13	20.24	22.14	21.20
CD (P = 0.05)	3.90	3.91	3.89	3.03	3.06	3.04	12.25	12.17	12.22	6.21	6.48	6.44

three splits of fertilization (10, 30, 75 DAS) where N, P and K efficiency was 15.40, 28.93 and 41.48 kg SCY, respectively. On the other hand, almost significantly lowest FUE of N, P and K was noticed in case of S_7 and S_8 treatments where N_{120} and K_{40} were applied in four splits (10,30, 45, 60 DAS or 10,30, 45, 75 DAS). It is apparent from these results that the full dose of N and K should be applied earlier in three splits within 60 days of plant growth. Because in case of four splits, the applied doses are curtailed for each split for actively growing plants as compared to the total three splits. Secondly in case of four splits, the fourth split approaches late after 75 days of sowing, by that time the N and K requirement of plants might have slightly reduced. Thus, the fertilizer use efficiency in the fourth split is eventually reduced as compared to the complete dose finished upto three splits. Similar observations have also been reported by Bhatia and Singh (2015).

The fertilizer use efficiency with respect to either N, P, K in its percentage or in terms of its seed cotton yield, in both cases, were found to be non-significant due to mode and timings of split application of N and K fertilizers. Due to similar dose of NPK fertilizers in all the treatment combinations, the reasoning for their interactions to come significant does not arise. Logically or fundamentally, the interaction between mode x timings of split application of fertilizers should not be upto the significant extent because both the such type of treatment are to play their role always on a positive direction.

Uptake of nutrients

The uptake of N, P and K nutrients was found to deviate significantly due to timings of fertilizer application, whereas only K uptake was significantly affected due to mode of fertilizer application, Phosphorus and K uptake was highest when the fertilizer was applied in 4 equal doses at sowing, 30, 45 and 60 DAS (Bhatia and Singh, 2015). That means N and P uptake due to mode of application was statistically identical. The reason behind this may be ascribed with the fact that the mode of the fertilizer application in case of N_{120} and P_{60} was the same in F_1 and F_2 treatments, whereas the uptake of K was significantly higher in F_2 treatment (229.20 kg ha^{-1}) when K was splitted as against 211.5 kg ha^{-1} in F_1 where full dose of K was applied as basal. The splitting of K at different timings proved more advantageous because of increased availability of K as per requirement of the speedily growing plants. Those results have also been supported by Bhatia and Singh (2015) The splitting of N and K fertilizers at different timings, i.e., 2, 3 or 4 times during growth period influenced significantly upon the uptake N, P and K nutrients. The treatments S_4, S_5 and S_7 comprising 3 to 4 splits, where N and K fertilizer doses were finished within 60 days growth period of plants, proved to be the best wherein maximum N, P and K nutrients were taken up by the plants. This might be attributed to improved utilization of N in the presence of K. Similar positive effect of potassium application on N uptake was reported by Senthivel and Paloniappan (1985). Superiority of split application of N and NK might be attributed to availability of nutrients at growth stage when cotton crop starts growing faster. This may be due to prevention of loss through leaching.

In case of N, it ranged from 184.89 to 210.01 kg ha^{-1}, in case of P 71.66 to 78.58 kg ha^{-1}. On the other hand, the N, P and K uptake was comparatively lower when N and K fertilizers were splitted upto 75 days growth period as

Table 2: PUE (kg SCY kg^{-1} nutrient applied) and P uptake by *Bt* cotton hybrid NSPL-999 as influenced by N and K supply at different timings of split application (2010-11 and 2011-12)

Treatments	Seed cotton yield (q ha^{-1})			PUE (kg SCY kg^{-1} P nutrient applied)			P, uptake (kg ha^{-1})			Phosphorus use efficiency (%)		
	2010-11	2011-12	Mean	2010-11	2011-12	Mean	2010-11	2011-12	Mean	2010-11	2011-12	Mean
(A) Mode of fertilizer application												
F_1 N_{120} (P_{60} K_{40} basal)	18.15	18.92	18.53	25.11	25.59	25.35	60.64	71.12	65.84	14.41	15.97	15.19
F_2 N_{120} K_{40} (P_{60} basal)	19.02	19.79	19.40	27.65	28.07	27.86	62.41	72.98	67.70	15.44	17.00	16.22
CD (P = 0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
(B) Timings of split applications												
S_1 2 splits (10 & 45 DAS)	18.50	19.28	18.88	25.50	26.14	25.83	54.09	64.66	59.37	11.55	13.11	12.33
S_2 3 splits (10, 45 & 75 DAS)	16.97	17.73	17.35	24.83	25.25	25.04	56.98	67.55	62.27	14.70	16.26	15.48
S_3 3 splits (10, 30 & 45 DAS)	17.74	18.50	18.12	24.36	24.78	24.57	62.71	73.12	67.83	18.65	20.21	19.43
S_4 3 splits (10, 30 & 60 DAS)	20.81	21.58	21.19	30.98	31.40	31.19	70.61	81.02	75.73	14.28	15.84	15.06
S_5 3 splits (10, 45 & 60 DAS)	20.77	21.54	21.15	30.89	31.31	31.10	73.32	83.87	78.59	14.36	15.92	15.14
S_6 3 splits (10, 30 & 75 DAS)	16.70	17.46	17.08	28.72	29.14	28.93	54.98	65.55	60.43	16.34	17.90	17.12
S_7 4 splits (10, 30 45 & 60 DAS)	19.77	20.53	20.15	22.08	22.50	22.29	66.38	76.95	71.66	12.13	13.69	12.91
S_8 4 splits (10, 30, 45 & 75 DAS)	17.43	18.19	17.81	23.68	24.10	23.89	53.12	63.69	58.41	17.39	18.95	18.17
CD (P = 0.05)	3.90	3.91	3.89	5.69	5.63	5.66	7.10	7.08	7.08	2.13	2.46	2.77

observed in treatments S_2, S_6 and S_8. This was the overall trend which indicates that the fertilizers should be splitted only upto 60 days of plant growth period. Beyond this period, the supply of nutrients decreased against the requirement or demand of the plants. Moreover, out of the above mentioned various treatments, N uptake was maximum (210.01 kg ha^{-1}) when N K fertilizers were applied at 10, 30 and 60 days stages (S_4). In case of P uptake, it was maximum (78.58 kg ha^{-1}) when N K were applied at 10, 45 and 60 DAS (S_5), being very close to S_4. However, in case of K uptake, S_4, S_5, S_6 and S_7 performed equally better removing 232.75 to 243.59 kg ha^{-1} K from the soil in case of splitting almost upto 60 days of growth period. The interaction between mode and time of fertilizer application also proved significant with respect to each of the nutrient under study (Table 3). The combination of the best mode as well as time of fertilizer application further encouraged the nutrients uptake synergistically which was eventual and logically truth full. This might be attributed to improved utilization of N in the presence of K. Similar positive effect of potassium application on N uptake was reported by Senthivel and Paloniappan (1985).

Fertilizer use efficiency

The data presented here reveal that nitrogen and potassium use efficiency was increased significantly (26.89 and 106.13%, respectively) when N_{120} K_{40} was applied as split and P_{60} as basal (F_2). This was against the treatment F_1 when only N_{120} was applied as split and P_{60} K_{40} as basal (22.48 and 49.50%, respectively). The significant impact of F_2 treatment might be due to the fact that nitrogen and potassium both were applied as split doses, whereas in case of F_1, only nitrogen was applied as split doses. In case of phosphorus, its full dose is applied as basal dose. It is not applied as split doses as in case of nitrogen and potassium which remain mobile and available in the soil system. The phosphorus use efficiency did not deviate to significant extent because of the fact that P_{60} was applied as basal both in F_1 and F_2 treatments. It is a well known phenomenon that 2/3 of the applied phosphorus, is generally, fixed in the soil and only 1/3 is available to the crop plants. Therefore, this situation prevailed in both the treatments.

The timings of split application of N and K fertilizers exerted significant impact upon the N, P and K use efficiency in Bt cotton hybrid. The treatment S_6 and S_7 having three or four splits exhibited the highest N use efficiency (29.35 to 29.36%), whereas the significantly lowest was in case of S_1 having two splits (16.54%). However, the P use efficiency was found in the higher range (18.17 to 19.43%) in S_3 and S_8 treatments having three or four splits. The significantly lowest P use efficiency (12.33%) was recorded in case of S_1 having only two splits of fertilizer application. As in case of P use efficiency, the K use efficiency was also found highest (103.8%) from S_3 treatment having three splits. Thus, S_3 timings of split application proved the best for maximum P and K use efficiency. The equally higher K use efficiency (97.36 to 98.26%) was also obtained from S_5 and S_6 treatments having three splits. The significantly lowest K use efficiency was recorded from S_8 treatment having four splits (48.88%). The overall trend in K use efficiency indicate that splitting of N and K fertilizers upto 45 DAS brought about the maximum K use efficiency, whereas splitting of fertilizers beyond 45 DAS tended to reduce the same.

Water use efficiency

The water use was found maximum under F_2 and S_4 treatments as well as with their combination F_2S_4. The result trend was almost the same as observed in case of fertilizer use efficiency because water use efficiency is maximized with the maximization of fertilizer use efficiency. Hence, FUE

Table 3: KUE (kg SCY kg^{-1} nutrient applied) and WUE (kg cm^{3-1}) of *Bt* cotton hybrid NSPL-999 as influenced by N and K supply at different timings of split application (2010-11 and 2011-12)

Treatments	Seed cotton yield (q ha^{-1})			KUE (kg SCY kg^{-1} K nutrient applied)			K, uptake (kg ha^{-1})			Potassium use efficiency (%)			Water use efficiency (kg cm^{-3})		
	2010-11	2011-12	Mean	2010-11	2011-12	Mean	2010-11	2011-12	Mean	2010-11	2011-12	Mean	2010-11	2011-12	Mean
(A) Mode of fertilizer application															
F_1 N_{120} (P_{60} K_{40} basal)	18.15	18.92	18.53	35.48	36.72	36.10	206.26	216.88	211.55	48.65	50.35	49.50	18.06	19.30	18.68
F_2 N_{120} K_{40} (P_{60} basal)	19.02	19.79	19.40	39.12	40.36	39.75	223.91	234.48	229.20	105.28	106.98	106.13	18.68	19.92	19.30
CD (P = 0.05)	NS	NS	NS	NS	NS	NS	13.89	13.23	13.28	20.45	21.23	21.19	NS	NS	NS
(B) Timings of split applications															
S_1 2 splits (10 & 45 DAS)	18.50	19.28	18.88	36.19	37.43	36.81	206.11	216.68	211.39	66.37	68.07	67.22	16.54	17.78	17.16
S_2 3 splits (10, 45 & 75 DAS)	16.97	17.73	17.35	35.04	36.28	35.66	179.61	190.18	184.90	56.79	58.49	57.64	17.77	19.01	18.39
S_3 3 splits (10, 30 & 45 DAS)	17.74	18.50	18.12	34.30	35.54	34.92	192.46	203.20	197.74	102.95	104.65	103.80	18.10	19.34	18.72
S_4 3 splits (10, 30 & 60 DAS)	20.81	21.58	21.19	44.24	45.48	44.86	233.18	243.75	238.47	75.99	77.69	76.84	20.57	21.81	21.19
S_5 3 splits (10, 45 & 60 DAS)	20.77	21.54	21.15	44.10	45.34	44.72	238.31	248.88	243.60	97.41	99.11	98.26	19.06	20.30	19.68
S_6 3 splits (10, 30 & 75 DAS)	16.70	17.46	17.08	40.86	42.10	41.48	237.04	247.61	242.32	96.51	98.21	97.36	17.41	18.65	18.03
S_7 4 splits (10, 30 45 & 60 DAS)	19.77	20.53	20.15	30.41	31.65	31.03	227.47	238.05	232.76	71.63	73.33	72.48	19.02	20.26	19.64
S_8 4 splits (10, 30, 45 & 75 DAS)	17.43	18.19	17.81	33.28	34.52	33.90	206.53	217.10	211.81	48.03	49.73	48.88	18.47	19.71	19.09
CD (P = 0.05)	3.90	3.91	3.89	8.79	8.78	8.79	39.39	40.46	38.57	42.76	43.17	43.23	3.03	3.06	2.62

and WUE both are complimentary to each other. A similar trend as for yield was observed for water productivity. According to Devkota *et al*. (2013) the average water productivity of cotton was 0.88 kg m^{-3} in respective of the tillage method.

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Manuscript recived on 11.12.2018

Manuscript accepted for publication on 3.3.2019

Journal of Eco-friendly Agriculture **14**(2): 28-33. 2019

Bio-enhancers for soil, plant health and insect pest management in organic production of horticultural crops

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ABSTRACT

Use of liquid preparations has been an age old practice in ancient India. As alternative, organic farmers had devised plant growth boosters on their own knowledge based on local experiences and given specific names such as *Amritpani, Panchagavya, Bijamrita, Jeevamrita* etc. Similarly in biodynamic farming, few effective preparations such as BD-500, BD-501, Cow Pat Pit, biodynamic liquid manures/bio-pesticides and in Homa Organic Farming: *Agnihotra* ash enriched water and Biosol are effective preparations are being used by number of farmers and organizations in India. It is interesting to note that in all these preparations, the basic ingredients are cow based products and studies done on bio-enhancers indicated that there is immense scope of their promotion in organic production of various crops.

Key words: Biodynamic, *amritpani, jeevamrita, panchagavya, Azospirillum*, biodynamic liquid pesticides

Imbalanced use of agro chemicals has resulted in some regions manifesting adverse effects on the environment, polluting soil and ground water resources. Soil quality, especially which of organic matter and micro-nutrients deficiencies are becoming ubiquitous and threatening sustainability, quality of produce and impacting on nutritional security. Further, indiscriminate use of agro-inputs specially chemical fertilizers and pesticides, has led to development of resistance to pests to pesticides, while destroying irretrievably the beneficial creatures viz., honey-bees, pollinators, parasitoids, predators, besides causing harmful pesticide residues in the end product adversely impacting productivity and food safety. Looking at the impact of green revolution, awakening has been created in many countries of the world to minimize the use of harmful agro-chemicals in horticultural crop production. In organic production there is always shortage of quality agri inputs for nutrient and insect pest management. There are number of organic farming systems such as Biodynamic agriculture, Natural farming, Natueco farming, Panchagavya farming, *Rishi Krishi*, *Homa* Organic farming, etc. emerged in different parts of the country. In these farming systems, some preparation are prepared and used in various crop productions. After closely working with Organic Farming Systems over a decade, we are of the view that "Bio enhancers" could be a cheap and alternative tool to resolve many issues including a cheap and effective alternative for fertigation.

"Bio-enhancers" are organic preparations, obtained by active fermentation of animal & plant residues over specific duration. These are rich source of microbial consortia, macro, micronutrients and plant growth promoting substances including immunity enhancers. In general, these are utilized to treat seeds/ seedlings, enhance decomposition of organic materials thereby enrich soil and induce better plant vigour".

It is pertinent to mention that 'Cow' plays key role in most of the organic farming systems prevalent in India and elsewhere (Pathak, et *al.*, 2010). Use of cow dung and cow urine as bio-control agents for curing plant and human diseases has long history. The five products of cow (dung, urine, milk, ghee and curd) are used in different organic systems.

BIO-ENHANCERS

Vrikshayurveda (science of plant life) deals with growing and nourishing plants with liquid manures (prepared with plant and animal products). There are several verses (e.g., *Brahatasamhita* of Varahamira, Surpala's *Vrikshayurveda* and *Upavanavinoda* of Sharangadhara), which provide information on irrigation methods using water mixed with herbal products obtained from different plant species and animal products to increase crop production (Sadhale, 1996). *Kunapajala* is a combination of two words 'Kunapa' which means dead or decaying matter and 'Jala' means water derived from this dead matter according to Sanskrit dictionaries. *Kunapajala* generally promotes flowering, fruiting and vegetative growth and is also used for plant protection measures (Majumdar, 1935).

Characteristic of bio-enhancers

- Potent source for macro and micro nutrients

- Presence of plant growth promoting factors
- Immunity enhancer
- Pesticidal & fungicidal properties
- Efficacy is influenced by inputs used and method of preparation
- Used for seed/seedling treatment, enhancing waste decomposition, improving soil fertility and productivity
- An effective and potent tool for fertigation

In general bio-enhancers are of two types and salient features are described as under:

(i) **Plant based:** These are prepared from whole tender plants and leaves viz; sun hemp, dhaincha (*Sesbania*), *Erythrina* and other legumes as potent source of nitrogen, leaves of neem, *pongamia*, Subabul (*Leucaena leucocephala*), glyricidia, *lantana, Calotropis* and other local plants having pesticidal properties, weeds viz; *Parthenium*, stinging nettle, *Cassia tora,* etc. are also used.

(ii) **Animal based:** These are prepared using cattle dung, sheep and goat droppings, fish manures (Anonymous, 2006). Combinations of plant and animal byproducts have better impacts on crop production (FAO, 2006). Liquid manures and liquid fertilizer, preparations are obtained by active fermentation of animal and plant residues over specific duration (Anonymous, 2006) are important. Organic liquid manures play a key role in promoting growth and providing immunity to plant system (Sreenivasa *et al.*, 2010).

There is increasing trend for naturally derived formulations for sustainable production in organic farming system (Suthar, 2010). Many formulations of liquid manures are being used by the farmers of different states. Few important and widely used formulations are discussed as under. On the basis of preparation, bio-enhancers can be grouped into simple and special preparations. Brief account of these has been dealt below:

Special bio-enhancers

Numbers of cow based bio-enhancers alone or in combination with few other products have been developed in different organic farming systems, and their impact has been recorded. Salient features of few of the selected bio-enhancers and their impact has been discussed here.

(i) Biodynamic preparation (502-507)

In biodynamic farming, preparations are used in compost production. These preparations are rich in microbial load and harness cosmic energy in compost heap. These compost preparations are produced with the use of different herbs. These are biodynamic preparation- 502, 503, 504, 505, 506 and 507.

Microbial analysis of these biodynamic preparations was done and enumeration of different beneficial microbial populations *viz.,* bacteria, fungi, actinomycetes, *Pseudomonas*, gram positive bacteria, gram negative bacteria, p-solubilzing bacteria, *Rhizobium, Azotobacter and Azospirillum* were carried out. Thirty six microbial cultures were tested for PGPR activities and one showed HCN production, 8 showed chelating and another 8 showed IAA production property (Ram and Singha, 2017).

(ii) Cow horn manure (BD-500)

Microbial analysis of biodynamic preparations was done and enumeration of different beneficial microbial populations viz. bacteria, fungi, actinomycetes, *Pseudomonas*, gram positive bacteria, gram negative bacteria, p-solubilzing bacteria, Rhizobium, *Azotobacter and Azospirillum* were carried out by standard methods (Ram *et al*., 2010). With regular application of preparation- 500 provide all the characteristics in the soil as summarized below:

- Strong humus formation
- Improved crumb structure and soil tilth
- Increase in bacterial population
- Increase in rhizobacteria activity (nodulation) in all legumes, e.g., gram, pea, moong, sun hemp *etc*
- Increase in phosphate solubilzing bacteria
- Increase in earthworm's activity
- Water absorption and retention power of the soil increased. (NB International research has found that BD-500 applied in soils requires 25% less irrigation than conventional soils)
- Plants develop deep root system

Cow horn silica (BD-501)

Using cow horn for making BD-501 employs an ideal focal device to concentrate the earthly or cosmic stream of forces on the material filled in the horn. Microbes isolated from cow horn silica are known microbes beneficial for soil and plant health management (Ram *et al*., 2010).

(iii) Cow Pat Pit (CPP)

CPP is a strong soil conditioner and a concentrated source of beneficial organisms. In a study, CPP showed highest bacterial load (4.8×10^6) per g, *Rhizobium* (1.9×10^6),

Azospirillum (0.2 x 10^6), *Azotobacter* (8.0 x 10^5) and fungi (2.5 x 10^6) (Ram *et al*., 2010). It also contained highest amount of *B. subtilis* (1.9 x 10^6) responsible for disease tolerance (Proctor, 2008) in plants.

CPP contained three plant growth hormones such as Indole Acetic Acid IAA (28.6 mg kg^{-1}), Kinetin (7.6 mg kg^{-1}) and Gibbrerllic acid (23.6 mg kg^{-1}) (Perumal *et al.,* 2006). CPP provides nutrients and stimulate plant growth by enhancing microbial population and protecting against fungal diseases (Perumal *et al.,* 2006).

Biodynamic liquid manure/pesticide

Biodynamic liquid manure/pesticide can easily be prepared by using cow dung, urine and leaves of leguminous tree, neem leaves, fish waste, caster leaves and other medicinal plant parts. Besides cow dung, cow urine and one set of BD-preparations (502-507) are also incorporated. Besides, pesticidal property biodynamic liquid manure/pesticide contains beneficial microbes and nutrients. In an experiment mango hopper management with biodynamic liquid pesticides was effectively done (Ram *et al.*, 2017) Fig.1.

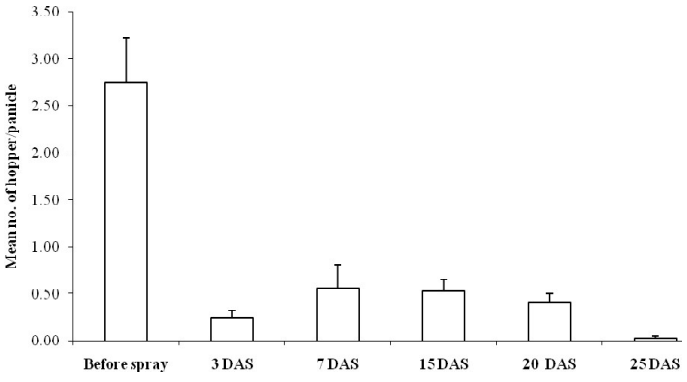

Fig 1: Mean mango hoppers population in experimental mango orchard before and after spray

(i) *Panchagavya*

It is a special bio-enhancer prepared from five products obtained from cow, i.e. dung, urine, milk, curd and ghee. In beginning, pioneer work was done by a medical doctor Natrajan (2003). Composition of *panchagavya* was investigated by Patnaik *et al*. (2012) and they observed the presence of aerobic heterotrophic bacteria, lactic acid bacteria, yeast, fungi and anaerobic bacteria. In a study, highest microbial load was recorded in 7 days old preparation.

The spray of *panchagavya* on chillies produces dark green coloured leaves within 10 days and its role has been reported by Sreenivasa *et al*. (2009). The effective micro organisms in *panchagavya* were the mixed culture of naturally occurring, beneficial microbes, mostly lactic acid bacteria (*Lactobacillus*), yeast (*Saccharomyces*), actinomycetes (*Streptomyces*), photosynthetic bacteria (*Rhodopsuedomonas*) and certain fungi (*Aspergillus*). Systematic microbial analysis of *panchagavya* from 0 to 25 days suggests that *panchagavya* contains maximum microbial load on 18th day of preparation. Therefore, use of *panchagavya* on 18th days will be more effective than other days Nutritional analysis of *panchagavya* revealed that it possesses almost all macro and micro-nutrients and growth promoting hormones (IAA, GA) required for plant growth (Ram *et al*., 2018 and Selvaraj *et al*., 2006). Other than beneficial microbial load, bio-enhancers contains major and micro nutrient also (Pathak *et al*., 2010 and Ram and Kumar, 2018).

(ii) *Dasgavaya*

As name indicates *Dasagavya* is a mixture of ten products, consisting of *panchagavya* and certain plant extracts. The leaf extracts of five commonly available weed plants, viz., *Artemisia nilagirica, Leucas aspera, Lantana camera, Datura metal* and *Phytolacca dulcamera* are obtained by soaking the plant materials separately in cow urine in 1:1 ratio for ten days. The extract is collected, mixed well with *panchagavya* and left for 25 days (Selvaraj, 2012).

Table 1: Nutrient analysis (% on dry weight basis) of different bio enhancers (liquid 50 ml^{-1})

Preparations	N (%)	P (%)	K (%)	Ca (%)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Na (%)
Cow pat pit	2.10	3.85	0.42	4.25	160	62	2595	309	0.30
BD 500	1.26	1.32	0.57	0.45	100	55	1945	173	0.20
Vermi wash	0.27	0.64	1.73	0.69	60	31	485	28	0.75
Neem based biodynamic pesticide	0.29	1.09	1.47	3.25	40	34	630	24	1.07
Castor based biodynamic pesticide	2.10	1.83	5.87	2.06	50	37	530	57	1.00
Karanj based biodynamic pesticide	2.04	2.06	5.95	6.57	70	44	2620	58	1.12
Calotropis based biodynamic pesticide	2.25	1.86	6.30	6.76	65	50	2460	83	1.02
Lantana based biodynamic pesticide	3.20	1.51	5.87	2.97	35	36	360	51	5.87
Amritpani	2.37	4.91	6.45	3.35	65	33	1680	109	6.45
Panchagavya	0.007	0.01	0.06	-	2.9	2.4	1.7	25.8	trace

Dasagavya has potential to promote growth and boost immunity in the plant system against pests and diseases. Its' regular use at a concentration of 3 percent solution has been found very effective in large number of crops pests and diseases such as leaf spot, blight, mildew, and rust of vegetables. Besides this, treated plants were found to exhibit inhibitory effects against sucking pests like aphids, thrips, white flies and mites and also foliar caterpillar (Selvaraj, 2006).

(iii) *Jeevamrita*

Jeewamrita is prepared by fermenting cow dung, urine, jaggery, pulse flour and virgin soil by simple facilities created in the village with minimum expenditure. Jeevamrita is a rich bio-formulation contains consortia of beneficial microbes. Systematic microbial analysis of Jeevamrita from the 0 day to 20 days of preparation suggests that formulation should be used within 6-9 days for maximum benefits (Ram *et al*., 2018).

(iv) *Beejamrita*

It is special bio-enhancer prepared with locally available materials for seed and seedlings treatment. As preparation is very cheap and cost effective, it can easily be prepared and used by small and marginal farmers. It is advisable to prepare fresh *Beejamrita* and use it for treatment of seeds/ seedlings and other plant parts before sowing/ planting/transplanting. Microbial analysis of Beejamrita showed that it should be used on 7th day of preparation (Ram *et al*, 2018).

(v) *Amritpani*

Use of Amritpani along with 250 g rhizospheric soil of *Ficus benghalensis* tree and organic mulching in organic production of guava resulted in maximum return (INR 1,27,746/ha) and benefit cost ratio (4.4) compared to INR 1,20,820 and 3.7 with application of 350 g N, 150 g P_2O_5 and 350 g K_2O/ tree (Ram and Verma, 2017). Systematic microbial analysis of Amritpani from 0-20 days suggests that Amritpani should be utilized with in 6-9 days of preparation for maximum effectiveness. It contains beneficial microbes viz; nitrogen fixing, phosphorus solubilizing and bio agents (Ram *et al*., 2018). After application in soil it improves humus content, earthworm activity and thus soil fertility and crop productivity.

(vi) Vermiwash

Vermiwash is a liquid leachate obtained by excess water to saturate the vermi compost substrate. It is a collection of excretory products and mucus recreations of earthworm along with nutrients from the soil organic molecules. In fact vermi wash is an enriched bio-enhancer prepared from the heavy population of earthworms reared in earthen pots/ plastic or cement containers. Microbiological study of vermiwash revealed that it contains nitrogen-fixing bacteria like *Azotobactre*r sp., *Agrobacterium* sp. and *Rhizobium* sp. and some phosphate solublizing bacteria. Vermi wash micro flora contains *Azotobacter, Agrobacterium and Rhizobium* and phosphate solubilzing microbes. Besides these, vermiwash contains total heterotrophs i.e.; *Pseudomonas* (0.01 x 10^7), Phosphate solubilzing microbes (0.06 x 10^6), *Rhizobium* (0.07x 10^5), *Azotobacter* (0.14 x 10^6) and *Azospirillum* (0.007 x 10^6) (Pathak and Ram, 2013).

(vii) Biosol

A special bio formulation developed by Gloria and named as "Gloria Biosol" from Peru (Weir, 2009). Biosol is superior to vermi wash as it contains high numbers of beneficial microorganisms and energy from Homa atmosphere (Yadav, 2009). Using Homa methods, it is possible to provide complete nutrition to the plants, which contains optimum concentration nutrients as macro elements, oligo elements and others. Biosol provides plant special medicinal and nutritional qualities. It is prepared after a series of operations and processes that lead to the biodegradation of organic matter, worm humus, fresh cattle manure and water, until reaching mineral grade. It is powerful bio food and bio fertilizer for the plants with high level of macro and micronutrients. It is powerful restorative and directly assailable through the membrane of the root cells of the plants. It is rich in enzymes, beneficial microorganisms, phyto hormones and other special useful components for the plant and improves fertility and health of the soil (Table 2).

Table 2: Different microbial populations in Biosol

Micro organism	Microbial population (cfu/ml)
Bacteria	31.39 x 10^8
Fungi	3.0 x 10^3
Actinomycetes	33.1 x 10^6
Gram positive bacteria	6.13 x 10^7
Gram negative bacteria	Colony not found
Pseudomonas	10.56 x 10^6
Rhizobium	1.22 x 10^6
Phoshate solublizing bacteria	2.96 x 10^4
Azotobacter	1.10 x 10^5
Azospirillum	Colony not found

Strategies for promotion of bio-enhancers

- From the aforesaid information, it is clear that Bio-enhancers have immense potential to improve soil fertility, crop productivity and pest management.

- It is a paradox to record that most of the information on these preparations has been experienced by Indian farmers since ancient time but number of apprehensions are persisting for use of bio- enhancers which require initiation of systematic research for further explanations.
- Comparative evaluation of bio-enhancers prepared through ingredients from similar origin and these scientific explanation for their nutrient status, microbial consortia and other associated scientific information can resolve many apprehensions.
- Impact and role played in package of practices will help for their acceptance in promotion of organic farming.
- These can be prepared with little support and skill up gradation trainings.
- There is a need for delineation of nutrient status (macro and micro nutrients), plant growth promoting factors, immunity enhancer ability, etc. for their quick acceptance by the scientific and farming community.
- After proper filtration, bio-enhancers can be used through drip/sprinkler as fertigation.
- Comparative evaluation of aforesaid bio-enhancers for their nutritive value and impact will help for their preparation and use.
- There is a need to work out their contribution in organic production and frequency of their use in different crops.

Based on above enumeration, it can be concluded that bio-enhancers could be a potent source to improve soil fertility, crop productivity and produce quality. Bio-enhancers could also be a potential alternative for fertigation which is becoming common in most of the crops. But care should be taken that bio-enhancers, which are used in limited quantities, cannot meet the entire nutrient requirement of the crop. These simply catalyze quick decomposition of organic wastes into humus, hence incorporation of enough bio-mass preferably combination of mono cot and legumes duly supplemented with animal wastes will be helpful in quality production of humus, which is prerequisite for improving soil fertility and crop productivity. Combined with manures and frequent use of bio-enhancers can address many challenges of agriculture and will be helpful to show a way for sustainable production through organic resources.

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Manuscript recived on 13.11.2018

Manuscript accepted for publication 1.2.2019

Journal of Eco-friendly Agriculture **14**(2): 34-37. 2019

Cultivation of high value vegetable crops and their economic feasibility under polyhouse condition in subtropics

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ABSTRACT

Protected cultivation has gained popularity in India in recent past as it enhances productivity by protecting the plants against biotic and abiotic stresses. Farmers from different agro-climatic regions are adopting these technologies for higher production and productivity. Cultivation of vegetables such as capsicum, brinjal and tomato is recommended in net houses and copsicum, coloured capsicum, tomato and cucumber (exotic) is recommended in polyhouse/green house structures.

Key words : Protected cultivation, vegetables, green house, polyhouse, net house

Rising population and urbanisation and decrease in agricultural land have posed a herculean task to the agriprenures and policymakers to develop or adopt modern farming practices for producing more fresh and disease free produce. The demand to have cropping techniques in which the micro-climate around the plant is controlled partially or fully as per the requirements of crops for their healthy growth besides its availability is increasing. The answer to such task is known as protected cultivation practices in which the crop is administered with desired needs for having its optimum produce with improved quality parameters that could fetch a better market price of the produce. These practices cover greenhouses/polyhouses, net houses, plastic tunnels/low tunnels, crop covers and plastic mulching, raised beds, trellising and microirrigation techniques for crop cultivation. These practices can be used to provide the favourable micro-climate to the plants for their healthy growth against the biotic and abiotic stresses as compared to open field conditions.

Advantages of protected cultivation

- Enhancing productivity per unit area along with maximizing water productivity with minimum/no weed infestation. Round the year production of floricultural crops and off-season production of vegetables and fruit crops.
- Protecting the plants against abiotic factors such as temperature, excess/deficit water, hot and cold waves, and biotic factors such as pest and disease attack, etc. besides minimizing the harmful chemicals use in crop production.
- Promotion of high value quality horticultural produce.
- Propagation of planting material to improve germination percentage, healthy, uniform, disease-free planting materials and better hardening.
- Genetically superior transplants can be produced continuously.

Limitation of protected cultivation

- Higher initial investment for creation of infrastructure (capital cost).
- Operation and maintenance of the infrastructure with desired skill-set.
- Non-availability of critical inputs such as design aspects suited to agro-climatic zones and trained manpower.
- Marketing of produce with assured remuneration for encouraging the growers towards adoption of these applications.

Scope and importance

With the coordinated efforts of the centre and state governments, protected cultivation has gained popularity in India in recent past. Combined efforts of the national agencies and flagship schemes such as Mission for Integrated Development of Horticulture (MIDH), National Horticulture Mission (NHM), National Horticulture Board (NHB) and Rashtriya Krishi Vikas Yojana (RKVY) have created awareness and are providing financial assistance to the farmers to adopt protected farming techniques for cultivation of horticultural crops so as to suffice the national food basket with higher availability and food sufficiency.

In the recent past, the protected cultivation technologies are gaining momentum all over the world but the level and extent of their use may be different among different countries.

As the production process of vegetables are protected from adverse environmental conditions such as temperature, hails, scorching sun, heavy rains, snow and frost the farmers from different agro-climatic regions are showing interest in adopting these technologies. Cultivation of vegetables such as capsicum, brinjal and tomato is recommended in net houses and capsicum, coloured capsicum, tomato and cucumber (exotic) are generally recommended in poly-house/greenhouse structures.

Efforts have been made to promote protected cultivation to increase cropping intensity, productivity and improve produce quality for higher income generation and remunerative market cost of the produce. Availability of off-season produce can fetch higher net returns, achieving greater degree of food and nutritional security and widen livelihood opportunities for the rural people. However, to ensure sustainable agricultural development of hill regions, together with generating additional income opportunities through on-farm and off-farm avenues, protected cultivation needs to be promoted and practised on a large scale.

Options in protected farming practices are many but important ones include polyhouses, net houses, walk-in tunnels, and plastic mulching coupled with micro-irrigation and fertigation. With the advancement of science and technology as well as institutional innovations, there is greater scope for promoting profitable protected cultivation in the country on a larger scale.

Protected cultivation technologies/applications

Greenhouse: Greenhouses are framed or inflated structures covered with a transparent/translucent material in which crops can be grown under controlled or partially controlled environment. This technology provides better space utilization and maintain conducive environment for crops against vagaries of climate such as heavy rainfall.

Advantages

- Higher yield with better quality.
- Helps to raise early and off-season crops.
- Helps hardening of tissue cultured plants.
- Controls pests and diseases.
- A greenhouse, depending upon the transparency of the glazing material, admits sunlight which is absorbed by the crop, equipment, structural members and floor. These objects in turn emit thermal radiation which is only partially transmitted out of the greenhouse. Resultantly, a part of solar energy is retained in the greenhouse giving rise to a temperature increase. This natural temperature rise in the greenhouse is utilized during winter to grow crops with or without supplementary heat. During summers, the greenhouses are needed to be cooled as per the crop requirements.

Walk-in tunnels: It is a plastic covered greenhouse like structure which utilizes solar heat and natural ventilation for temperature and humidity control. These are most suitable for growing vegetables during winters.

Advantages

- Suitable for all type of crops, flowers and vegetables.
- Off-season cultivation is possible.
- Improves crop health and growth.
- Increased production with limited area and low cost.

Plastic Tunnels: Plastic tunnels are structures producing greenhouse like effect. These structures facilitate the entrapment of carbon dioxide (CO_2), thereby enhancing the photosynthetic activity of the plants and hence the yield. These structures protect the plants from high winds, rain and snow. These are less expensive, easy to construct and dismantle. The right selection of cladding material is important in creating favourable microclimate for the plants.

Advantages

- Provides microclimate for seedlings and grafts.
- Creates favourable condition to enhance photosynthetic activity and create a condition for better nutrients uptake by the plants.
- Ease of construction and dismantling as well as mobility.
- Disease free and healthy saplings for nursery are developed.

Plant protection nets: These are used to protect vegetable and fruit crops from solar radiation, insects, birds, hail, wind and frost.

Advantages

- These are flexible and low weight.
- Protects crop/plants from birds, insect, hail, etc.
- UV stabilized and resistant to bacteria, chemical agents, etc.
- Can be placed with or without structure.

Shadenet house: These are framed or inflated structures covered with nets to protect the plant and fruits from insects, birds, animals and extreme weather conditions. Depending

upon the use, these nets are available in different mesh size and shade factors between 15 to 90 per cent. Presently shade nets are available in white, black, red, blue, yellow and green having response on crop/plant yield attributes. It provides partially controlled environment by reducing the light and temperature favourable to crop growth.

Advantages

- Used for raising and hardening nursery of fruits, flowers and vegetables during summer season.
- Helps in quality drying of various agro products.
- Helps to enhance yield.
- Helps in creating favourable micro environment for vegetable cultivation.

Crop covers: They provide protection against frost, cold, insects and pests to the crop. It can be used as fruit cover or crop cover to save fruits from insect attacks while allowing air and sunlight to pass through, thereby improving the yield as well as the quality of the fruit. It is lightweight and cost effective.

Advantages

- Protects from hailstorms, frost, and heavy gusts of wind.
- Controls insects and pests.
- Protects crop against heavy rains.
- Better yield with improved quality of produce.
- Extends growing season.

Tomato

Crop duration	October – May
Crop varieties	Heemsona, Naveen, Arkasamrat, Arkarakshak, Abhilash, Shahenshah, NS-524, Red beauty (Cherry tomato), etc.
Crop spacing (R x P)	60 cm x 45 cm
Irrigation & Fertigation scheduling	Daily irrigation through drip 2-3 l m^{-2} day^{-1} with dripper discharge rate of 2 lph, emitters spaced at 30 cm. Fertigation: Twice a week starting from 25 days after transplanting. The crop is sprayed with micro nutrient mix solution containing ferrous, zinc, copper, manganese, boron and molybdenum (3 gl^{-1}) two to three times at 30 days interval starting from 60 days after transplanting.
Fertilizer requirement	NPK- 120:80:100

Capsicum

Crop duration	September – April
Crop variety	Bharat, Indra, Yellow Wonder, Swarna, etc.
Crop spacing (R x P)	45 cm x 30 cm
Irrigation & Fertigation scheduling	Daily irrigation through drip 2-3 l m^{-2} day^{-1} with dripper discharge rate of 2 lph, emitters spaced at 30 cm Fertigation: Twice a week starting from 25 days after transplanting.
Fertilizer requirement	NPK- 120:80:100

Cucumber

Crop duration	January – April, July – October, October – January
Crop variety	Isatis, Kian, Infinity (Parthenocarpic cucumber varieties)
Crop spacing (R x P)	60 cm x 60 cm
Irrigation & Fertigation scheduling	Daily irrigation through drip 2-3 l m^{-2} day^{-1} with dripper discharge rate of 2 lph, emitters spaced at 30 cm Fertigation: Twice a week starting from 15 days after transplanting.
Fertilizer requirement	NPK- 120:80:100

Production of vegetable and economic detail under naturally ventilated greenhouse (1000 sq m)

Crop	Crop period	Production (quintal 1000 m^{-2})	Cost benefit ratio
Tomato	November – May	75 – 100	1 : 2.5
Capsicum	October – April	20 – 25	1 : 2.0
Cucumber	July – April (three crops)	50 – 60	1 : 2.5

Approx. production cost (Rs m^{-2}) of vegetable (tomato, capsicum and cucumber) in greenhouse : 105; Payback period : 23-24 months

Under the climate change scenario, protected cultivation may possibly provide ample scope for 'off' season production of high value vegetable crops. Among the high value crops, tomato, cucumber and capsicum are most important and rich sources of neutraceutical values. The package of practice for cultivation of these crops is as follows:

Economic feasibility

- Cost of polyhouse structure (Low cost : Rs 250 – 400 m^{-2}, Medium cost : Rs 500 – 1000 m^{-2}, High cost : Rs 1000 – 2000 m^{-2})
- Quality of material for polyhouse used
- Crops/variety used
- Location/season and market

Cost benefit ratio will vary under different economic situations, depending upon the subsidy rate.

Production of vegetable and economic detail (1000 m^2) under medium cost ranging in greenhouse (fan pad system)

Crop	Production (quintal) approx		Increased production (%)	Total return (Rs.)		Benefit (%)	Employment generation (man days)	
	Open field	Green house		Open field	Green house		Open field	Green house
Tomato	20	150	650	12000	80000	567	250	1500
Cucumber	12	60	400	12000	70000	483	250	1200
Capsicum	10	40	300	10000	80000	700	400	1600

Extract from MIDH operational guidelines on protected cultivation component

Intervention	Cost norms	Pattern of assistance
Plastic tunnels	Rs. 60.00 m^{-2} and Rs. 75.00 m^{-2} for hilly areas	50% of cost limited 1000 m^{-2} per beneficiary
Walk-in-tunnels	Rs. 600.00 m^{-2}	50% of the cost limited to 5 units per beneficiary
Shade Net House structure		
(i) Tubular structure	Rs. 710.00 m^{-2} and Rs. 816.00 m^{-2} for hilly areas	50% of cost limited to 4000 m^{-2} per beneficiary
(ii) Wooden structure	Rs. 492.00 m^{-2} and Rs. 566.00 m^{-2} for hilly areas	50% of cost limited to 20 units per beneficiary
(iii) Bamboo structure	Rs. 360.00 m^{-2} and Rs. 414/sq m for hilly areas	50% of cost limited to 20 units per beneficiary
Green House structure		
(i) Fan and Pad system	Rs. 1650.00 m^{-2} (up to area 500 sq m^2) Rs. 1465.00 m^{-2} (>500 up to 1008 sq m^2) Rs. 1420.00 m^{-2} (>1008 up to 2080 sq m^2) Rs. 1400.00 m^{-2} (>2080 up to 4000 sq m^2) Above rates will be 15% higher for hilly areas.	50% of cost for a maximum area of 4000 m^{-2} per beneficiary
Naturally ventilated system		
(i) Tubular structure	Rs. 1060.00 m^{-2} (up to area 500 sq m^2) Rs. 935.00 m^{-2} (>500 up to 1008 sq m^2) Rs. 890.00 m^{-2} (>1008 up to 2080 sq m^2) Rs. 844.00 m^{-2} (>2080 up to 4000 sq m^2)	50% of cost limited 4000 m^{-2} per beneficiary
(ii) Wooden structure	Rs. 540.00 m^{-2} and Rs. 621/sq m^{-2} for hilly areas	50% of the cost limited to 20 units per beneficiary
(ii) Bamboo structure	Rs. 450.00 m^{-2} and Rs. 518/sq m for hilly areas	50% of the cost limited to 20 units per beneficiary
Anti Bird/Anti Hail Nets	Rs. 35.00 m^{-2}	50% of cost limited to 5000 m^{-2} per beneficiary.
Cost of planting material & cultivation of high value vegetables grown in poly house	Rs. 140.00 m^{-2}	50% of cost limited to 4000 m^{-2} per beneficiary
Cost of planting material & cultivation of Carnation & Gerbera under polyhouse/shade nethouse	Rs. 610.00 m^{-2}	50% of cost limited to 4000 m^{-2} per beneficiary
Plastic mulching	Rs. 32,000.00 ha^{-1} and Rs. 36,800.00 ha^{-1} for hilly areas	50% of the total cost limited to 2 ha per beneficiary

The cost norms and financial assistance varies from time to time and state to state

Manuscript recived on 4.12.2018
Manuscript accepted for publication 23.3.2019

Journal of Eco-friendly Agriculture **14**(2): 38-40. 2019

Advances in genomics for fruit improvement

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ABSTRACT

The omic tools are being used for chancing the quality and nutritional composition of fruit, besides they also play a significant role in resistance breeding, shelf life enhancement and productivity. The use of genomics, proteomics, transcriptomics and metabolomics provide insights to the molecular mechanisms of flowering, fruit development, ripening, insect resistance, herbicides tolerance, etc. Genomics and ascompanying technologies enable systems biology approach toward deciphering complex interactions between genes, proteins and metabolites for resulting phenotype.

Key words: Genomics, proteomics, metabolomics, molecular mechanism, phenotype

Fruit crops are key to economic empowerment of the agrarian society, and human health. Malnutrition and hunger reports from the world and global fruit market growth patterns, highlight need for concerted efforts in area of fruit improvement including biofortification(Younis *et al*., 2015). Changing consumer preferences drive fruit variety improvement for agronomic and economic traits, as international trade is increasing with enhanced logistic support apart from domestic market. Breeding of perennial fruit trees is hampered by lengthy breeding cycles from seed to fruit bearing, long juvenile period, high cost of raising individuals to maturity in the field due to large plant size, and high heterozygousity occasionally preventing theoretical breeding approaches (Bally *et al*., 2009). Manipulation at the gene level has emerged as a viable tool to mitigate these boundaries, and increasing efforts have been directed to enhancing the quality, resistances to the stresses, postharvest and nutritional properties of fruits (Yamamoto, 2016) using biotechnological tools.

Genome sequence information in fruit crops

Development and advent of next generation rapid sequencing technologies, facilitated whole genome sequencing for major fruit tree species which offers scope for development and use of molecular markers for marker-assisted selection. Rapid advances in omics studies (transcriptomics, proteomics, metabolomics, hormonomics, ionomics and phenomics) allows genome-wide association studies, and genomic selection in fruit species for improving speed and precision in fruit breeding. Grapevine (*Vitis vinifera*) was amongst the first fruit genomes to be sequenced by Sanger shotgun sequencing (Jaillon *et al*., 2007) and followed quickly by other fruit species like papaya, apple, strawberry, banana and pear. There are also several instances of *de novo* genome assembly that employed Next-Gen data alone, such as sequencing of sweet orange and kiwifruit. In Rosaceae fruit species, two important international research programs, the USA-based international research program RosBREED (www.rosbreed.org) and the European research program Fruit Breedomics (www.fruitbreedomics.com/), have provided molecular and bioinformatics tools, superior pre-breeding materials, and valuable resources. Genome information of fruit trees such as banana, citrus, papaya are available in the public domain (www.phytozome.com) while that of guava and mango, are yet to be open in public domain. Tree fruit Genome Database Resources (www.tfgdr.org) is dedicated to Rosaceae, *Citrus*, and *Vaccinium* bioinformatics resources and software tools (Wegrzyn *et al*., 2012) and are being used by fruit breeders. At present, genome information of guava cv. Zhenshu, which has been submitted by Hainan University, is available at https://www.ncbi.nlm.nih.gov/assembly/GCA_002914565.1/#/st. In mango, genome sequencing initiatives have been fruitful to the stage of draft genomes for mango cv. Tommy Atkins (US/Israel), Amrapali (India) and Kensington Pride (Australia), yet it has to be published in public domain. A list of genomes which has been completed sequenced among fruit crops is presented in Table 1.

Comprehensive study of gene expression using transcriptomics

RNA-Seq has been recognized as a powerful tool to analyze the transcriptome of an organism that has not been completely sequenced (Wang *et al*., 2009). The sequencing of RNA has long been recognized as an efficient method for gene discovery and remains the gold standard for annotation of both coding and non-coding genes (Adams *et al*., 1991). Furthermore, the RNA-Seq method offers a holistic view of the transcriptome, and the information obtained from this wide analysis can be used to study specific pathways or physiological situations in a given species, in order to obtain

useful information. RNA-Seq experiments have been primarily oriented in fruit trees to the high-throughput sequencing of the messenger RNA for important traits like fruit quality including flavour, nutrition, appearance and postharvest processing, many of which are a causality to yield. As far as fruits are concerned flavour quality can be considered a paradigm for improvement of many multigenic traits involving multiple metabolic pathways (Klee, 2010). Some of the crops in which flavour research has given insights is apple, muskmelon, strawberry and grape wine. At the same time, many genes for disease and pest resistance have been reported by MetaQTL analysis for scab resistance in apple (Calenge *et al.,* 2004), plum pox virus resistance in apricot (Zuriaga *et al.,* 2018) ; QTL analysis in brown rot resistance in peach, and downy and powdery mildew resistance in grapevine (Buonassisi *et al.,* 2017). Various QTLs controlling fruit quality traits (e.g., harvest time, fruit skin color, fruit weight, sugar content) have also been identified in apple, pear, peach, and grapevine (Devoghalaere *et al.,* 2012). Association studies with candidate genes have been conducted in apple, and contributed to finding a gene controlling fruit flesh firmness using GWAS and more recently by GS. Functional genomics approaches are now beginning to be utilized for controlling the juvenile–adult phase transition by inducing a flowering gene or/and silencing a floral repressor towards enhancing breeding cycle in case of Citrus and such studies have applications in mango and olive breeding. The papaya genome sequence gave insights on the primitive XY sex-chromosome system, where the Y chromosome contains a male-specific region (MSY) approximately 8 Mb in length.

Mass Spectrometry-Based Proteomic Analysis

Mass spectrometry-based methods allow for the direct, comprehensive analysis of expressed proteins and their quantification among different conditions. However, in general identification of proteins by assigning experimental mass spectra to peptide sequences of proteins relies on matching mass spectra to theoretical spectra derived from genomic databases of organisms (Luge and Sauer, 2016). This conventional approach limits the applicability of proteomic methodologies to species for which a genome reference sequence is available. Recently, RNA-sequencing (RNA-Seq) became a valuable tool to overcome this limitation by de novo construction of databases for organisms for which no DNA sequence is available, or by refining existing genomic databases with transcriptomic data. Several high-throughput technologies such as mass spectrometry (MS)-based techniques such as Tandem-MS and gel-based techniques such as differential in-gel electrophoresis (DIGE) and their databases (UniProtKB, IntAct, Reactome and PRIDE) have allowed in depth identification and quantification of proteins (Feng *et al.,* 2016). These technologies are pivotal to understanding proteome level responses in fruit development and key to fruit quality preservation.. Recent advances in NMR spectroscopy for quantifying metabolites that are central to primary metabolism has paved way for corroboration of metabolic responses of genotypes to varied environments and agrosytems. Further researches combining proteomic, transcriptomic, and metabolic approaches are also needed to characterize mechanisms involved in interactions among fruits, pathogens and environmental conditions.

Table 1: Published genomes of important fruit crops

Scientific name	Common name	Year	Chr (#)	Size (Mb)	Assembl. (Mb)	Assem (%)	Gene (#)	Repeat (%)	Scaffold N50 (kb)	Contig N50 (kb)	Sequencer types
Vitis vinifera	Grape	2007	19	475	487	103	30,434	41	2,065	66	Sa
Carica papaya	Papaya	2008	9	372	370	99	28,629	43	1,000	11	Sa
Malus x domestica	Apple	2010	17	742	604	81	57,386	67	1,542	13	Sa,4
Theobroma cacao	Cocoa	2011	10	430	327	76	28,798	24	473	20	Sa,4,I
Fragaria vesca	Strawberry	2011	7	240	210	87	34,809	23	1,361	NA	4,S,I
Phoenix dactylifera	Date palm	2011	18	658	381	58	28,890	40	30	6	I
Musa acuminata malaccensis	Banana	2012	11	523	472	90	36,542	44	1,311	43	Sa,4,I
Prunus mume	Chinese plum	2012	8	280	237	85	31,390	45	578	32	I
Pyrus bretschneideri	Pear	2013	17	527	512	97	42,812	53	541	36	I
Prunus persica	Peach	2013	8	265	227	86	27,852	37	27,400	214	Sa
Durio zebithinus	Durian	2017	28	738	712	96.88	45,355	54.8	22,700	-	Pa
Lotus japonicus	Lotus	2008	6	472	315	67	30,799	56	NA	NA	Sa
Citrullus lanatus	Watermelon	2012	11	425	354	83	23,440	45	2380	26	I
Cucumis melo	Melon	2012	12	450	375	83	27,427	NA	4680	18	Sa,4,I

Abbreviations: Sa, Sanger; 4, Roche/454; S, SOLiD; I, Illumina; Pa, PacBio NA, not reported in primary publication; kb, kilobases; Mb, megabases; Chr, chromosome (Adapted and Modified from Michael and Jackson, 2013).

The arsenal of omic tools are being used for enhancing the quality, taste, and nutritional composition of fruit, besides playing a significant role in resistance breeding, shelf life and productivity. Through the use of genomics, proteomics, transcriptomics, and metabolomics, the precision in fruit breeding have been improved, and these provide insights to the molecular mechanisms of flowering, fruit development, ripening, insect resistance, herbicides tolerance, etc. Genomics and accompanying technologies enable systems biology approach toward deciphering complex interactions between genes, proteins, and metabolites for resulting phenotype. This integrated approach relies heavily on bioinformatics, and computational analysis, the advances in which are leading genomics research.

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Manuscript recived on 2.12.2018

Manuscript accepted for publication 5.3.2019

Journal of Eco-friendly Agriculture **14**(2): 41-44. 2019

Assessment of different irrigation levels on quality production of headed back guava cv. Lalit under high density plantation

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ABSTRACT

In guava (*Psidium guajava* L.) low production of crop due to crowding and encroachment of tree branches leading to inefficient light penetration is a general problem in older orchards. The internal bearing capacity of trees also declines with time due to overshadowing. To overcome this problem heading back of unproductive trees to the extent of 1.5 meter height above the ground level to facilitate the sprouting of new shoots below the cut point and allowing the development of productive canopy is important. In this context a field experiment was conducted during 2014-2018 with the objective of improving yield and quality of headed back (1.5 m height) guava cv. Lalit (fifteen year old) under high density planting (6.0 x 3.0 m), accommodating 555 plants per hectare. The experimental plot was irrigated with a drip irrigation system having four irrigation levels with one ring basin irrigation method (control). The black polyethylene mulching (100 µ thickness) was used to cover 40 per cent area of tree canopy. Fertigation with recommended dose of fertilizer was applied at different growth stages. Maximum water saving (47.52%) was recorded at 80 per cent pan evaporation through drip irrigation and polyethylene mulching as compared to control. Enhancement in flowering in tune of 60 to 85 per cent and maximum fruit yield (24.0 kg tree^{-1}) at 80 per cent irrigation level with polyethylene mulching and minimum yield (15.1 kg tree^{-1}) in control was recorded. Drip irrigation coupled with polyethylene mulching resulted in better quality of fruits in terms of increased TSS (13.4 °Brix), total sugar (6.65%) and ascorbic acid (181.3 mg 100 g^{-1}) without any significant change in acidity (0.24%) as compared to minimum TSS (11.0 °Brix), total sugar (6.50%) and ascorbic acid (160.6 mg 100 g^{-1}) in control.

Key words: Guava, Lalit, headed plant, irrigation level, flowering and fruiting

Guava (*Psidium guajava* L.), a member of Myrtaceae family, is an important fruit crop of tropical and subtropical regions. The fruit is rich source of vitamin C, pectin and minerals like calcium, phosphorus and iron. Water is one of the most important inputs essential for the crop production. In northern India, guava fruiting occurs twice in a year during rainy and winter seasons. Guava tree bears flowers and fruits on the current season matured shoots either from lateral buds on older wood or shoot terminals (Pratibha and Goswami, 2013). Comparatively, good quality fruits are available only in winter season, whereas the rainy season fruits are poor in quality as well as insipid in taste. As the global water consumption is doubling every 20 years and projected increase in food demand will have to be met by irrigation. Appropriate scheduling of irrigation increases the water use efficiency along with water saving for other purposes. Surface irrigation system is most common method of irrigation. The present water management practices through surface irrigation results in water logging and salinity in many parts of the country. It is therefore, essential to formulate an efficient, reliable and economically viable water management strategy in order to irrigate more land area with existing limited water resources and enhance economic returns. It is established that drip irrigation and plastic mulching improve the fruit quality in many other crops. Many workers have reported that there is 50 to 70 per cent saving in irrigation water and 10 to 70 per cent increase in yield of fruit and vegetable crops through drip irrigation (Srivastava *et al*., 1994; Cetin *et al*., 2004). The drip irrigation system also provides opportunity to apply appropriate amount of nutrients and chemicals along with water, which reduces leaching losses and enhances yield, quality, water and nutrient use efficiency (Zhang *et al*., 2004). The improvement in physical and biological health of soil as a result of mulching has been reported (Garg *et al*., 2007). The response of guava to the combined effect of drip, with different levels of irrigation, in conjunction with polyethylene mulch and their economic feasibility in headed back high density guava are not well known. Except some preliminary study (Singh *et al*., 2007), water requirement under high density, has not been worked out. Hence, the present study was conducted to evaluate crop water requirement and improvement in yield and quality of guava under drip irrigation with polyethylene mulching.

MATERIALS AND METHODS

An orchard of guava located at ICAR-Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow (elevation of 128 m above sea level and situated between 26.55 °N latitude and 80.59 °E) was selected to study the

different irrigation levels on quality production of headed back guava cv. Lalit under high density plantating during 2014-18. The soil is coarse loam, mixed hyperthermic family of Typic Ustochrepts, pH 7.8, low total N <0.5 per cent, high available P >12 ppm and low K <80 ppm. Observations on meterological parameters, *viz.* pan evaporation, temperature and soil moisture were recorded by the weather station located adjacent to the experimental plot. Plant growth parameters, *viz.* plant height (m), yield (kg tree^{-1}), fruit weight (g) and quality attributes, *viz.*, TSS (°Brix), acidity (%), vitamin C (mg 100 g^{-1} FW) and total sugar (%) were recorded at appropriate stages. High density plantation of guava cv. Lalit at a spacing of 6.0 x 3.0 m (555 tree ha^{-1}) was maintained since 2014. The plants were irrigated through drip irrigation system having 2 emitters per plant of 8 LPH capacity and mulched with UV stabilized black polyethylene (100 micron thick) during the months of October - November. Each tree was headed back uniformly at the height of 1.5 m during the month of January. In the experiment the drippers were placed equidistant at 50 per cent distance of canopy radius. There were four irrigation levels (60%, 80% and 100% PE replenishment) and one ring basin irrigation method with 100 per cent PE replenishment (control), under mulch and without mulched conditions. The treatments were replicated thrice in a randomized block design. The water received through rain was accommodated in irrigation schedule in successive days in all the treatments but ignored in control plants. One square meter canopy area with one mm evaporation replenishment was equal to one liter of drip irrigation (Dinesh *et al.*, 2008). The time of drip operation was determined by the total discharge rate. The details of eight treatments are given below:

T1 = Drip irrigation with 60% PE + polyethylene mulch
T2 = Drip irrigation with 80% PE + polyethylene mulch
T3 = Drip irrigation with 100% PE + polyethylene mulch
T4 = Drip irrigation with 60% PE + without mulch
T5 = Drip irrigation with 80% PE + without mulch
T6 = Drip irrigation with 100% PE + without mulch
T7 = Basin irrigation with 100% PE + polyethylene mulch (Control)
T8 = Basin irrigation with 100% PE + without mulch (Control)

The fertilizer NPK @ 350:240:200 g plant^{-1} along with 50kg of FYM was applied in all the plants. The single super phosphate was applied as soil application in September and urea along with muriate of potash was applied through fertigation in 10 split doses.

RESULTS AND DISCUSSION

All the experimental trees produced flowers and fruits two times. The spring season flowering began during February-March and the fruits were harvested during July-September (rainy season). Likewise, monsoon season flowering produced fruits during the months of November-January. The plant height was not significantly affected by drip irrigation coupled with mulching that might be due to regular concurrent pruning. Plant height, fruit number, fruit weight and yield were recorded for consecutive years from 2015-2018 (Tables 1 to 4). The fruit yield was calculated on individual tree basis. Similar and increasing trends were found in subsequent years due to drip irrigation along with polyethylene mulch at 80 per cent PE. Drip irrigation at 80 per cent PE level and polyethylene mulching (T2**)** showed significantly higher yield (17.5 kg tree^{-1}), per cent flowering (86.1 tree^{-1}), and fruit weight (177.6 g) compared to minimum yield (10.3 kg tree^{-1}), per cent flowering (53.4 tree^{-1}) and fruit weight (147.2 g) in control trees (Table 4). Improvement in fruit quality in terms of higher TSS (13.4 °Brix), total sugar (6.65%), ascorbic acid (181.3 mg 100 g^{-1}) and reduced acidity

Table 1. Effect of drip irrigation and polyethylene mulching on growth of guava cv. Lalit under high density planting (6.0 x 3.0 m)

Treatment	Details	Tree height (m)			
		2015-16	2016–17	2017-18	Mean
T1	60% PE +PM	1.91	3.21	4.30	3.14
T2	80% PE +PM	1.95	3.30	4.46	3.23
T3	100% PE +PM	1.84	3.24	4.31	3.13
T4	60% PE + WPM	1.71	3.10	4.19	3.00
T5	80% PE + WPM	1.75	3.15	4.22	3.04
T6	100% PE + WPM	1.79	3.09	4.20	3.02
T7	100% PE basin irrigation + PM	1.71	3.03	4.14	2.96
T8	100% PE basin irrigation + WPM	1.69	3.01	4.09	2.93
CD (p=0.05)		0.095	0.088	0.029	-

PE- Pan evaporation, WPM- Without polyethylene mulching

Table 2. Effect of drip irrigation and polyethylene mulching flowering of guava cv. Lalit high density planting (6.0 x 3.0 m)

Treatment	Details	Flowering (%)			
		2015 - 16	2016 – 17	2017 - 18	Mean
T1	60% PE +PM	78.2	65.3	65.8	69.7
T2	80% PE +PM	88.1	83.1	87.1	86.1
T3	100% PE +PM	73.4	72.2	76.1	73.9
T4	60% PE + WPM	70.2	58.5	63.2	63.9
T5	80% PE + WPM	78.8	70.8	75.3	74.9
T6	100% PE + WPM	67.2	68.0	70.0	68.4
T7	100% PE basin irrigation + PM	59.3	64.3	65.5	63.0
T8	100% PE basin irrigation + WPM	48.1	50.1	62.0	53.4
CD (p=0.05)		11.32	2.35	3.36	-

PE- Pan evaporation, WPM- Without polyethylene mulching

Table 3. Effect of drip irrigation and polyethylene mulching on fruit weight of guava cv. Lalit under high density planting (6.0 x 3.0 m)

Treatment	Details	Fruit weight (g)*			
		2015 - 16	2016 – 17	2017 - 18	Mean
T1	60% PE +PM	129.7	189.7	161.6	160.3
T2	80% PE +PM	134.5	210.2	188.2	177.6
T3	100% PE +PM	126.2	192.3	175.3	164.6
T4	60% PE + WPM	138.1	166.8	169.5	158.1
T5	80% PE + WPM	142.3	172.5	196.1	170.3
T6	100% PE + WPM	128.2	178.2	182.3	162.9
T7	100% PE basin irrigation + PM	128.4	175.4	171.2	158.3
T8	100% PE basin irrigation + WPM	125.8	155.8	160.1	147.2
CD (p=0.05)		6.72	6.85	8.77	-

PE- Pan evaporation, WPM- Without polyethylene mulching
* mean of 10 fruits

Table 4. Effect of drip irrigation and polyethylene mulching on yield of guava cv. Lalit under high density planting (6.0 x 3.0 m)

Treatment	Details	Fruit yield (kg tree^{-1})			
		2015 - 16	2016 –17	2017 - 18	Mean
T1	60% PE +PM	9.08	12.86	17.3	13.1
T2	80% PE +PM	10.89	17.97	24.0	17.5
T3	100% PE +PM	8.03	15.71	20.2	14.5
T4	60% PE + WPM	6.22	10.58	15.1	10.6
T5	80% PE + WPM	6.81	14.92	18.4	13.3
T6	100% PE + WPM	7.10	13.24	17.5	12.6
T7	100% PE basin irrigation + PM	7.78	12.83	16.3	12.3
T8	100% PE basin irrigation + WPM	6.56	9.82	14.6	10.3
CD (p=0.05)		0.113	1.77	1.39	-

PE- Pan evaporation, WPM- Without polyethylene mulching

(0.24%) in T2 was recorded as compared to less TSS (11.0 °Brix), total sugar (6.50%), ascorbic acid (160.6 mg 100g^{-1}) and high acidity (0.33%) in control trees (100% PE + basin irrigation) (Table 5). Water saving to the tune of 18.33 to 47.52 per cent through mulching along with drip irrigation was observed against unmulched drip irrigated trees (28.02%) *vis-à-vis* unmulched basin irrigation (Table 6). The results are in conformity with the findings of Srinivas (2005) under Bangalore conditions, Dixit *et al.* (2005) under Raipur conditions Shirgure *et al.* (2004) under Nagpur conditions. Singh *et al.* (2015) reported maximum canopy volume and fruit yield with irrigation equivalent to 0.8 PE in guava under ultra high density planting system. Drip irrigation provides a consistent moisture regime in the soil due to which root

Table 5. Effect of drip irrigation and polyethylene mulching on fruit quality of guava cv. Lalit under high density planting (6.0 x 3.0 m)

Treatment	Details	TSS (°Brix)	Ascorbic acid (mg 100 g^{-1} FW)	Total sugar (%)	Acidity (%)
T1	60% PE +PM	11.8	167.1	6.56	0.26
T2	80% PE +PM	13.4	181.3	6.65	0.24
T3	100% PE +PM	12.4	170.2	6.54	0.27
T4	60% PE + WPM	11.2	161.1	6.51	0.29
T5	80% PE + WPM	12.2	175.2	6.54	0.26
T6	100% PE + WPM	11.8	163.3	6.57	0.30
T7	100% PE basin irrigation + PM	11.6	164.2	6.53	0.29
T8	100% PE basin irrigation + WPM	11.0	160.6	6.50	0.33
CD (p=0.05)		0.83	0.55	0.31	0.19

PE- Pan evaporation, WPM- Without polyethylene mulching

Table 6. Water saving percentage in polyethylene mulched and unmulched guava plants calculated on the basis of unmulched basin irrigation (as 0 value)

Treatment	Water saving (%)	
	Drip	Basin
Polyethylene mulching	47.52	18.33
Without mulching	28.02	-

remains active throughout the season resulting in optimum availability of nutrient and its proper translocation which accelerates the fruit growth and development in guava. Coelho and Borges (2004) and Singh *et al.* (2013) emphasized the importance of drip irrigation in fruit crops for better yield and quality.

The soil moisture increased 20-35 per cent with increasing the drip irrigation regimes in 0-40 cm, i.e., the effective root zone in the guava plants. The maximum available soil moisture content was recorded with drip irrigation at 100 per cent PE + polyethylene mulching which was at par with 80 per cent PE + PM during successive months. Minimum soil moisture was recorded in control. Similar findings were reported by Shirgure *et al.* (2003), Garg *et al.* (2007), Pratibha *et al.* (2013) and Singh *et al.* (2013). In case of drip irrigation, water is made available in the root zone thereby reducing the water stress pressure near roots (Bankar *et al.*, 1993). Similar results were also reported by Singh *et al.* (2015), Panigrahi *et al.* (2010), Kumar *et al.* (2008), Dixit *et al.* (2003) and Shirgure *et al.* (2004) in different fruit crops. It may be concluded from the present study that guava should be irrigated at 80 per cent PE coupled with polyethylene mulching in headed back 6.0 x 3.0 m crop density for increasing yield and quality.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, ICAR-Central Institute for Subtropical Horticulture, Lucknow for providing necessary facilities and to the National Committee on Plasticulture Applications in Agriculture and Horticulture (NCPAH), Ministry of Agriculture, New Delhi for providing necessary budgetary support during the course of this investigation.

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Manuscript recived on 17.12.2018

Manuscript accepted for publication 20.2.2019

Journal of Eco-friendly Agriculture **14**(2): 45-46. 2019

Efficacy of different management practices on the incidence of guava bark eating caterpillar, *Indarbela* sp.

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ABSTRACT

Bark eating caterpillar is a polyphagous insect pest causing considerable damage to various fruit crops. Very high incidence of this pest has been noticed in unmanaged guava orchards. Keeping in the view the importance of this pest, different management practices were evaluated under field conditions at ICAR-Central Institute for Subtropical Horticulture, Rehmankhera farm Lucknow. Among the treatments tested, swabbing of insecticide acephate on guava trunk reduced the bark eating caterpillar infestation effectively with no new ribbon formation by the caterpillar, it was followed by swabbing of profenophos, *Beaveria bassiana* and combination of dichlorovos sealer cum healer (IIHR product) +/- copper oxychloride, respectively. The results of the effect of *Beaveria bassiana* were most significant in the experiment as it not only reduced the incidence but also colonized in the guava trunk.

Key words: Guava, bark eating caterpillar, *Indarbela*, management

Bark eating caterpillar is a polyphagous insect pest that damages many fruit crops, avenue trees and ornamental trees (Ali *et al*., 2007). The incidence of this pest could be observed by the presence of an elongated zigzag ribbon like messy web or galleries made of fragments of bark pieces and silk excreta, near the stem or branch angles (Patil and Deshpande, 1990). Two species of bark eating caterpillar, i.e., *Indarbela quadrinotata* walker and *I. tetraonis* Moore, are commonly recorded on guava (Haseeb, 2005). The incidence of this pest varied from 56 to 82 per cent in orchards surveyed in guava growing regions of Uttar Pradesh (Anonymous, 2000, 2001, 2002, 2003). The life cycle of this pest is annual. The larvae is the damaging stage which bores into the trunk or branches, usually at forks or angles, to a depth of 15-25 cm. Stem damage by the pest results in an interruption in the translocation, leading to the inhibition of growth and adverse effects on fruiting capacity of the tree, while severe cases of infestation lead to complete death of young trees (Butani, 1979). The occurrence of bark eating caterpillar on guava is regular and its infestation is found throughout the year (Verma and Khurana, 1978). Under Gujarat conditions, the initial incidence of pest was reported during the first week of July, peak incidence was observed during the second week of November (Patel and Patel, 2008). Whereas in Uttar Pradesh (Lucknow) conditions, its initial incidence was observed during the second week of April and its peak incidence was during the first week of October (Haseeb, 2005). Population of bark eating caterpillar was found negatively correlated with the minimum temperature (Patel and Patel, 2008). Relatively high incidence of this pest has been recorded in the subtropical conditions in recent years. Different management practices were also developed for this pest by various workers, however, studies on effect of botanicals and entomopathogenic fungi for management of bark eating caterpillar in guava are scanty. Hence, this study was undertaken to evaluate different management practices against guava bark eating caterpillar under field conditions.

MATERIALS AND METHODS

The study was conducted at the experimental farm of ICAR-Central Institute For Subtropical Horticulture, Rehmankhera, Lucknow. The 10 years old guava trees cv. Allahabad Safeda were selected for the study. The experiment was conducted in randomized block design with 15 treatments replicated thrice. The treatments were, *viz*., T_1- swabbing of dichlorvos (5 ml L^{-1}) + Sealer cum healer (s/h) + copper oxychloride (3g L^{-1}) (COC) on trunk, T_2- swabbing of chlorpyriphos (2 ml L^{-1}) on trunk, T_3-swabbing of profenophos (2 ml L^{-1}) on trunk, T_4- swabbing of dichlorovos (5 ml L^{-1}) + COC (3g L^{-1}) on trunk, T_5- swabbing of acephate (2g L^{-1}) on trunk, T_6- swabbing of chlorpyriphos (2 ml L^{-1})+ s/h + COC (3g L^{-1}) on trunk, T_7- swabbing of profenophos (2ml L^{-1}) + s/h + COC (3g L^{-1}) on trunk, T_8- swabbing of dichlorovos alone, T_9- swabbing of sealer cum healer alone, T_{10} – swabbing of COC (3g L^{-1}) alone on trunk, T_{11}- swabbing pine oil alone on trunk, T_{12}- swabbing of linseed oil (3 ml L^{-1})+ neem oil (5ml L^{-1}) on trunk, T_{13}- swabbing of neem oil (5ml L^{-1}) alone, T_{14}- swabbing of entomopathogenic fungi *Beauveria bassiana* (2×10^8) @ 5g L^{-1} on trunk and T_{15} – control. The treatments were imposed during the last week of October 2015, when the peak incidence of the pest was observed. The observations were recorded before imposition of the treatments and 30, 45 and 60 days after the treatments. The number of ribbons formed by the caterpillar on the guava trunk was visually counted and recorded at each interval. After each observation, ribbons formed on trunk were

destroyed by hand to avoid the confusion of old ones with new ribbon formation. The data was subjected to analysis of variance and treatment means were compared by Tukey's honesty test.

RESULTS AND DISCUSSION

The effect of different treatments on the incidence of guava bark eating caterpillar was significantly different at 30 days after the treatment. Least number of ribbon formation by the caterpillar was recorded in the treatment where profenophos was swabbed on the trunk (0.33 ribbons tree^{-1}), which was followed by chlorpyriphos and acephate with mean number of ribbon formation per tree was 0.67. The findings are in agreement with the previous studies on organophosphate insecticides like demeton methyl, quinalphos and monocrotophos (Singh *et al*., 1988; Mote and Tambe, 1990; Patil and Deshpande, 1990; Verghese and Jayanthi, 2001). After 45 days of treatment the significant difference was observed in the level of incidence of the bark eating caterpillar. On the acephate treated trunk no incidence of bark eating caterpillar was observed, which was followed by profenophos and chlorpyriphos+ s/h + COC with 0.33 ribbon formation per tree. Dichlorovos (0.5%) along with s/h (IIHR product) and copper oxychloride (3%) reduced the bark eating caterpillar infestation significantly (Anonymous, 2015). At 60 days after the treatment swabbing of Acephate on the trunk stands superior in comparison to other treatments, which was followed by profenophos and *Beauveria bassiana.* The results are in corroboration with the findings of Fasih and Srivastava (1988), they reported that entomogenous fungus *Beauveria bassiana* killed the caterpillars and provide natural control of the pest in guava orchards. Application of conidial suspension of naturalis – L (0.40%) in the borer hole also proved effective in bringing down (up to 81.48%) the incidence of this pest (Anonymous, 2001).

Table 1: Bio-efficacy of different treatments on the incidence of guava bark eating caterpillar

Treatments	Mean number of new ribbons formed tree^{-1}			
	Pre-count	30 DAT*	45 DAT*	60 DAT*
Dichlorvos + s/h + COC	2.33 ± 0.88	1.33 ± 0.33	1.00 ± 0.00	0.67 ± 0.33
Chlorpyriphos	0.67 ± 0.67	0.67 ± 0.33	1.33 ± 0.33	1.00 ± 0.00
Profenophos	0.67 ± 0.67	0.33 ± 0.33	0.33 ± 0.33	0.33 ± 0.33
Dichlorovos + COC	1.33 ± 0.88	1.33 ± 0.88	1.00 ± 0.58	1.33 ± 0.88
Acephate	1.67 ± 1.20	0.67 ± 0.67	0.00 ± 0.00	0.00 ± 0.00
Chlorpyriphos + s/h + COC	2.00 ± 0.58	1.33 ± 0.67	0.33 ± 0.33	1.67 ± 0.33
Profenophos + s/h + COC	4.00 ± 0.00	1.00 ± 0.00	0.67 ± 0.33	1.33 ± 0.33
Dichlorovos	3.33 ± 1.33	3.67 ± 2.73	2.67 ± 1.76	2.00 ± 0.58
Sealer cum Healer (s/h)	2.67 ± 1.45	2.67 ± 1.20	3.67 ± 0.33	2.00 ± 0.58
COC	3.00 ± 1.00	6.67 ± 0.33	4.33 ± 1.20	3.33 ± 0.88
Pine oil	4.00 ± 2.08	3.00 ± 1.73	3.33 ± 1.76	1.67 ± 0.33
Linseed oil + Neem oil	1.67 ± 1.20	1.67 ± 1.20	2.00 ± 1.00	1.00 ± 0.58
Neem oil	1.67 ± 0.33	2.33 ± 0.33	1.67 ± 0.33	2.33 ± 0.33
B.bassiana	5.00 ± 2.08	1.67 ± 0.67	1.67 ± 1.20	0.33 ± 0.33
Control	1.33 ± 0.33	2.33 ± 0.88	3.00 ± 0.58	2.33 ± 0.88
F	1.37 NS	2.24*	2.33*	2.98**

*DAT-Days after treatment.

The study concludes that treating the guava trunk with acephate or entomopathogenic fungi *B. bassiana* will serves as the best management practice against guava bark eating caterpillar.

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Manuscript recived on 6.11.2018

Manuscript accepted for publication 11.1.2019

Journal of Eco-friendly Agriculture **14**(2): 47-49. 2019

Efficacy of botanical insecticides against pod borer (*Maruca vitrata*) on cowpea (*Vigna unguiculata*)

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ABSTRACT

The field experiment was conducted during kharif 2016-17 to evaluate botanical insecticides against pod borer on cowpea. Two sprays of insecticides were done at fifteen days interval. The most effective treatment against pod borer was Chlorpyriphos 20 EC@ 300g a.i. ha^{-1} after first spray (1.97, 1.74 and 1.53 larvae m^{-2} at 3, 5, and 7 days after spray, respectively) and second spray (1.51, 1.54 and 1.93 larvae m^{-2} at 3, 5 and 7 days after spray, respectively) followed by Neem oil @ 2 per cent first spray (4.30, 4.08 and 3.52 larvae m^{-2} at 3, 5 and 7 days after spray, respectively) and second spray (3.00, 2.85 and 3.28 larvae m^{-2} at 3, 5 and 7 days after spray, respectively); and NSKE @ 5 per cent after first spray (4.62, 4.18 and 3.81 larvae m^{-2} at 3, 5 and 7 days after spray, respectively) and second spray (3.12, 3.09 and 3.37 larvae m^2 at 3, 5 and 7 days after spray, respectively). The maximum larval population was found in untreated plot. The highest green pod yield of cowpea was recorded in plants sprayed with Chlorpyriphos 20 EC @ 300 g a.i. ha^{-1} (53.12 qha^{-1}) followed by Neem oil @ 2 per cent (43.53 qha^{-1}) and NSKE @ 5 per cent (42.11 qha^{-1}), while lowest yield was recorded in untreated control plot (32.16 qha^{-1}).

Key words: Evaluation, cowpea, pod borer, botanical insecticides

Cowpea (*Vigna unguiculata*), an important legume crop, belonging to family Leguminaceae, is known as vegetable meat due to its high content of protein in the grain with better biological value on dry weight basis. The grain contains 26.61 per cent protein, 3.99 per cent lipid, 56.24 per cent carbohydrates, 8.60 per cent moisture, 3.84 per cent ash, 1.38 per cent crude fibre, 1.51 per cent gross energy, and 54.85 per cent nitrogen free extract (Owolabi *et al*., 2012). The pod borer complex poses serious threat to cowpea cultivation which includes *Maruca vitrata* (Fabricius), *Lampides boeticus* (L.), *Helicoverpa armigera* (Hubner), *Etiella zinckenella* Treitsche, *Adisura atkinsoni* Moore and *Exelastis atomosa* (Walsingham). Application of highly toxic chemical insecticides at short intervals against insect pests has resulted in many deleterious effects such as residual toxicity, insecticide resistance, pest resurgence, destruction of natural enemies and environmental pollution. In this context, the use of botanicals and newer chemicals for managing pod borer complex assumes greater significance. Azadirachtin containing formulations are effective in reducing the larval population of pod borers and contribute to a higher yield (Singh and Yadav, 2006). The major constraint in the cultivation of cowpea is insect pest attack which has been observed to have caused up to 70 per cent grain yield loss (Adipala *et al*., 2005).

Among the insecticides, the botanical insecticides are naturally occurring, often slow-acting crop protectant and minimal residual effects. Therefore, the plant pesticides have been recommended as a suitable alternative of plant protection with minimum negative risks (Isman, 2006). The present study was undertaken to evaluate the efficacy of botanical insecticides against spotted pod borer on cowpea (*Vigna unguiculata*).

MATERIALS AND METHODS

The field experiment was conducted at the farm of College of Agriculture and Research Station, Boirdadar, Raigarh during *Kharif* 2016-17. The experiment was laid out in randomized block design (RBD) with eight treatments (Table 1) and three replications. The plot size was 5x4 meter (m) and the seeds of cowpea were sown at 45x30 centimeter (cm) spacing during the last week of July. Cowpea variety Gomti was used for experiment. All agronomic practices except plant protection were followed as and when required. The sprays of botanical and conventional insecticides were applied at the initial incidence of pod borer and two sprays were given. All the sprayings were done by using knapsack sprayer at 15 days interval. The one m^2 area was randomly selected and plants were marked to count the number of pod borer larvae before spray, 3, 5 and 7 days after each spray. The green pod yield was recorded per plot and converted into quintal per hectare. The calculated data of pod borer larvae were transformed into square root values $\sqrt{x + 0.5}$ as per the standard requisites (Gomez and Gomez, 1984).

Per cent reduction in larval population over control was calculated by using following modified formula given by Henderson and Tilton (1955).

Per cent reduction over control =

$$\left(1-\frac{\frac{Larval\ population}{in\ contorl\ before\ treatment}\times\frac{Larval\ population}{in\ treated\ plot\ after\ treatment}}{\frac{Larval\ population}{in\ contorl\ after\ treatment}\times\frac{Larval\ population}{in\ treated\ plot\ before\ treatment}}\right)\times 100$$

Table 1: Treatment details

Treatment	Treatment details
T_1	Neem oil @ 2%
T_2	NSKE @ 5%
T_3	Karanj oil @ 2%
T_4	Karanj seed powder @ 30 kg ha^{-1}
T_5	Chilli + Garlic solution @ 9 kg ha^{-1}
T_6	Chilli solution @ 10 kg ha^{-1}
T_7	Chlorpyriphos 20 EC @ 2 ml^{-1}
T_8	Untreated control – plain water spray

RESULTS AND DISCUSSION

The result presented in Table 2 revealed that the larval population of pod borer in cowpea was significantly reduced in treated plants as compared to untreated plants after each of the sprays. No significant difference was observed in pre-treatment count of larvae of pod borer before first and second sprays.

After the first spray, among different botanical and conventional insecticides, the minimum number of larval population of pod borer in cowpea was found in chloropyriphos 20 EC @ 2 ml litre^{-1} water, i.e., 1.97, 1.74 and 1.53 larvae m^{-2} at 3, 5 and 7 days after spray (DAS), respectively followed by botanical insecticide neem oil @ 2 per cent, i.e., 4.30, 4.08 and 3.52 larvae m^{-2} and NSKE @ 5 per cent, *i.e.*, 4.62, 4.18 and 3.81 larvae m^{-2} at 3, 5 and 7 DAS, respectively. The maximum larval population of 8.11, 9.39 and 9.53 larvae m^{-2} at 3, 5 and 7 DAS, respectively, was recorded in control (untreated) plots. The overall per cent reduction over control treatment was highest in chloropyriphos 20 EC @ 2 ml litre^{-1} water, i.e., 80.84 per cent (1.75 larvae m^{-2}) followed by neem oil @ 2 per cent, i.e., 54.0 per cent (3.97 larvae m^{-2}) and NSKE @ 5 per cent, i.e., 53.54 per cent (4.20 larvae m^{-2}).

In second spray, the minimum larval population was found in chloropyriphos 20 EC@ 2 ml litre^{-1} water, i.e., 1.51, 1.54 and 1.93 larvae m^{-2} at 3, 5 and 7 DAS followed by neem oil @ 2 per cent (3.00, 2.85 and 3.28 at 3, 5 and 7 DAS, respectively) and NSKE @ 5 per cent (3.12, 3.09 and 3.37 at 3, 5 and 7 DAS, respectively). The overall per cent reduction over control treatment was highest in chloropyriphos 20 EC @ 2 ml litre^{-1} water, i.e., 79.56 per cent (1.66 larvae m^{-2}) followed by neem oil @ 2 per cent, i.e., 59.38 per cent (3.04 larvae m^{-2}) and NSKE @ 5 per cent, i.e., 57.6 per cent (3.19 larvae m^{-2}). The chloropyriphos 20 EC @ 2 ml litre^{-1} water was effective to minimize larval population in cowpea. In the present study, botanical insecticides performed well to reduce the larval population of pod borer in cowpea. The effectiveness of NSKE against *M. distalis* infesting green gram (Irulandi and Balasubramaniam, 2000) and *H. armigera*

Table 2: Efficacy of botanical insecticides against larval population of pod borer in cowpea during *Kharif* 2016

Treatment	Pre-treatment (larvae m^{-2})	Larval population at different DAS after first spray (larvae m^{-2})					Pre-treatment (larvae m^{-2})	Larval population at different DAS after second spray (larvae m^{-2})				
		3DAS	5DAS	7DAS	Mean	PROC		3DAS	5DAS	7DAS	Mean	PROC
T_1	16.61	4.30	4.08	3.52	3.97		13.14	3.00	2.85	3.28	3.04	
	(4.12)	(2.19)	(2.14)	(2.00)	(2.11)	54.00	(3.69)	(1.87)	(1.83)	(1.94)	(1.88)	59.38
T_2	17.40	4.62	4.18	3.81	4.20		13.21	3.12	3.09	3.37	3.19	
	(3.23)	(2.26)	(2.16)	(2.08)	(2.17)	53.55	(3.70)	(1.90)	(1.90)	(1.97)	(1.92)	57.60
T_3	20.76	5.67	5.16	5.14	5.32		12.43	3.53	3.47	3.97	3.66	
	(4.59)	(2.49)	(2.38)	(2.37)	(2.41)	50.68	(3.59)	(2.01)	(1.99)	(2.11)	(2.04)	48.30
T_4	17.87	5.25	5.02	4.90	5.06		13.88	3.47	3.36	3.83	3.55	
	(4.04)	(2.40)	(2.35)	(2.32)	(2.36)	45.51	(3.79)	(1.99)	(1.96)	(2.08)	(2.01)	55.09
T_5	18.79	4.94	4.70	4.24	4.63		13.23	3.27	3.22	3.45	3.31	
	(4.38)	(2.33)	(2.28)	(2.18)	(2.26)	52.58	(3.71)	(1.94)	(1.93)	(1.99)	(1.95)	56.07
T_6	20.70	5.97	5.56	5.73	5.75		13.95	3.64	3.63	4.17	3.81	
	(4.60)	(2.54)	(2.46)	(2.50)	(2.50)	46.54	(3.80)	(2.04)	(2.03)	(2.16)	(2.08)	52.05
T_7	17.58	1.97	1.74	1.53	1.75		14.26	1.51	1.54	1.93	1.66	
	(4.24)	(1.57)	(1.50)	(1.43)	(1.50)	80.84	(3.84)	(1.42)	(1.43)	(1.56)	(1.47)	79.56
T_8	17.34	8.11	9.39	9.53	9.01		13.66	6.15	8.01	9.18	7.78	
	(4.22)	(2.94)	(3.13)	(3.16)	(3.08)	-	(3.76)	(2.58)	(2.91)	(3.11)	(2.86)	-
Sem	-	0.04	0.08	0.05	0.06		-	0.04	0.05	0.06	0.05	
CD at 5%	NS	0.11	0.25	0.14	0.17		NS	0.13	0.16	0.17	0.15	

Note: Figure in parenthesis is square root transformed value, DAS: Days after spraying, PROC: Percent reduction over control

infesting green pod of Indian bean (Dalwadi *et al*., 2008) have been reported, the data obtained here supports these findings. The neem based extract reduce pod borer and protect the cowpea plants. It might have been absorbed by the flowers/pods through osmotic pressure causing the insect to stop feeding (Oparaeke *et al*., 2005).

Table 3: Botanical insecticidal impact on total green pod yield of cowpea

Treatment	Green pod yield (q ha^{-1})	Increased yield over control (q ha^{-1})
T_1	43.53	11.37
T_2	42.11	9.95
T_3	40.08	7.92
T_4	41.44	9.28
T_5	41.86	9.70
T_6	38.59	6.43
T_7	53.12	20.96
T_8	32.16	-
Sem	1.37	
CD at 5%	4.15	

The data presented in Table 3 indicates that with the application of treatments the green pod yield of cowpea was significantly increased as compared to untreated control. The highest green pod yield was recorded in chloropyriphos treatment (53.12 q ha^{-1}) followed by Neem oil @ 2 per cent (43.53 q ha^{-1}) and NSKE @ 5 per cent (42.11 q ha^{-1}). The lowest yield was recorded in untreated plot (32.16 q ha^{-1}) followed by chilli solution 10 kg ha^{-1} (38.59 q ha^{-1}). These findings are in agreement with the results obtained by Dalwadi *et al*. (2008) in Indian bean.

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Manuscript recived on 13.12.2018

Manuscript accepted for publication 12.2.2019

Journal of Eco-friendly Agriculture **14**(2): 50-53. 2019

Dissipation of imidacloprid residues in mango orchard soil quantified by HPLC

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ABSTRACT

Imidacloprid, a neonicotinoid insecticide, is widely used in mango ecosystem to control mango hopper at pre-bloom stage. Persistence of imidacloprid in soil is well reported. While spraying any insecticide to a tree, some portion does come in contact with rhizosphere soil. Hence after spraying at 0.005 per cent to mango (cv. Dashehari) trees, its residue was analyzed in rhizosphere soil by HPLC. Soil samples were extracted with acetonitrile by vortexing followed by ultrasonic solvent extraction without any cleanup. Imidacloprid dissipated from its initial deposit of 0.760 mg g^{-1} at zero day to 0.044 mg g^{-1} after 60 days of spraying in mango orchard soil (Sandy loam). After 60 days of application, 94.21 per cent of imidacloprid degradation was recorded in soil. The dissipation rate followed pseudo first-order kinetics in soil with calculated half-life (DT_{50}) value of 17.5 days. Imidacloprid has been found a persistent insecticide in mango orchard soil.

Key words: Imidacloprid, persistence, mango orchard soil

Mango (*Mangifera indica* L.) is a commercially important fruit worldwide which grows in various states of India. Uttar Pradesh is one of the leading mango producing states in India and Lucknow region is famous for the production of Dashehari mango, which is unique in taste, aroma and flavor. It is mainly used for table purpose and generally not used for processing. Hopper (*Amritodus atkinsoni* or *Idioscopus niveosparsus*), the most damaging insects for Dashehari mango, attacks mostly at flowering stage. It is a sucking insect and imidacloprid [1-(6-chloro-3-pyridinyl methyl)-N-nitro-2-imidazolidinimine] is found effective in controlling hopper even at lower doses of 0.2 ml/L (Verghese 2000). It is a broad-spectrum systemic insecticide from neonicotinoid group used to control sucking insects in many fruit and vegetable crops. It has been reported that imidacloprid is an extremely persistent insecticide in soil and can cause damage to soil health by depleting the number of soil invertebrates which play major role in maintaining soil structure and nutrient cycling (Goulson, 2013). In different countries persistence of imidacloprid varies depending on types of soil, *viz*. 48-190 days half-life in sandy loam soil in Australia (Baskaran *et al*., 1997), 990-1230 days half-life in red brown soil in Australia (Baskaran *et al*., 1999), half-life of 445-518 days in sandy loam soil and 233-366 days in silty clay loam soil in Spain (Fernández-Bayo *et al*., 2009), 29-48 days half-life in alluvial, lateritic and coastal alkaline soils in West Bengal, India (Sarkar *et al*., 2001) and 39-50 days half-life in black, sandy loam, clay and red soils of Gujarat, India (Samnani *et al*., 2013). In another literature a half-life of about 156 days for imidacloprid, a relatively polar, non-volatile insecticide with high solubility in water, in soil has been reported (Jeschke *et al*., 2011). HPLC was found to be the simple, sensitive and cost effective method for the analysis of imidacloprid in various matrices like soil and water (Samnani *et al*., 2011; Baig *et al*., 2012), fruits (Mohapatra *et al*., 2011; Bhattacherjee, 2013) and vegetables (Gajbhiye *et al*., 2004; Mandal *et al*., 2010). However, most of these studies have been conducted under laboratory conditions. The present investigation was, therefore, undertaken to study the persistence of imidacloprid in rhizosphere soil under mango cultivation after spraying to mango trees to control hopper.

MATERIALS AND METHODS

Technical grade standard of imidacloprid (99% purity) was procured from Sigma-Aldrich, USA. Formulation of imidacloprid (MediaÒ 17.8 SL, Dhanuka Agritech Ltd., Jammu & Kashmir) was collected from the local market. Analytical and HPLC grade solvents were procured locally. Soil sample under cultivation of mango cv. Dashehari was chosen for the present investigation because Dashehari was frequented by imidacloprid application to control mango hopper.

Imidacloprid formulation was sprayed to Dashehari mango trees at 0.005 per cent dose during flowering stage. The deposition of imidacloprid to rhizosphere soil after spraying was considered for residues analysis in this study, because spraying of insecticide to fruit trees would invariably led to its deposition in the rhizosphere soil beneath the canopy. Soil samples (500 g) were collected at 0 (1 hr after spraying), 10, 20, 30, 40, 50 and 60 days after the spray from

0-15 cm upper layer. It was then crushed to powder with the help of pestle and mortar, air dried at room temperature and sieved to remove exogenous materials. The soil under Dashehari cultivation at the Institute farm at Rehmankhera, Lucknow is sandy loam type soil with 7.30 pH. Soil physico-chemical properties are given in Table 1.

Table 1. Physico-chemical properties of soil used in the present investigation

Parameters	Amount
Type of soil	Loam to fine sandy loam
pH	7.30
Organic carbon	0.68%
Phosphorus (P)	9.23 mg kg^{-1}
Potassium (K)	81.25 mg kg^{-1}
Sodium (Na)	63.75 mg kg^{-1}
Calcium (Ca)	1400 mg kg^{-1}
Zinc (Zn)	1.28 mg kg^{-1}
Copper (Cu)	1.94 mg kg^{-1}
Manganese (Mn)	24.76 mg kg^{-1}
Iron (Fe)	32.86 mg kg^{-1}

Ten gram soil sample (each for three replications, prepared by quartering method) was taken in 50 ml culture tube and 25 ml of acetonitrilie was added to it. The extraction was done by vortexing for 5 min followed by ultrasonic solvent extraction (USE) for 20 min and filtration. The process was repeated again with 25 ml acetonitrile and the pooled filtrate was evaporated in a rotary vacuum evaporator at 55° C to near dryness. The residue was immediately dissolved in 5 ml of HPLC grade acetonitrile for analysis by HPLC. The USE method of extraction is an effective and single step method which does not require any clean-up and thereby substantially reducing time and saving money as mentioned by Pan *et al*. (2008).

A Shimadzu make HPLC (model LC10 ATVP) coupled with photodiode array (PDA) detector and reverse phase mBondapak™ C_{18} column (300 x 3.9 mm i.d., 10m film thickness, 125Å porosity) was employed for residue analysis of imidacloprid in soil. The mobile phase was acetonitrile : water (35 : 65, v/v) run isocratically with a flow-rate of 0.8 ml/ min (Bhattacherjee, 2013). The detection wavelength and injection volume were 270 nm and 20 ml, respectively. All the samples were filtered through a sample clarification kit using nylon membrane filter (Millipore, 0.45 mm thickness, 13 mm diameter) before injecting through Rheodyne injector having 20 ml loop.

Stock solution of imidacloprid (400 mg L^{-1}) was prepared by dissolving accurately weighed 10 mg of imidacloprid in 25 ml of HPLC grade acetonitrile. Working solutions of 0.01 to 10 mg L^{-1} were prepared in the same solvent by subsequent dilution. The calibration curve for imidacloprid was found linear in this range. The calculation of limit of detection (LOD) and limit of quantification (LOQ) at signal to noise ratio of 3:1 and 10:1, respectively, was done to check the precision of the HPLC technique. Similarly, to judge the accuracy of the USE method, recovery study was conducted by fortifying soil samples at three concentration levels (0.02, 0.2 and 1 mg L^{-1}). The residual half-life value (DT_{50} in days) was calculated as per the method described in literature (Hoskins, 1961).

RESULTS AND DISCUSSION

Dissipation of imidacloprid in mango orchard soil

The recovery of imidacloprid from soil at three fortification levels ranged between 90.50 to 93.52 per cent (Table 2). The limit of detection and limit of quantification of imidacloprid in soil were observed as 0.01 and 0.02 mg kg^{-1}, respectively. Samnani *et al*. (2011) have also reported recoveries of imidacloprid from black, red, sandy loam and clay soil as 95.18, 94.66, 95.27 and 94.78 per cent, respectively. They found LOD and LOQ of 0.006 and 0.02 mg kg^{-1}, respectively, for imidacloprid in soil analyzed by HPLC with UV detector. Sarkar *et al*. (2001) have reported an average recovery of 92.0- 94.1 per cent for imidacloprid in alluvial, lateritic and coastal alkaline soils using HPLC-UV.

The initial soil concentration of imidacloprid residues at 0-15 cm depth was recorded as 0.760 mg kg^{-1} after spraying at mango trees @ 0.005 per cent dose. It dissipated to 0.044 mg/kg after 60 days of spraying (Table 3), which meant that residues persisted in soil up to 60 days. During the course of study the degradation of imidacloprid was 76.97 and 94.21 per cent after 30 and 60 days of application, respectively. The rate of dissipation followed pseudo first-order kinetics in soil with initial rate of dissipation up to 30 days was

Table 2. Recoveries of imidacloprid from mango orchard soil by HPLC-PDA

Fortification level (mg kg^{-1})	Amount recovered (mg kg^{-1})			Recovery (%)			Average recovery (%) ± SD
	R_1	R_2	R_3	R_1	R_2	R_3	
0.02	0.0182	0.0174	0.0187	91.00	87.00	93.50	90.50 ± 3.279
0.2	0.1832	0.1893	0.1815	91.60	94.65	90.75	92.33 ± 2.051
1.0	0.9123	0.9362	0.9571	91.23	93.62	95.71	93.52 ± 2.242

SD = Standard deviation

faster as compared to later period, which was quite slow (Figure 1). The residual half-life value in soil was calculated as 17.50 days from this indirect application.

Table 3. Dissipation of imidacloprid in mango orchard soil after indirect application

Days after application	Residues (mg kg^{-1})			Mean residues (mg kg^{-1}) ± SD	Dissipation (%)
	R_1	R_2	R_3		
0	0.803	0.767	0.711	0.760 ± 0.046	–
10	0.692	0.606	0.528	0.609 ± 0.082	19.87
20	0.45	0.44	0.387	0.426 ± 0.033	43.95
30	0.172	0.181	0.171	0.175 ± 0.005	76.97
40	0.128	0.15	0.132	0.137 ± 0.012	81.97
50	0.097	0.083	0.094	0.091 ± 0.007	88.06
60	0.029	0.046	0.056	0.044 ± 0.014	94.21
DT_{50}				17.50 days	

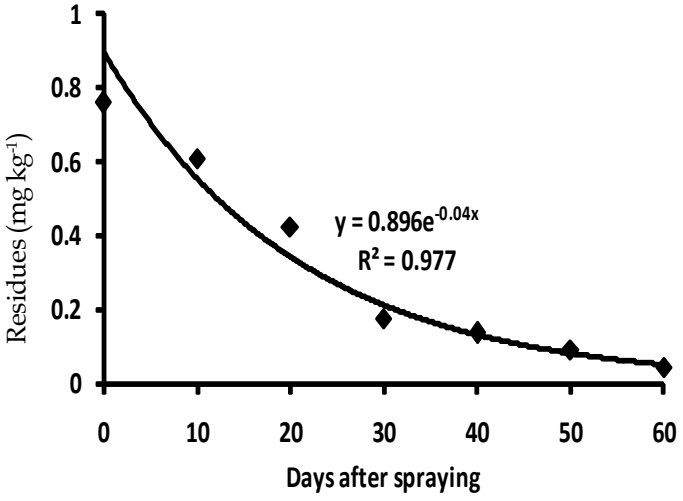

Figure 1: Exponential curve for dissipation of imidacloprid residues in mango orchard soil

Dissipation of imidacloprid in three different types of soils of West Bengal (Gangetic alluvial soil of Kalyani, lateritic soil of Jhargram and coastal alkaline soil of Canning) followed first-order kinetics with DT_{50} values ranging from 28.7 to 47.8 days (Sarkar *et al*., 2001). After 40 days of drenching apple tree basin at 890 and 1780 g a.i. ha^{-1}, imidacloprid residue in apple orchard soil was reported to be 0.14-3.61 mg kg^{-1} (Dubey *et al*., 2006). Samnani *et al*. (2013) have reported that imidacloprid degradation under laboratory conditions was faster in black soil followed by clay soil, red soil and sandy loam soil with half-life values of 39.10, 42.74, 45.69 and 50.10 days, respectively. In Australia, it was noticed that half-life value of imidacloprid in red brown soil at termiticidal application rate of 50 mg kg^{-1} was quite higher (990-1230 days) than normal agricultural rate of 1 mg kg^{-1} (Baskaran *et al*., 1999) under laboratory conditions which followed first-order kinetics. Persistence of imidacloprid depends mainly on soil organic carbon content and insecticide concentration. Due to indirect application of insecticide to soil, half-life of imidacloprid is comparatively lower in mango orchard soil. In vegetated soil, half-life of imidacloprid was reported over 100 days, whereas, in non-vegetated soil it exceeded 180 days and more than 3 years in dry and aerobic conditions (Scholz and Spiteller, 1992). In greenhouse soil in Palestine, imidacloprid degraded with a half-life of 61 days under laboratory conditions (Haddad *et al*., 2014). In a pot experiment conducted at Chennai, Tamil Nadu, imidacloprid has been found persistent up to 7 days only in four different soils (sandy loam, loamy sand, sandy clay and clay) with half-life values ranging between 2.8 to 4.0 days (Pandiselvi *et al*., 2011). Soil samples collected at 15 days after the last application of combination products of â-cyfluthrin and imidacloprid to brinjal was noticed devoid of imidacloprid residues (Mandal *et al*., 2010). Similar observation regarding below detectable limit of imidacloprid residues has also been reported in soil under grape cultivation collected 105 days after the last treatment (Mohapatra *et al*., 2011).

The study concludes that imidacloprid is a stable insecticide in the soil environment and persists for two months in mango orchard soil (sandy loam) despite its indirect application. It's rate of dissipation followed pseudo first-order kinetics in soil with faster rate during initial period and slower one during later stages.

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Manuscript recived on 12.1.2019

Manuscript accepted for publication 22.3.2019

Journal of Eco-friendly Agriculture **14**(2): 54-56. 2019

Pests status of coconut in managed and unmanaged garden

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ABSTRACT

The major pests incidence and intensity on coconut was recorded during fixed plot survey. It was carried out at bimonthly interval from April, 2018 to February, 2019 at Regional Research Station, Bhatye, Ratnagiri jurisdiction. Two plots were selected for observations, one was well managed (good sanitation, use of fertilizes, etc.) and another unmanaged (poor sanitation, no use of fertilizers). The major pest status in managed garden was minimum which recorded rhinoceros beetle (25.73%), eriophyid mite (41.74%) and mite grade index 0.71. Whereas, maximum infestation of rhinoceros beetle, red palm weevil and eriophyide mite were observed to be 30.31, 0.37, 71.70 per cent, respectively and mite grade index was 1.57 (moderate) in unmanaged garden. The average data of two fixed plots revealed that the incidence of rhinoceros beetle was in the range of 7.64 to 48.00 per cent and maximum infestation was observed in the month of June, 2018 (48.00%), and minimum incidence was observed in February, 2019 (7.64 %). The infestation of red palm weevil and black headed caterpillar were 1.11 and 0.64 per cent only in February, 2019. The infestation of eriophyid mite was in the range of 41.65 to 64.98 per cent and maximum infestation (64.98%) was noticed in the month of April, 2018 and least incidence was observed in October, 2018 (41.65%). The mite damage grade index 1.39 (moderate) was recorded in February, 2019. However, lowest MGI (0.67) was observed in October, 2018.

Key wards: Coconut, rhinoceros beetle, red palm weevil, Eriophyid mite, black headed caterpillar

Coconut (*Cocos nucifera* Linn) is the most important versatile tree crop cultivated in the tropics providing livelihood and employment securities to the rural agrarian mass in the region. It is an important plantation crop which is being mainly cultivated by farmers of southern state and states like Goa, Maharashtra, Gujarat, West Bengal, Odisha, Assam, Andaman and Nicobar Islands, Lakshdweep and Minicoy islands are also having considerable area under coconut. Coconut is essentially a crop of marginal and small farmers in India. The low size of the land holding by coconut growers is a serious challenge to both profitability and sustainability of the crop. The coconut palm is attacked by a variety of pests. The majority attack the leaves, while others attack the trunk (stem), inflorescence, young nuts and roots. Some are major pests which cause considerable damage and yield loss, while some are less damaging. Most of these insects remain as minor pests, either because the environmental conditions are not favourable for their rapid multiplication or because they are adequately controlled by natural enemies. The coconut eriophyid mite *Aceria guerreronis* Keifer was first reported as a serious pest in Kerala during 1997-98. Subsequently, the devastating effect of these mites were noticed in Coimbatore and Theni districts of Tamil Nadu and Bangaluru in Karnataka (Sathiamma *et al*., 1998 and Mohanasundaram *et al*., 1999). Since extensive damage has been caused to coconut palms in the southern states of India (Nair *et al*., 2000), Mathen (1962) reported that the red palm weevil, *Rhynchophorus ferrugineus* Oliv, (RPW) (curculionidae, Coleoptera) is a serious pest attacking different species of palm trees (e.g., date palm, coconut palm and royal palm). Chalapathi Rao *et al*. (2018) found that per cent of leaf damage due to rhinoceros beetles ranged from 12.5 to 35.5 and spindle damage ranged from 33.3 to 45 per cent. By considering economic importance of pest and yield loss of palm, the present investigation was carried out for assessment of major pests in fixed plots during survey to know the exact status of coconut pests in Ratnagiri district of Konkan region of Maharashtra.

MATERIALS AND METHODS

A field survey was carried out as Entomological Experiment under Pest surveillance of coconut at AICRP (Palms), RCRS, Bhatye, Dist. Ratnagiri (M.S.) during 2018-19 for knowing the exact pests status on coconut. Two different plots at different locations were selected with well managed (good sanitation, use of fertilizers, etc.) and unmanaged (poor sanitation, no use of fertilizers). One plot has need based management treatment for major pests and other plot with no management aspect (natural control). Six observations were made per year at 2 months interval, *viz*., April, June, Aug., Oct., Dec. and February. Major pests like rhinoceros beetle, red palm weevil, black headed caterpillar and eriophyid mite were recorded by using following methods:

Rhinoceros beetle: Per cent of palm infested (out of 100 palms garden^{-1})- The top 10 fronds in each palm with single/ multiple cuts was observed. Per cent leaf damage (25 palms at random garden^{-1}) (infested/total number of leaves x 100).

Eriophyid mite: Per cent nut infested (mite infested nuts/total nuts) (25 palms at random garden^{-1}). Damage grade (mature bunch) (intensity 0-4 scale) (out of minimum 100 nuts garden^{-1}) using CPCRI scales were followed.

CPCRI Scale			
Per cent damage on nut surface	Scale	Grade Index	Intensity
Nuts with no mite damage	0	0	Nil
< 25%	1	0.1-1.0	Mild
25-50%	2	1.1-2.0	Moderate
50-75%	3	2.1-3.0	High
>75%	4	3.1-4.0	Severe

Red palm weevil: Per cent of palms infested (out of total palm (>100) garden^{-1}) with typical symptoms of RPW. Black headed caterpillar: Per cent leaf damage (infested leaf/total leaf) (50 palms garden^{-1}).

RESULTS AND DISCUSSION

The data depicted in Table 1 indicated that minimum per cent incidence of major pest status, *viz*., rhinoceros beetle (25.73%), eriophyid mite (41.74%) and mite grade index 0. 71 was noticed in managed garden. Whereas, maximum infestation of rhinoceros beetle, red palm weevil and eriophyid mite were observed 30.31, 0.37, 71.70 per cent, respectively with mite grade index of 1.57 (moderate) in unmanaged to be garden (Table 2).

The average data of two fixed plots are presented in Table 3. The data revealed that the incidence of rhinoceros beetle was in the range of 20.00 to 48.00 per cent and maximum infestation was observed in the month of June, 2018 (48.00 %) and minimum incidence was observed in December, 2018 (20.00 %). Chalapathi Rao *et al*. (2018) found that the per cent of leaf damage due to rhinoceros beetles ranged from 12.5 to 35.5 and spindle damage ranged from 33.3 to 45 per cent. The infestation of red palm weevil and black headed caterpillar were not recorded during these surveys. The infestation of eriophyid mite was in the range of 41.65 to 64.98 per cent. Similar results on eriophyid mite

Table 1: Extent of infestation by different pests in fixed plot (managed) during survey in Maharashtra (Plot No. 1)

Month	Rhinoceros beetle			Red palm weevil incidence (%)	Black headed caterpillar incidence (%)	Eriophyid mite	
	Incidence (%)	Leaf damage (%)	Spindle damage (%)			Infestation (%)	MGI
April,18	24.00	2.80	0.00	0.00	0.00	56.58	1.22
June,18	52.00	7.03	0.00	0.00	0.00	53.11	0.92
Aug.,18	36.00	4.00	8.00	0.00	0.00	37.82	0.50
Oct., 18	28.00	3.60	0.00	0.00	0.00	35.82	0.61
Dec., 18	08.00	1.60	0.00	0.00	0.00	30.88	0.54
Feb., 19	06.41	2.00	1.28	0.00	0.00	36.25	0.51
Mean ± SE	25.73 ± 7.72	3.50 ± 0.87	1.54 ± 1.43	0.00 ±0.00	0.00 ±0.00	41.74 ± 4.68	0.71 ± 0.13

Table 2: Extent of infestation by different pests in fixed plot (unmanaged) during survey in Maharashtra (Plot No. 2)

Month	Rhinoceros beetle			Red palm weevil incidence (%)	Black headed caterpillar incidence (%)	Eriophyid mite	
	Incidence (%)	Leaf damage (%)	Spindle damage (%)			Infestation (%)	MGI
April,18	25.00	6.00	0.00	0.00	0.00	73.39	1.53
June,18	44.00	7.20	0.00	0.00	0.00	76.09	1.78
Aug.,18	48.00	6.00	4.00	0.00	0.00	72.01	1.39
Oct., 18	24.00	4.40	4.00	0.00	0.00	47.49	0.74
Dec., 18	32.00	5.20	0.00	0.00	0.00	78.80	1.76
Feb., 19	8.88	3.20	3.33	2.22	0.00	82.42	2.27
Mean ± SE	30.31 ± 6.42	5.33 ± 0.62	1.88 ± 0.93	0.37 ± 0.40	0.00 ± 0.00	71.70 ± 5.56	1.57 ± 0.22

Table 3: Average per cent infestation by different pests in fixed plot survey in Maharashtra

Month	Rhinoceros beetle			Red palm weevil incidence (%)	Black headed caterpillar incidence (%)	Eriophyid mite	
	Incidence (%)	Leaf damage (%)	Spindle damage (%)			Infestation (%)	MGI
April,18	24.50	4.40	0.00	0.00	0.00	64.98	1.37
June,18	48.00	7.11	0.00	0.00	0.00	64.60	1.35
Aug.,18	42.00	5.00	6.00	0.00	0.00	54.91	0.94
Oct.,18	26.00	4.00	2.00	0.00	0.00	41.65	0.67
Dec., 18	20.00	3.40	0.00	0.00	0.00	54.84	1.15
Feb., 19	7.64	2.60	2.30	1.11	0.64	59.33	1.39
Mean ± SE	28.02 ± 5.44	4.41 ± 0.63	1.71 ± 1.16	0.18 ± 0.00	0.10 ± 0.00	56.71 ± 4.25	1.14 ± 0.13

infestation was also noticed by Alagar *et al.* (2019). Maximum infestation (64.98 %) was noticed in the month of April, 2018 and least incidence (41.65%) was observed in October, 2018. The mite damage grade index 1.37 (moderate) was recorded in April, 2018. However, lowest MGI (0.67) was observed in October, 2018. Present data was correlated with those of Levin and Mammooty (2003) indicated that most of the infested nuts were in the damage category of two and three and the percentage of mite damage was only 25.4 per cent. Desai *et at.* (2009) reported that the eriophyid mite infestation was higher in Thane district followed by Sindhudurg district. Gurav *et at.* (2018) observed that pest incidence and pest intensity trend showed that peak incidence was recorded during summer months, (April to May) while it decreases from September onwards and reaches to minimum in winter months, (November and December). Muyengi *et al.* (2015) showed that about 46.7 per cent of the farmers experienced the problem of rhinoceros beetle (*Oryctes monoceros*) in their farms and about 4.7 per cent problems with coconut mites (*Aceria guerreronis*). Vanderplank (1959), Bedford (1975), Paul (1985) and Seguni (2010) also reported the same results.

ACKNOWLEDGEMENTS

The authors are thankful to ICAR-AICRP (Palms) and ICAR-CPCRI, Kasaragod for providing funds, technical help and timely guidance for conducting the experiment.

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Manuscript recived on 5.11.2018

Manuscript accepted for publication 16.1.2019

Journal of Eco-friendly Agriculture **14**(2): 57-60. 2019

Management of bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae) in stored cowpea through plant extract and carbaryl

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ABSTRACT

Cowpea bruchid, *Callosobruchus maculatus*, is a major pest of stored cowpea in Eritrea. A comparative study was conducted on the effectiveness of plant extract and chemical as grain protectants against *C. maculatus* in Hamelmalo Agricultural College. Five hundred gram cowpea was treated with extract of neem leaf 5 per cent, *Lantana* leaf 5 per cent, wood-ash and carbaryl 2 per cent with three replicates. The plant extracts significantly reduced the population of *C. maculatus* at different days of intervals. At 14 DAT, the mortality by carbaryl was 8.21 per cent, while ash 7.67 per cent and control 7.34 per cent showed low mortality, respectively. The mean weight loss of control was 44.9 per cent, whereas it was low in ash (41.8%), lantana (38.4%), neem (34.2%) and carbaryl (19.5%). The treated seeds recorded higher germination percentage, while the control had the least germination percentage (40%). Among the botanical protectants neem was found to be effective against the storage pests.

Key words: Cowpea, *Callosobruchus maculatus*, neem, lantana, ash, carbaryl.

Cowpea crop (*Vigna unguiculata* L. Walp), member of the family Fabaceae, is a warm season, annual, herbaceous, widely cultivated legumes and is economically important legume crop in Eritrea. Its fresh or dried seeds, pods and leaves are commonly used as human food and for feed to livestock. Cowpea is highly susceptible to pulse beetles called bruchids. Bruchids cause a great damage in quality and quantity for matured crops as well as stored pulses. The bruchid problems, are high in Eritrea due inappropriate management practices such as misuse of pesticides, inadequate ventilation, poor storage, improper drying and chilling periods also affect the production and storage of the cowpea seeds. A current enhancement and well designation of adequate botanical treatments which are efficient, easily available and less expensive are needed to challenge the low management practices and poor knowledge in keeping storage seeds in Eritrean farmers. Its fresh or dried seeds, pods and leaves are commonly used as human food and feed for livestock. It is one of the grains that suffer post-harvest losses heavily from insects, both in the field and in storage condition. Like other crops, cowpea is attacked by a wide range of diseases and pests, and annual yields, longevity of the grain is greatly reduced by a complex insect pests. Yield reductions caused by insects can reach as high as 95 per cent, depending up on location, year, and cultivar (Adugna, 2007). They cause loss of weight, nutritional value and viability of stored grains. Bruchids are the main storage pests of the cowpea.

Callosobruchus spp. is an important pest of pulses in Eritrea. The two most widespread species of bruchid beetle are *Callosobruchus maculatus* and *Callosobruchus chinensis*. On-farm storage studies showed that staple grains of pulses produced by small farmers in Eritrea are attacked by different storage pests and the germination loss due to these pests range from 4 to 88% (Adugna, 2007). Adugna (2006) investigated that one of the main problems of storage in Eritrea is management of infestation in the stored areas and farmers in most areas keep old and new harvested grains in the same vicinity, which causes an easy migration of pests in new grains from the old grains. Farmers in Eritrea use different pest control methods, some are using chemicals and traditional methods like mixing of grain with ash, sand, chilly pepper, smoke and plant materials (Adugna, 2006). The renewed interest in plant materials as stored product protectants can be attributed to various factors including the development of resistance to synthetic insecticides, fears over their misuse and overuse (during application), and fears about the potential effect of insecticide residues on consumers, wildlife and the environment.

The present work has been undertaken to observe the efficacy of selected botanical powders on cowpea (*Vigna unguiculata* L. Walp) against *Callasobruchus maculatus* (Fab.), and to investigate the potential of these botanicals as sources of sustainable alternative protectants to synthetic insecticides for use in stored product protection using methods compatible with small-scale farmer practices in Eritrea.

MATERIALS AND METHODS

Site description

The experiment was carried out in the laboratory of the Department of Plant Protection of Hamelmalo Agricultural College (HAC) between February and May, 2018. HAC is located 13 km north of Keren. The altitude of the area is about 1330 m above sea level. The average rainfall and annual temperature of the area are 436 mm and 24°C, respectively.

Inoculation of bruchid in cowpea

The infested cowpea seeds were brought to the laboratory and were reared to reach sufficient population for inoculation of the treatments. Each treatment having 500 g of clean cowpea seeds were inoculated with 10 adults. Ten adults (1:1 sex ratio) of *C. maculatus* were introduced into each treatment in 1.5 l volume plastic vials. Observations were made at 2 week intervals. The number of adult bruchids that survived in each container was recorded every two weeks (14 days). The survival of the adult beetles was later calculated. And the number of exit holes per seed (weevil perforation index) was taken randomly from 100 seeds per container after two months (57 days) of treatment.

Treatments

The plant materials having insecticidal value against *C. maculatus*, like fresh leaves of neem and lantana were collected from Hamelmalo Agricultural College and dried under shade for a week, and wood-ash, carbaryl as well as control were used as a treatments. Five per cent of the botanicals and ash and 2 per cent of carbaryl were used for the treatments. The treatments were applied and mixed thoroughly and uniformly with cowpea seeds.

Data collection

Data were collected on bruchid mortality and natality, grain damage, weight loss and seed germination. Adult mortality was recorded at 14 days interval after application of the treatments. Insects that failed to respond to three probing using blunt dissecting probe were assumed dead and were included in the counts (Onu and Baba, 2003).

$$\%\ mortality = \frac{No\ of\ dead\ insects}{Total\ no\ of\ insects} \times 100$$

Grain damage in per cent was assessed after a month of bruchid inoculation and is calculated as

$$\%\ grain\ damage = \frac{No\ of\ perforated\ grains}{Total\ no\ of\ insects\ Total\ no\ of\ grains\ counted} \times 100$$

Germination test of treated and untreated seeds was tested after three months from each treatment. Five seeds were selected randomly from the experimentally treated grains and control groups from each plastic vial.

$$\%\ germination = \frac{Number\ of\ seeds\ that\ germinated}{Total\ number\ of\ seeds\ planted} \times 100$$

Statistical analysis

The statistical software, Genstat 4th edition was used for the analysis of variance (ANOVA) under the experimental design CRD–complete randomized design with three replication each treatment. The analysis was performed at 5 per cent level of significance.

RESULT AND DISCUSSION

Effect of treatments in the mortality of adult *C. maculatus*

The result of mortality of *C. maculatus* is given in Figure 1. Results of this study showed that percentage of adult mortality was significantly higher at second two weeks of application of the botanicals than that of first two weeks after treatment. Percentage mortality progressively increased with time of exposure. The treatments were highly significant in the mortality of adult *C. maculatus*. At 14 DAT, there was significant difference in mortality in all the treatments and control but carbaryl recorded high mortality (8.21%), while ash (7.67%) and control (7.34%) showed low mortality. At 28 DAT, the mortality of *C. maculatus* was significantly reduced. The control showed significant mortality at 9.6 per cent. At 42 DAT, the treatments and control were significantly different from each other. However, the least *C. maculatus* adult mortality occurred in the control treatments for the 28 DAT, 42 DAT and 56 DAT. The present finding is in agreement with Ahmad *et al.* (2015) that storage of faba bean and cowpea treatment with different physical, neem seed powder and sesame oil were found significantly managing the bruchid beetle in laboratory conditions. The

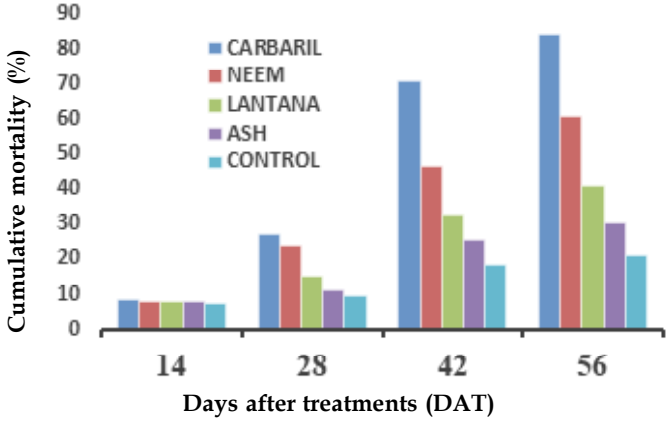

Figure 1: Effect of treatments on *C. maculatus* mortality

insecticidal potential of *A. indica* could be attributed to the presence of azadiratins, which is toxic to stored product insect pests (Onu and Baba, 2003). It showed that cowpea seeds treated with plant powders, ash and carbaryl caused a significant mortality within 28 DAT and effectiveness increased up to 60 DAT. *A. indica* and carbaryl powders caused more than 50 per cent mortality of adult bruchid beetle at different concentrations in laboratory (Ileke, 2012).

Effect of treatments on quality of cowpea

Plant extracts significantly affected the bruchid beetle and reduced its damage potentiality stored seeds in laboratory. At 14 DAT, percentage damage of treated seed was not significantly different from the control (13.33%) (Fig. 2). At 28 DAT, per cent damage in all the treated seed and control was significantly different from each other. Moreover, the percentage damage in all the treated seed and control was highly significant to each other at 42 and 56 DAT. The damage caused to control was due to low mortality and high progeny emergence implying that the insect numbers were increasing thereby causing more damage to grain. There was a significant reduction in damage by the bruchid to the treated cowpea seeds compared with the control and that is why the weight loss of treated samples was low compared to the control. There was a corresponding reduction in the number of exit holes in treated cowpea seeds as a result of limited contact of the bruchids with the treated seeds especially those treatments such as carbaryl, neem, lantana and ash, sequentially.

Assessment of weight loss and germination test

The result of germination of the cowpea seeds is presented in Figure 3. Germination percentage in the pre-

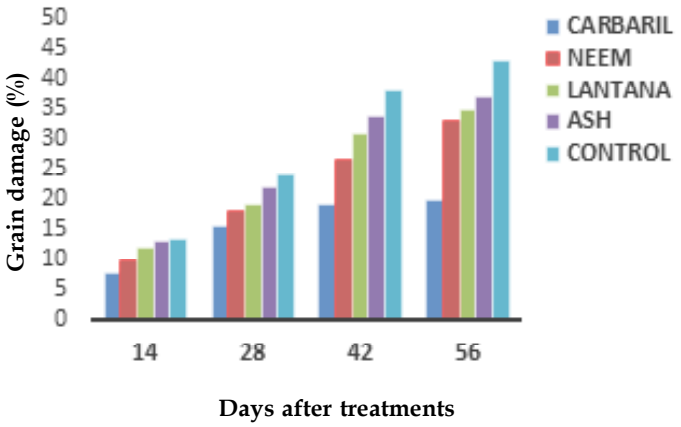

Figure 2: Per cent grain damage by cowpea beetle

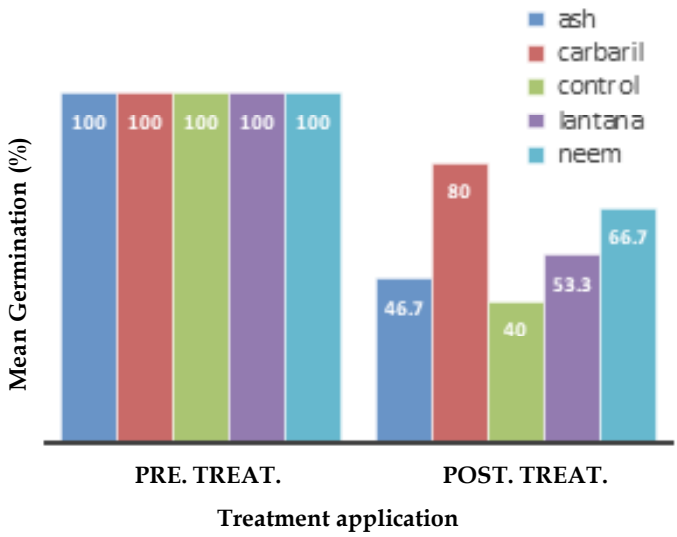

Figure 3: Per cent damage and germination test

treatment was not significantly different among the treatments and control. However, the germination percentage in the treated seeds (post-treatment) was highly significant. The treated seeds recorded higher germination percentage while the control had the least germination percentage (40%). This result showed that the bruchid attack altered the germination of the control treatment. It also showed that plant materials tested against *C. maculatus* did not show any adverse effect on germination capacity of the cowpea seeds. Weight loss of stored cowpea seeds was not significantly affected by the treatments. At 56 DAT, mean weight loss in different treatments were significantly different from each other. The mean weight loss in control was 44.9 per cent, whereas it was 19.5, 34.2, 38.4 and 41.8 per cent in seeds treated with carbaryl, neem, lantana and ash, respectively.

The results conduded that most of the botanical powders had insecticidal activity similar to synthetic chemicals were effective in the control of *C. maculatus*. The treatments significantly achieved high mortality of the adult *C. maculatus* and significantly reduced weight loss due to its ability to inhibit oviposition by adult *C. maculatus* and hatching ability of the eggs. Most botanicals have anti-feedant and repellent capability to control the insect pests in cowpea and reduce the seed damage and weight loss of the seed. Moreover, the local availability of these botanicals makes it easy for smallholder farmers and reduces the cost of cowpea seed production. Hence, the botanicals significantly lower number of progeny emerged. Thus, reduces further infestation on the crop.

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Manuscript recived on 26.2.2019

Manuscript accepted for publication 28.3.2019

Journal of Eco-friendly Agriculture **14**(2): 61-62. 2019

Molecular characterization of *Sclerotium* spp in Meghalaya

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ABSTRACT

Sclerotium delphinii and *S. rolfsii* are the major soil borne plant pathogens infecting many plant species worldwide. The fungus *S. delphinii* closely resembles *S. rolfsii* and causes similar symptoms. The pathogen *S. rolfsii* differs from *S. delphinii* in few aspects but still differentiation based on morphology alone is difficult. Isolations were done from the infected soybean plants showing collar rot symptoms. Molecular identification of *Sclerotium* isolates using 28s rDNA region revealed that both *Sclerotium rolfsii* and *S. delphinii* are present in this region.

Key words: *Sclerotium rolfsii*, *Sclerotium delphinii*, 28s rDNA

The genus *Sclerotium* includes three species, *viz*., *S. rolfsii*, *S. delphinii and S. coffeicola* (Gawande *et al*., 2013). They have a wide host range, mostly infecting dicotyledonous plant and few monocotyledonous species. The soil borne fungus *S. delphinii* is a serious pathogen on many herbaceous plants worldwide. It does not produce spores but forms highly melanised sclerotial bodies that help in long-term survival (Mukherjee *et al*., 2015). The pathogen *S. rolfsii* differs from *S. delphinii* in optimal growth temperature, host range, colony morphology and size of sclerotia (Punja and Damiani, 1996). Molecular markers play a major role in analyzing genetic basis of genotypic variation among fungal population (Prasad *et al*., 2010).

MATERIALS AND METHODS

Infected soybean samples exhibiting collar rot symptoms were collected from experimental plots of ICAR, Research Complex for NEH Region, Umiam. Diseased samples (stem) were collected from soybean field and isolation was done following the method described in Saveinai *et al.* (2017) with minor modifications (surface sterilization using 0.1% mercuric chloride ($HgCl_2$) solution for ~30 seconds then the samples were washed three times with sterile distilled water to eliminate excess mercuric chloride and again blotted dry using sterile blotting paper). Pure cultures were obtained by cutting hyphal tips of the 3-day old cultures and were transferred to PDA slants. Isolates were designated as SU, SL1, SL2, IVT17, IVT18, IVT25 and IVT26 for use in further studies. Microscopic observations were performed using Olympus BX 53 microscope equipped with a digital camera DP 72 (Olympus) and image analysis software (cellSens Standard 1.5, Olympus) on soybean isolates for confirmation of the fungus as *Sclerotium* spp. based on hyphal characters like clamp connections.

Molecular identification of *Sclerotium* spp

The DNA was extracted from the cultures grown on PD broth following the method (Phenol-Chloroform extraction method) as mentioned in Mahendra *et al*. (2016). PCR was used for amplification with universal fungal primers for 28s rDNA region (LR0R (forward) ACCCGCTGAACTTAAGC, LR5 (reverse) TCCTGAGGGAAACTTCG (Vilgalys and Hester, 1990). Components of PCR master mix were as mentioned in Mahendra *et al*. (2016). Fifty microliter reaction volume was added to the PCR tubes and PCR was done for 35 cycles with cycling conditions (initial denaturation 95°C for 5 min; denaturation 95°C for 30 sec; annealing 50°C for 40 sec; extension 72°C for 1 min and final extension 72°C for 7 min). After PCR, amplified products were checked through electrophoresis using ethidium bromide and the samples were sent for sequencing using the same primers. The sequences were deposited in Genbank and BLASTn was used for similarity search for all the sequences. The phylogenetic analysis was done using Maximum likelihood (ML) method in MEGA 6.0 (Tamura *et al*., 2013). The ML analysis was done using the Subtree pruning and regrafting algorithm (SPR-5). One thousand bootstrap replicates were used for calculating nodal support (Felsenstein, 1985). The sequence of *Amyloathelia crassiuscula* (DQ144610) was used as root.

RESULTS AND DISCUSSION

Hyphal characters like clamp connections were observed for all the seven isolates of *Sclerotium* spp using light microscopy.

Amplified PCR products were obtained by using primers LR5 and LR0R for four isolates, *viz*., SL1, SU, IVT25 and IVT17 (band size ~900bp). The resulting 28s nrDNA

(partial) sequences have been deposited in Genbank (SL 1 seq – KY172987, SU seq – KY172988, IVT 25 seq – KY172989 and IVT 17 seq – KY172990). Similarity, searches for all the sequences using BLASTn showed 99 per cent similarity with the *Sclerotium* spp. sequences (AY635773, JN543692, etc.). For further confirmation, phylogenetic analysis using maximum likelihood was used which clustered the sequence KY172987 (SL1) with *Sclerotium rolfsii* sequences with 83 per cent bootstrap support and the sequences KY172988 (SU), KY172989 (IVT 25) and KY172990 (IVT 17) clustered with *S. delphinii* with 85 per cent bootstrap support as shown in Fig. 1. Phylogenetic analysis was also conducted for confirmation after doing similarity searches since only similarity criteria can be misleading sometimes.

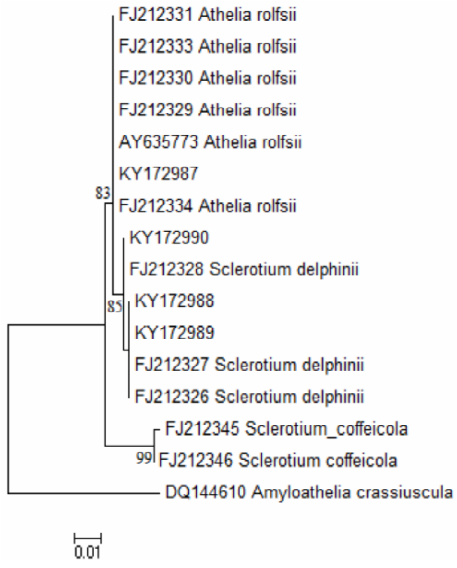

Fig. 1. Phylogenetic analysis of *Sclerotium* spp

Xu *et al*. (2010) have used the primers designed by Vilgalys and Hester (1990) for amplifying 28s rDNA from *Sclerotium* spp. for phylogenetic studies.

This study confirms the presence of *S. delphinii* along with *S. rolfsii* in this region and warrants the need of detail investigation related to the management caused by these pathogens.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, ICAR Research Complex for NEH Region, Umiam, Meghalaya for providing facilities and to the Dean, CPGS (Central Agricultural University), Meghalaya for support.

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Manuscript recived on 28.11.2018
Manuscript accepted for publication 6.1.2019

Journal of Eco-friendly Agriculture **14**(2): 63-68. 2019

Host plant resistance against *Rhizoctonia solani* AG 1-IB causing foliar blight of soybean in Meghalaya

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ABSTRACT

The present investigation was carried out to determine the host plant resistance against *Rhizoctonia solani* causing foliar blight of soybean in Meghalaya. Twelve isolates were used for molecular characterization using specific primers to determine anastomosis groups and sub-groups. The isolates RL1, Rim_1, RL2, RL4, RL5, RL6, RL7, RL8, RLR, RL9 and RL10 belonged to AG 1-IB. Only one isolate RL3 (product size ~ 265 bp) belonged to AG 1-IA. In advanced varietal trial (AVT) 1, varieties/lines KDS 780 and DSb 28-3 had low natural incidence (2.5 and 7.5%, respectively) and low disease severity (25 and 23.3%, respectively), while varieties/lines JS 20-98 and JS 97-52 had high natural incidence (35 and 52.5%, respectively) and high disease severity (62.7 and 80.9%, respectively). In AVT 2, varieties/lines SL 955 and SL 983 had low natural incidence (2.5 and 5%, respectively) and low disease severity (5 and 15%, respectively), while varieties/lines Himso 1685 and JS 97-52 had high natural incidence (35 and 47.5%, respectively) and high disease severity (63.6 and 65.1%, respectively). Twenty varieties/lines from AVT 1 and twelve varieties/lines from AVT 2 were used for in vitro screening against *R. solani*. In AVT 1 (leaves) varieties/lines JS 20-96 and DSb 28-3 were less susceptible (Area under disease progress curve AUDPC = 53.6 for both). In AVT 2 (leaves) varieties/lines DSb 25 and VLS 86 were less susceptible (AUDPC = 37.0 and 49.6, respectively). In AVT 2 (pods) varieties/lines VLS 86 and DSb 23-2 were less susceptible (AUDPC = 119.3 and 101.1, respectively).

Key words: Foliar blight, *Glycine max*, AG 1-IB, *Rhizoctonia solani*

Soybean (*Glycine max*) belonging to family Fabaceae includes beans, peas, chickpeas, peanuts, etc. Soy protein is a major component of the diet of domesticated animals and is now increasingly becoming important in the human diet (Friedman and Brandon, 2001). Dry seeds of local soybean are used as fermented, edible and flavoured products in Northeast India (Tamang *et al*., 2009).

Diseases such as rust, Rhizoctonia foliar blight, Rhizoctonia root and stem rot, charcoal rot, anthracnose and bacterial blight are responsible for causing yield losses in case of soybean. Even bird damage is also reported specially during seedling stage (Firake *et al*., 2016). Rhizoctonia foliar blight/ aerial blight/ web blight, caused by *Rhizoctonia solani* can cause 60-70 per cent yield losses (Meyera *et al*., 2006). The pathogen *R. solani* is a soil-borne, phytopathogenic, basidiomycetous fungus which can cause extensive yield losses (Gonzalez *et al*., 2006). Rhizoctonia foliar blight often occur during late vegetative growth stages on the lower portion of the plant. Leaf symptoms appear as greyish green water soaked lesions that become tan to brown later. Lesions can appear on infected pods, petioles, stems and leaves. High relative humidity and a temperature of 25 - 32°C favours disease development (Chattopadhyay *et al*., 2015).

The pathogen, *R. solani* has been divided into 14 anastomosis groups. Out of these 14 AG groups, AG 1 isolate causes seed and hypocotyl rots, aerial blight and web blight, AG 2 isolate causes root cankers and root rots of conifers, AG 3 isolate causes seed rots of potato and barley, AG 4 isolate causes seed and hypocotyl rots of different angiosperm species (Gonzalez *et al*., 2006; Guillemaut *et al*., 2003).

Natural incidence and disease severity help in assessing the disease and estimating the yield losses. These can also be used in comparative epidemiological studies for understanding disease-population dynamics (Seem, 1984). Even approaches like use of area under disease progress curves, can be important in making strategic decisions on management aspects (Jeger, 2004). Resistance against *R. solani* in soybean is rare but some cultivars have moderate resistance hence identification and use of these cultivars/lines will help in managing this disease (Chattopadhyay *et al*., 2015). Keeping the above mentioned points in view, the present investigation to determine anastomosis groups and subgroups of *R. solani* from soybean using molecular characterization and to determine host plant resistance against *R. solani* in cultivars/lines of soybean was conducted. Natural incidence and severity of foliar blight on cultivars/ lines of soybean was also recorded.

MATERIALS AND METHODS

All the experiments were carried out in the laboratories and experimental field (Plant Pathology) of ICAR Research Complex for NEH Region, Umiam, Meghalaya.

Infected samples showing typical symptoms of foliar blight were collected from Plant Pathology field. These samples were used for pathogen isolation following the method of Mahendra *et al.* (2016) with minor modifications. Water Agar used for isolation was amended with the antibiotic, Chloramphenicol @ 50 mg l^{-1} (antibiotic was suspended in 10 ml of 95 per cent alcohol, then it was mixed with one litre of media before sterilization). The petri dishes were then incubated at 27±1°C for 3 days. Purification of cultures and microscopic observations were performed according to Mahendra *et al.* (2016).

Two to three days old cultures on PD broth were used for DNA extraction. The phenol-chloroform extraction method was used for DNA extraction. The DNA was dissolved in 60 µl of Tris-EDTA (TE) buffer and it was used for making suitable dilutions. The PCR was done for amplification using specific and universal fungal primers with minor modifications (Mahendra *et al.,* 2016). The amplified products were checked through electrophoresis using ethidium bromide. Presence of specific bands was used for identification of the anastomosis groups and subgroups of *R. solani*. Amplified product (ITS1 - 5.8s - ITS2) of one isolate, *i.e.,* Rim_1, obtained using universal primers was also sequenced. The sequence was deposited in Genbank and phylogenetic analysis was also conducted using MEGA 6 (Tamura *et al.,* 2013). The Maximum Parsimony (MP) analysis was done using the Subtree pruning and regrafting algorithm in which the initial trees were obtained with the random addition of sequences and nodal support was calculated using one thousand bootstrap replicates (Tamura *et al.,* 2013; Felsenstein, 1985). Tree was rooted using the ITS sequence of *Athelia rolfsii* (AY684917).

To assess natural incidence and disease severity twenty-seven soybean varieties from AVT 1 (Advanced varietal trial) and twenty varieties from AVT 2 in Plant Pathology field were screened. The sowing for the experiment AVT 1 and AVT 2 was done on 18.06.15 and 22.06.15, respectively, and the fertilizer (N, P, K) dose used was 40:60:40 in both the experiments (R-R 45 cm, P-P 10 cm). Each experiment had two replicates with two rows of 3 m length. Maximum and minimum temperatures (°C), rainfall (mm) and average relative humidity (%) during the screening the month (September, 2015), were also obtained from the Agricultural Engineering Division of ICAR Research Complex for NEH Region umiam. The natural incidence was recorded in terms of percent incidence using following formula:

$$\text{Per cent disease incidence} = \frac{\text{No. of plants infected}}{\text{Total number of plants}} \times 100$$

The disease severity was determined by visually assessing the per cent area infected and the readings were averaged. On the basis of disease severity the varieties/lines were categorized as moderately resistant (<30%), susceptible (31-60%) and highly susceptible (>61%). Scale used was a modification of already proposed scale (Muyolo *et al.,* 1993).

For determining the host plant resistance of varieties/lines of soybean against *R. solani*, the detached leaf method was used (Rahayu, 2014). Apparently healthy leaves from twenty varieties/lines from AVT 1 and leaves and pods from twelve varieties/lines from AVT 2 were used for *in vitro* screening against *R. solani*. Initial pathogenicity test was carried out using isolate Rim_1. This isolate was inoculated on healthy leaves of soybean and it was found to be virulent. Virulent isolate Rim_1 was used for inoculation on detached healthy leaves and pods of different varieties/lines. Apparently, healthy soybean leaves of approximately similar size were washed with sterile distilled water and blotted dry. The leaves were then placed in moist chambers made of petri dishes lined with moist filter paper on the upper lid. A 5 mm disc of mycelium of Rim_1 isolate was used for inoculation. These moist chambers were then incubated at 27±1°C in a BOD incubator. Three replicates of each variety/line were maintained. The humidity was maintained in the moisture chambers by adding sterile distilled water, as and when required.

Apparently healthy pods were also collected from AVT 2 trial and the pods were kept in plastic trays, the mycelial discs were kept on one end of the pods, these trays were then covered with moist muslin cloth, an additional layer of blotting paper was also added above it. These trays were also incubated at 27±1°C. Three replicates per variety were used. The humidity was maintained by sprinkling water on the top and on the sides of the muslin cloth covering the trays, as and when required.

Observations were taken on leaves and pods on different days to assess the per cent area infected. The area under disease progress curve (AUDPC) was calculated as it helps to indicate the spread of the disease over time. Data from three separate days were used for calculating AUDPC. In case of AVT 1, leaves data was recorded on 4^{th}, 5^{th} and 7^{th} day, in AVT 2 (leaves) on 2^{nd}, 3^{rd} and 4^{th} day and in AVT 2 (pods) it was recorded on 5^{th}, 6^{th} and 9^{th} day.

Area under disease progress curve was calculated by using the following formula.

$$AUDPC = \sum_{i=1}^{k} \frac{1}{2}(S_i + S_{i-1})(t_{i+1} - t_1)$$

Where, Si = disease severity at the end of day l, k = number of successive evaluations of disease severity and, t_i = number of days after first observation

Statistical analysis was carried out using SPSS 20.0 software. The values were transformed when required before analysis. Mean separation was done using REGWQ test (Ryan/Einot/Gabriel/Welsch Procedure) (á = 0.05).

RESULTS AND DISCUSSION

Molecular characterisation

The isolates were grouped under AG 1-IA (band size ~265 bp) and AG 1-IB (band size ~300 bp) based on amplification with specific primers and band size. Twelve isolates could be identified by using specific primers, except one belonged to AG 1-IB. The isolate Rim_1, which was used for inoculation on the screened varieties/lines against *R. solani,* also belonged to AG 1-IB. The ITS sequence of isolate Rim_1 has been deposited in Genbank (KX132898). The Blastn search using this sequence revealed a very high similarity (99%) with sequences of AG 1-IB. Phylogenetic analysis carried out using the Maximum Parsimony method clustered the sequence with AG 1-IB clade with a bootstrap support of 98 per cent. This provided additional support and confirmation that the isolate Rim_1 belonged to AG 1-IB.

The findings confirmed the detection of AG subgroups by the use of specific PCR primer designed from 28S ribosomal DNA of *R. solani* by (Matsumoto, 2002). It was reported that the specific primers developed for AG 1-IA, AG 1-IB, AG 1-IC, AG 2-1, and AG 2-2 were able to detect isolates of the same anastomosis group. AG 1 IA on rice and maize AG 1 IB on soybean and marigold has been identified by Baiswar *et al.,* (2012 and 2014) by using the primers designed by (Matsumoto, 2002; Sayler and Yang, 2007).

Natural incidence and disease severity

The average maximum temperature recorded during the month of September, 2015, when observations were recorded, was 27°C and the average minimum temperature was 17.9°C. The average rainfall and the relative humidity recorded were 16.9 mm and 82.6 per cent, respectively.

In AVT 1, among all the varieties/lines screened, the varieties/lines KDS 780, RVS 2007-6, DS 3101, RVS 2008-24, AMS 1002, DSb 28-3 and KDS 753 showed low natural incidence ranging from 2.5 to 7.5 per cent, varieties/lines JS 20-96, VLS 89, JS 20-98 and JS 97-52 had high natural incidence, (30-52.5%), whereas varieties/lines KDS 869, RSC 10-46, MAUS 706, EC 241778, EC 241780, MACS 1442, DSb21, RKS 18, RVS 2008-8, MACS 1460, PS 1556, AMS 1003, NRC99, SL 1028, Bragg, JS 20-87 showed moderate incidence ranging from 10-27.5 per cent (Table 1). Least disease incidence was observed in KDS 780, RVS 2007-6 (2.5%) followed by DS 3101, RVS 2008-24 (5%). Maximum incidence was observed in JS 97-52 (52.5%).

In terms of disease severity in AVT 1, it was found that varieties/lines DSb 28-3, DS 3101, KDS 780 and MAUS 706 had low disease severity of 23.3 to 29 per cent and were classified as moderately resistant. Whereas, 18 lines, viz., KDS 869, RVS 2007-6, KDS 753, RVS 2008-24, RSC 10-46, SL 1028, AMS 1003, MACS 1460, PS 1556, EC 241780, JS 20-96, EC 241778, MACS 1442, VLS 89, JS 20-89, NRC99, Bragg and AMS 1002 had moderate severity ranging from 30 to 59.2 per cent and categorised as susceptible, the varieties/lines JS 20-98, DSb 21, RVS 2008-8, RKS 18 and JS 97-52 showing very high disease severity (62.7-80.9%) were

Table 1: Natural disease reaction of soybean lines/varieties against foliar blight in AVT 1

Variety	Average incidence (%)	Average disease severity (%)
MACS 1460	22.5	46.7
KDS 869	10.0	30.0
MAUS 706	12.5	29.0
AMS 1003	25.0	45.6
JS 20-87	27.5	54.8
NRC99	25.0	55.4
JS 20-98	35.0	62.7
RVS 2008-8	20.0	67.1
MACS 1442	17.5	51.5
SL 1028	25.0	45.4
DSb 28-3	7.5	23.3
DS 3101	5.0	25.0
KDS 780	2.5	25.0
JS 20-96	30.0	49.4
PS 1556	22.5	47.5
VLS 89	32.5	52.1
RVS 2007-6	2.5	30.0
KDS 753	7.5	33.3
RSC 10-46	10.0	40.0
AMS 1002	7.5	59.2
RVS 2008-24	5.0	36.7
DSb21	20.0	65.8
EC 241778	17.5	50.5
EC 241780	17.5	48.0
JS 97-52	52.5	80.9
RKS 18	20.0	67.5
Bragg	27.5	57.9

categorised as highly susceptible. The varieties/line DSb 28-3 showed least disease severity (23.3%) followed by DS 3101 (25%) the highest severity was recorded on JS 97-52 (80.9%) (Table 1).

In AVT 2, the varieties/lines SL 955, SL 983, DSb 25, KDS 726 and MACS 1370 had low natural incidence (2.5 to 7.5%) varieties/lines RKS 18, Himso 1685, MACS 1410 and JS 97-52 had high natural incidence (30 to 47.5%) and JS 20-79, VLS 86, RVS 2002-4, DSb 21, EC 241778, MACS 1407, DSb 23-02, JS 20-53, EC 241780 and JS 20-89 showed moderate incidence ranging from 10 to 25 per cent. The varieties/lines SL 955 showed least incidence (2.5%) followed by SL 983, DSb 25, KDS 726 (5%) whereas, JS 97-52 had highest incidence (47.5%) followed by MACS 1410 (42.5%) and Himso 1685 (35%). In terms of disease severity in AVT 2, the varieties/lines SL 955**,** KDS 726, SL 983, JS 20-79, MACS 1407 and RVS 2002-4 having low disease severity of 5 to 28.3 per cent were considered as moderately resistant, the varieties/lines MACS 1370, JS 20-53, EC 241780, MACS 1410, DSb 25, VLS 86, Bragg, EC 241778, RKS 18 and DSb 23-02 with moderate disease severity (32.5-59.2%) were considered as susceptible and Himso 1685, JS 97-52, JS 20-89 and DSb 21 with high disease severity (63.6-70.8%) were categorised as highly susceptible. The varieties/lines SL 955 showed least disease severity (5%) followed by KDS 726 (7.5%). Highest disease severity was recorded on DSb 21 (70.8%) followed by JS 20-89 (65.6%) (Table 2).

Natural incidence has been used as a criteria to screen soybean varieties to check resistance against *R. solani* causing root rot disease of soybean (Haq *et al.,* 2012). The varieties William-82, NARC-2 and FS-85 were reported to be less susceptible as compared to other screened varieties. Disease severity has been used as a criteria to screen soybean varieties against web blight and it was found that variety NK S57-11 was moderately resistant (Stetina *et al.,* 2006).

Table 2: Natural disease reaction of soybean lines/varieties against foliar blight in AVT 2

Variety	Average incidence (%)	Average disease severity (%)
VLS 86	15.0	39.0
SL 983	5.0	15.0
DSb25	5.0	37.5
SL 955	2.5	5.0
DSb 23-02	20.0	59.2
MACS 1370	7.5	32.5
Himso 1685	35.0	63.6
MACS 1410	42.5	37.2
JS 20-79	10.0	20.0
JS 20-53	20.0	33.0
JS 20-89	25.0	65.6
DSb 21	17.5	70.8
EC 241778	17.5	51.7
EC 241780	20.0	33.8
RKS 18	30.0	52.5
JS 97-52	47.5	65.1
Bragg	25.0	51.3
KDS 726	5.0	7.5
MACS 1407	17.5	22.9
RVS 2002-4	15.0	28.3

Host plant resistance

Disease reaction of selected soybean lines/varieties against foliar blight using detached leaf technique (AVT 1 leaves)

In AVT 1, the varieties/lines JS 20-96 and DSb 28-3 were found to be least susceptible with AUDPC – 53.6 followed by varieties/lines PS 1556 (77.1), MAUS 706 (77.3), KDS 753 (77.4), KDS 869 (78.3) and RKS 18 (87.1) and were statistically at par (Table 3). SL 1028 was found highly susceptible with AUDPC – 210.5 followed by Bragg (182.0) and RVS 2007-6 (157.7).

Table 3: Disease reaction of selected soybean lines/varieties against foliar blight using detached leaf technique (AVT 1 leaves)

Variety	AUDPC*
MACS 1460	120.5^{abcd}
KDS 869	78.3^{ab}
MAUS 706	77.3^{ab}
JS 20-89	90.9^{abc}
NRC 91	100.1^{abc}
JS 20-98	127.8^{bcd}
RVS 2008-8	137.9^{bcd}
SL 1028	210.5^{e}
DSb 28-3	53.6^{a}
DS 3101	131.3^{bcd}
JS 20-96	53.6^{a}
PS 1556	77.1^{ab}
VLS 89	119.9^{abcd}
RVS 2007-6	157.7^{cde}
KDS 753	77.4^{ab}
RSC 10-46	112.1^{abc}
AMS 1002	111.8^{abc}
JS 97-52	99.1^{abc}
RKS 18	87.1^{ab}
Bragg	182.0^{de}

*Arcsine transformed values

Means with same letters are not significantly different according to REGWQ test (p=0.05). (Ryan/Einot/Gabriel/Welsch procedure)

Disease reaction of selected soybean lines/varieties against foliar blight using detached leaf technique (AVT 2 leaves)

In AVT 2 (leaves), the varieties/lines DSb 25 (AUDPC-37.0), JS 97-52 (39.3), VLS 86 (49.6) and JS 20-53 (50.6) were found least susceptible and statistically at par followed by

EC 241778 (58.0) and DSb 21 (73.5) (Table 4). The variety DSb 23-02 (122.7) was highly susceptible followed by MACS 1370 (110.8). The varieties/lines DSb 21 (73.5), MACS 1407 (80.7), JS 20-89 (85.1) and RVS 2002-4 (85.9) were at par and appeared to be moderately susceptible.

Table 4: Disease reaction of selected soybean lines/varieties against foliar blight using detached leaf technique (AVT 2 leaves)

Variety	AUDPC*
DSb 23-02	122.7^{c}
DSb 25	37.0^{a}
RVS 2002-4	85.9^{abc}
JS 20-53	50.6^{a}
VLS 86	49.6^{a}
MACS 1407	80.7^{abc}
JS 97-52	39.3^{a}
DSb 21	73.5^{abc}
EC 241778	58.0^{ab}
Bragg	90.3^{abc}
MACS 1370	110.8^{bc}
JS 20-89	85.1^{abc}

*Arcsine transformed values
Means with same letters are not significantly different according to REGWQ test (p=0.05). (Ryan/Einot/Gabriel/Welsch procedure)

Detached leaf assay was also used to evaluate resistance to web blight in common beans caused by *R. solani* (Gonzalez-Martinez and Annette, 2008). Two groups of bean lines were screened with isolates from AG 1-1F, AG 1-1E and AG 1-1A using the detached leaf technique and was reported that all lines were susceptible to all isolates.

Disease reaction of selected soybean lines/varieties against foliar blight using detached leaf technique (AVT 2 pods)

In AVT 2 (pods), the varieties/lines DSb 23-02 (AUDPC- 101.1) was least susceptible followed by VLS 86 (119.3) and RVS 2002-4 (138.6) while, JS 20-89 (252.5) was highly susceptible followed by MACS 1407 (250.7), JS 97-52 (239.2) and EC 241778 (230.8) which were statistically at par. (Table 5). Correlation analysis revealed that a weak negative correlation (-0.372) exists in the degree of susceptibility between leaves and pods.

In case of AVT 2, a weak negative correlation (-0.372) was detected between AUDPC for leaves and pods. As can be seen in the case of variety/line DSb 25, which showed less susceptibility in terms of natural incidence and severity had also low disease severity in case of *in vitro* screening but it was highly susceptible considering pod screening. Variety/line DSb 23-02 in AVT 2 showed a high degree of susceptibility in case of leaves but a low degree of susceptibility in case of pods. This difference may be due to the difference in tissue susceptibility between leaves and pods. The results also indicate that the susceptibility of leaves as well as pods could be checked, which will give a better overall indication of susceptibility of a line or variety. Only line VLS 86 showed less susceptibility in case of leaves and pods.

Table 5: Disease reaction of selected soybean lines/varieties against foliar blight (AVT 2 pods)

Variety	AUDPC *
DSb 23-02	101.1^{a}
DSb 25	219.2^{cd}
RVS 2002-4	138.6^{abc}
JS 20-53	216.8^{cd}
VLS 86	119.3^{ab}
MACS 1407	250.7^{d}
JS 97-52	239.2^{d}
DSb 21	200.0^{bcd}
EC 241778	230.8^{d}
Bragg	179.8^{abcd}
MACS 1370	195.1^{bcd}
JS 20-89	252.5^{d}

*Arcsine transformed values
Means with same letters are not significantly different according to REGWQ test (p=0.05). (Ryan/Einot/Gabriel/Welsch procedure)

The study revealed that most of *R. solani* isolates used for molecular characterization causing foliar blight in soybean in Meghalaya belonged to AG 1-IB based on identification using specific primers. Hence, screening of genotypes was advised to be done using AG 1-IB isolates of *R. solani*. Among all the varieties/lines screened (in AVT 1 and AVT 2), DSb 28-3, MAUS 706, KDS 869 and VLS 86 performed better under *in vivo* and *in vitro* screening. Therefore, these varieties/lines can be cultivated in the areas where foliar blight is highly prevalent and can further be used as a source for resistance breeding in the future.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, ICAR Research Complex for NEH Region, Umiam, Meghalaya for providing facilities and to the Dean, CPGS (Central Agricultural University), Meghalaya for support.

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Manuscript recived on 29.11.2018
Manuscript accepted for publication 13.2.2019

Journal of Eco-friendly Agriculture **14**(2): 69-71. 2019

Efficacy of sterilized culture filtrate of *Trichoderma harzianum* Rifai against mango wilt pathogen, *Ceratocystis fimbriata* Ellis and Halst

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ABSTRACT

Mango wilt disease caused by *Ceratocystis fimbriata* has become a serious constraint in most mango growing areas in Uttar Pradesh and in several states in India. Management of mango wilt has been achieved by the application of cultural practices and the foliar and soil application of fungicides. In this study, the sterilized culture filtrate of TH-12 was also evaluated for their efficacy against *C. fimbriata* through food poison technique under laboratory conditions. All the concentrations of culture filtrate of TH-12 were found effective up to 20 days but no difference in growth of control and 0.15 per cent concentration was recorded at 30th day after inoculation. The maximum suppression of *C. fimbriata* was found at 10 per cent concentration on 30th day after inoculation. Concentrations of filtrate used in the range from 0.15 to 10 per cent significantly reduced the growth of *C. fimbriata* in comparison to control. The suppressive potential of the sterilized culture filtrate of TH-12 bio-control agent indicated the possibility of development of bio-formulation for the management of mango wilt under field conditions.

Key words: Mango, wilt, *Ceratocystis fimbriata, Trichoderma harzianum,* management

Mango (*Mangifera indica* L.) is the world's sixth most important fruit crop. Global fruit production of mango was estimated at 46.51 million MT during 2016-17 (Statista, 2017). In India, it is cultivated over an area of 2.26 million hectares with production of 19.69 million tonnes (NHB, 2016-17). *Ceratocystis* spp. responsible for causing mango wilt is now one of the most important pathogen of mango (Van Wyk *et al*., 2007; Shukla *et al*., 2018). The fungus was first recorded in the 1930s on the trees suffering from severe "seca" or "murcha" disease (Ploetz, 2003). Till 1960, the wilting of mango trees was considered a disease of minor importance, when Ceratocystis wilt was reported by Iton during 1960. Some other pathogens (*Botryosphaeria, Diplodia, Lasiodiplodia, Fusarium, Fusicoccum, Physalospora, Phytophthora, Pythium, Rhizoctonia* and *Sclerotium*) have also been considered associated with mango wilt and decline (Ploetz and Prakash, 1997; Misra, 2004, 2006; Al Adavi *et al*., 2006; Saeed *et al*., 2010; Shukla *et al*., 2018). However, the *Ceratocystis fimbriata* is established as the major causal organism of mango wilt and decline in India (Shukla *et al*., 2018). Gradually, the wilt of mango has become a great threat to mango production in Bangladesh, Brazil, Oman, Pakistan, Spain and very recently in India also (Bastos and Evans, 1978; Jiskani, 2002; Al-Yahani *et al*., 2005; Fateh *et al*., 2006; Deadman *et al*., 2007; Masood *et al*., 2009; Shukla *et al*., 2018).

Mango wilt disease management strategy has been developed by adopting cultural, physical and chemical means (Shukla, 2017), however, no recommendation could be made with bio-control agents. *Trichoderma* spp. have been considered as potential antagonistic fungi and their secondary metabolite production had been found involved in bio-control activity against pathogenic fungi (Dennis and Webster, 1971a,b; Reino *et al*., 2008). *Trichoderma* species produce both volatile and non-volatile metabolites that adversely affect growth of different fungi (Eziashi, 2006; Khan *et al*., 2017; Somu *et al*., 2018). Keeping in view the bio-control efficacy of *Trichoderma* spp against pathogenic fungi, the efficacy of the sterilized culture filtrate of *Trichoderma harzianum* isolated from wood tissue of wilted mango tree was evaluated against mango pathogen, *C. fimbriata*.

MATERIAL AND METHODS

Seven different concentrations of *Trichoderma harzianum* (TH-12) sterilized metabolite filtrate were evaluated by poisoned food technique (Nene and Thapliyal, 1993). The cultures of CISH *Trichoderma harzianum* strain 12 (TH-12) were grown in petri-plates each having 20 ml PDA. After inoculation, petri-plates were maintained in BOD incubator at 27 ± 2^{o}C for 60 days and best sporulated cultures were harvested with media. Five gram culture was suspended in 50 ml sterilized distilled water and stirred for 30 minutes using magnetic stirrer at 6000 rpm. The suspension, thus prepared, was left and then filtered through (Whatman No.1) filter paper. Filtrate was used in the experiment at different concentrations. Potato Dextrose Agar medium was prepared and transferred into flasks. In order to prepare different

concentrations of the filtrate into the medium, 10, 5, 2.5, 1.25, 0.6, 0.3, 0.15 ml filtrate was transferred to 100 ml measuring cylinders and the volume was made up to 100 ml with fresh PDA medium prepared. The medium was then poured into 250 ml conical flasks and the flasks were then sterilized at 15 lbs inch^{-2} pressure for 20 minutes. After sterilization, PDA medium was poured into labelled petri-plates. The petri-plates were incubated for 48 hours to check contaminations and then inoculated with 5 mm disc of 15 day old *C. fimbriata* culture. Suitable control plates along with treated ones were also maintained without filtrate at 27 ± 2°C up to 30 days. The observations were made periodically for recording the radial growth of test fungus at different concentrations of the filtrate. The mean of growth parameters were subjected for analysis variance.

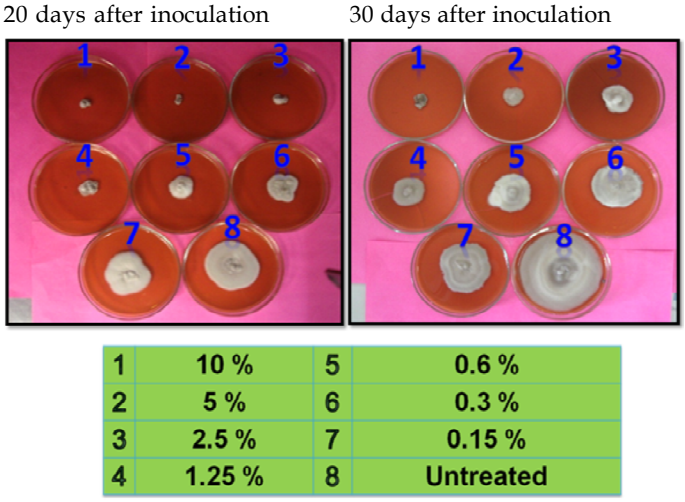
RESULTS AND DISCUSSION

The experiment was conducted to determine the efficacy of different concentrations of sterilized filtrate of *Trichoderma harzianum* strain 12 (TH-12) on the growth of *C. fimbriata* in *in vitro* conditions. All concentrations of culture filtrate of TH-12 were found effective to varying extents up to 20 days. Among the seven test concentrations, the highest one (10%) exhibited the highest suppression of growth (14.0 mm) of *C. fimbriata* up to 30 days followed by 5 ml (23.8 mm), 2.5 ml (41.0 mm), 1.25 ml (51.4 mm), 0.6 ml (54.8 mm), 0.3 ml (56.0 mm) and 0.15 ml (75.4 mm), (Table 1; Fig. 1). The control plates had 77.6 mm radial diameter of growth on 30th day. No difference in growth of control and 0.15 per cent (0.15 ml) concentration was recorded at 30th day after inoculation. At this stage, all the treatments significantly reduced the growth in comparison to control. Khan *et al.* (2017) and Somu *et al.* (2018) also reported *T. harzianum* effective against *C. fimbriata* causing wilt disease of pomegranate. Secondary metabolites were also found effective in similar studies on evaluation of secondary metabolites of *T. harzianum* by Mishra *et al.* (2018)

Table 1. Effect of CISH *Trichoderma harzianum* strain 12 metabolite filtrate on average radial growth of *Ceratocystis fimbriata* at different concentrations

Doses (ml)	Radial growth of *C. fimbriata* (mm)		
	10th Day	20th Day	30th Day
10	6.0	9.8	14.0
5	11.6	19.0	23.8
2.5	14.2	26.8	41.0
1.25	22.2	32.8	51.4
0.6	26.0	44.4	54.8
0.3	28.6	45.0	56.0
0.15	44.0	56.4	75.4
Control	57.0	76.2	77.6
$CD_{0.05}$	2.9	3.7	3.9

Fig. 1. Suppressive effect of CISH *Trichoderma harzianum* strain 12 sterilized culture filtrate on radial growth of *Ceratocystis fimbriata* at different concentrations



against *Fusarium oxysporum* f. sp. *capsici* and *Colletotrichum capsici*, and Eziashi *et al.* (2006) against *Ceratocystis paradoxa.*

From the study it can be concluded that the sterilized culture filtrate of TH-12 has potential to inhibit the fungus *C. fimbriata* and it is also heat resistant. The TH-12 culture is fast growing and sporulates within 7-8 days. The bio-control potential of TH-12 sterilized culture filtrate could be employed in developmenting a new bio-pesticide formulation, which may be incorporated into the integrated package of mango wilt disease management.

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Manuscript recived on 16.12.2018

Manuscript accepted for publication 23.2.2019

Journal of Eco-friendly Agriculture **14**(2): 72-74. 2019

Enzymatic and siderophore production behavior of fungal isolates from various biodynamic preparations

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ABSTRACT

Biodynamic farming refers to working with energies which create and maintain life. Basically there are two types of biodynamic preparations: biodynamic field spray (BD 500 – BD 501) and biodynamic compost preparations (BD 502 – BD 507). The BD sets are used in cow pat pit, BD compost, biodynamic liquid manure and biodynamic liquid pesticides. Enormous literature and supportive material justify the use of these farming systems to combat soil pollution created by use of various chemicals. However, when it comes to scientific explanation, the work is scanty. The present work reports the enzymatic and siderophore production potential of fungal isolates of these biodynamic preparations. Out of 25 fungal isolates from biodynamic preparations, high pectinase activity was exhibited by isolate no. BD 0-7 (0.939µ mole ml^{-1} min^{-1}) followed by BD 0-2 (0.894 µ mole ml^{-1} min^{-1}) and BD 0-8 (0.852 µ mole ml^{-1} min^{-1}) all these fungus isolates are from BD 500 biodynamic preparations. High cellulase activity was exhibited by isolate no. BD 6-1 (0.1107 µ mole ml^{-1} min^{-1}) followed by BD 0-5 (0.1053 µ mole ml^{-1} min^{-1}) and BD 5-4 (0.1044 µ mole ml^{-1} min^{-1}) from BD 506, BD 500 and BD 504 biodynamic preparations, respectively. High amylase activity was exhibited by isolate no. BD 3-4 (0.1284 µ mole ml^{-1} min^{-1}) followed by BD 3-3 (0.1092 µ mole ml^{-1} min^{-1}) and BD 4-1 (0.1053 µ mole ml^{-1} min^{-1}) from BD 503 and BD 504, respectively. High siderophore production efficiency (%) were exhibited by isolate no. BD 4-5 (285.71%) followed by BD 0-2 (200.00%) and BD 6-1 (190.0%) from BD 504, BD 500 and BD 506. High enzyme activity shown by various isolateds from different BD preparations explains the degradative powers associated with these preparations. The siderophore production indicates the iron chelating activity which is an indication of plant growth promoting power.

Key words: Biodynamic preparations, enzymatic activity, siderophore production

Rhizosphere is a specific region of plant root surface which harbours diverse group of microbes. Some of the bacteria can play important role in the plant growth, referred to as plant growth promoting rhizobacteria (PGPR). Latter can promote the plant growth by various direct and indirect mechanism such as phosphate solubilisation, nitrogen fixation, indole-3-acetic acid (IAA) production, siderophore production and repression of soil borne pathogens by production of hydrogen cyanide and antibiotics (Glick, 1995).

Iron is one of the important microelements for all living cells, which is usually present in the environment, particularly in soils. Despite being the most abundant element in earth's crust, the availability of iron is limited due to very low solubility of the dominant ferric iron (Fe^{3+}) in soil and it becomes unavailable to plants as a micronutrient (Thompson and Troeh, 1973). Some microbial isolate produce siderophore which in turn chelate iron from soil and make it available to plant.

The primary mechanism of plant disease suppression by biological control agents is by production of antimicrobial secondary metabolites like siderophores, antibiotics, volatile substances, etc. Siderophores are low molecular iron chelating compounds produced by fungi and bacteria under iron stress condition (Ghosh *et al*., 2017). The degradative properties of soil microorganism are also due to high enzymatic activity. Pectinase is an enzyme that breaks down pectin. Pectic substances are glycosidic macromolecules with high molecular weight. These form the major components of the middle lamella and primary plant cell wall (Puangsri *et al*., 2005). Amylases are starch degrading enzymes widely distributed in microbial, plants and animals kingdom. These degrade starch and related polymers to yield products characteristics of individual amylolytic enzymes (Aiyer, 2005). Cellulose is the most abundant carbohydrates present on earth and is commonly degraded by enzyme complex called cellulases. The enzyme is produced by several microorganisms including bacteria and fungi (Immanuel *et al*., 2006). Cellulase is an important and essential kind of enzyme for carrying out the depolymerization of cellulose into fermentable sugar (Xing-hua *et al*., 2009).

Biodynamic farming is said to improve soil health and quality of produce. However, the mode of action of its preparations is still not fully worked. Vaish *et al.* (2017) reported the presence of bacteria and fungi having high PGPR activity.

Our preliminary studies have indicated the presence of potential microbal isolates having high degrading potential. Current study reports some bacteria and fungus isolates from these biodynamic preparations for siderophore production and enzymatic behaviour.

MATERIAL AND METHOD

Siderophore production was conducted as per method described by Schwyn and Neilands (1987). CAS (chrome azoural S) agar medium was used for siderophore production test. Bacterial and fungal isolates were inoculated in the centre of the CAS agar medium plate and incubated at 32°C for 3 to 5 days for yellow zone formation.

For enzymatic analysis carbohydrate utilization broth with 1 per cent pectin/starch/carboxy methyl cellulose was inoculated with bacterial and fungal isolates and incubated at 28 °C for 72 hours. For bacterial enzyme estimation, culture broth was centrifuged at 10,000 rpm for 10 minutes and the supernatant was used for enzyme analysis and for fungus enzyme estimation, broth was filtered through G-1 glass crucible to remove the fungus growth and filtrate was used for enzyme precipitation.

One volume of sample (culture filtrate from bacteria / fungus) was added in 4 volume of cold acetone (1:4) mixture, kept at -20°C for 20 minutes and centrifuged at 10,000 rpm for 15 minutes at 4°C. The supernatant was discarded and the pellet was suspended in acetate buffer solution (0.2mM). The mixture was used for enzymatic analysis for cellulase, pectinase and amylase using carboxy methyl cellulose, pectin and starch as substrate as per method described by Miller (1972), Garg and Ashfaque (2010) and Wood and Bhat (1988), respectively. The enzyme activity was expressed as U of sugar released per ml per min. of incubation.

The microbial culture showing higher enzyme activities were identified on molecular basis as per method for bacterial culture using 16s r DNA based molecular technique (Boye *et al.,* 1999). Fungal cultures were identified using ITS region based molecular technique (Weisburg *et al.*, 1991, Drancourt *et al.*, 2000).

RESULT AND DISCUSSION

Out of 68 bacterial isolates, high pectinase activity was exhibited by isolate no. BD 504–3 (2.595 U ml^{-1} min^{-1}) followed by BD 500-1 (2.361 U ml^{-1} min^{-1}) and BD 502-5 (2.307 U ml^{-1} min^{-1}). High cellulase activity was exhibited by isolate no. BD 504-3 (0.308 U ml^{-1} min^{-1}) followed by BD 500-1 (0.287 U ml^{-1} min^{-1}) and BD 502-5 (0.230 U ml^{-1} min^{-1}). High amylase activity was exhibited by isolate no. BD 504-3 (0.418 U ml^{-1} min^{-1}) followed by BD 502-5 (0.376 U ml^{-1} min^{-1}) and BD 500-1 (0.180 U ml^{-1} min^{-1}). Among the three bacterial isolates, bacterial isolate no. BD 504-3 showed high pectinase, cellulase and amylase activity which was later identified on the basis of molecular characterization as *Bacillus licheniformis*.

Vengadaramana *et al.* (2011) reported the high á-amylase activity (32.95 U ml^{-1}) by *Bacillus licheniformis* ATCC 6346. Although, many microorganisms produce amylase enzyme, the ones most commonly used for their industrial production are *Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquifaciens* and *Aspergillus niger* (Brook *et al.,* 1969). Sivakumar *et al*. (2016) reported eight cellulolytic *B. licheniformis* strains from compost samples which were able to utilize xylan, cellobiose, mannose and carboxy methyl cellulose (CMC). Rehman *et al.* (2015) isolated *Bacillus licheniformis* KIBGE-IB21 with the GenBank accession number JQ 411812 from rotten vegetables that produced higher pectinase enzyme.

Out of 25 fungal isolates from biodynamic preparations, higher pectinase activity was exhibited by isolate no. BD500-7 (0.939 µM ml^{-1} min^{-1}) followed by BD500-2 (0.894 µM ml^{-1} min^{-1}) and BD 500-8 (0.852 µM ml^{-1} min^{-1}). High cellulase activity was exhibited by isolate no. BD506-1 (0.1107 µM ml^{-1} min^{-1}) followed by BD 500-5 (0.105 µM ml^{-1} min^{-1}) and BD505-4 (0.1044 µM ml^{-1} min^{-1}). High amylase activity was exhibited by isolate no. BD503-4 (0.1284 µM ml^{-1} min^{-1}) followed by BD 503-3 (0.1092 µM ml^{-1} min^{-1}) and BD504-1 (0.1053 µM ml^{-1} min^{-1}). High enzyme activity shown by various isolates from different BD preparations explained the degradative powers associated with these preparations.

Fungal culture that exhibited high pectinase activity BD 500-7, was identified as *Aspergillus fumigatus* strain BT7. Similar observation has been made by Ezugwu *et al.* (2014) regarding high pectinase production by *A. fumigatus* and *A. niger* in a submerged fermentation system.

The fungal isolate exhibiting high cellulase activity was BD 506-1 was identified as *Penicillium rubens* strain CBS 339.52. Chinedu and Okochi (2011) reported waste cellulosic materials (corncob, sawdust and sugarcane pulp) and crystalline cellulose induced cellulase production by wild strains of *Aspergillus niger, Penicillium chrysogenum* and *Trichoderma harzianum,* isolated from a wood-waste dump in Lagos, Nigeria. Highest enzyme activity was obtained from the culture broth of *P. chrysogenum* after 60 h incubation with an enzyme activity of 0.67 Units mg $protein^{-1}$.

High amylase activity was exhibited by BD 503-4 and it was identified as *Penicillium citrinum*. This observation was also supported by report stating *P. citrinum* HBF62 exhibited higher amylolytic activity in starch agar medium (Metin *et al.,* 2010). The isolate no. BD 504- 5 showing high

siderophore efficacy (285.71%) was identified as *Alternaria brassicae* isolate T5 using ITS primer. The other two isolates exhibited siderophore production are BD 500-2 and BD 506-1 with siderophore production efficiency 200 and 190 per cent, respectively. Pedras and Park (2015) also reported siderophores production in cultures of *A. brassicicola* containing low and high ferric ion concentrations.

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Manuscript recived on 23.12.2018

Manuscript accepted for publication 28.1.2019

Journal of Eco-friendly Agriculture **14**(2): 75-77. 2019

Food and nutrition behaviour of women in Hubli-Dharwad

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ABSTRACT

Nutrition, as one of the key factors that help an individual to attain one's full potential as an adult, depends to a great extent on the quantity and quality of food. The investigation, to assess the food behaviour and diet quality of women police personnels working in technical cadres of police department of Hubbali-Dharwad, was carried out during 2013-2014. The nutrient adequacy of women police varied for energy (81 to 157%), protein (55.66-96.54%), fat (109.85-379.60%), thiamine (96.00 to 211.00%) and magnesium (31.20-298.49%). The rest of the nutrient contents were lower than the RDA. More than 50 per cent of police women consumed fair quality diets (54.45%) followed by good (22.22%) and poor (18.89%) qualities. Only 4.44 per cent women police consumed very good quality diet.

Key words: Nutrition behaviour, diet quality, RDA, nutrient adequacy.

Indian civilization is one of the most ancient civilizations of the world, and so are its diverse systems and sub systems. The industrial revolution and the processes of liberalization and globalization have changed the position of women in India. Policing is considered to be one of the most masculinized occupations of the world. It has been described as one of the most "gendered" professions. It is a demanding job, which involves long and uncertain hours of duty. However, over the past few decades, the police workforce has grown much more diverse with regard to gender and race (Butler *et al*. 2003; Sklansky, 2006; Sahgal, 2007). An investigation was planned to evaluate the nutritional, food and nutrition behaviour and diet quality of women working in police department of Hubli-Dharwad.

MATERIALS AND METHODS

A total of 90 police women from 22 rural and urban police stations (including a women police cell) formed the study group. The intake of food was assessed by 24 hour recall method using a set of pre standardized vessels. Information on the actual ingredients used for preparation and quantity of cooked food consumed by each subject were recorded with the help of standard vessels. The raw weight of ingredients used for each measure of each cooked food was ascertained by cooking the food in the laboratory.

The raw food equivalents were computed from the standardized cups. The nutrient composition of foods was computed using Annapurna VAR.3 software developed by M. R. Chandrashekar (2003) of Bengaluru. The adequacy (%) of nutrient for each subjects was computed using the formula.

$$\text{Nutrient adequacy (\%)} = \frac{\text{Nutrient intake}}{\text{RDA of the nutrient}} \times 100$$

Diet quality of respondents was determined with the help of pretested scoring pattern suggested by Jirlimath (1983). The total marks allotted for diet quality pattern questionnaire was 10 marks. Based on the scores of respondents, diet quality was determined and classified as poor, fair, good or very good diet. Frequency and percentages were computed to interpret the demographic profile of the subjects. Mean and standard deviations were calculated for dietary and nutritional adequacy of subjects. The results obtained were analyzed employing following statistical methods (SPSS statistical package, version 16.0).

RESULTS AND DISCUSSION

Diet quality of women police

Nutrition is one of the key factors that helps an individual to attain one's full potential as an adult and it depends to a great extent on the quantity and quality of food they consume. Proper nutrition is important in improving the community health in general and of the risk groups in particular.

Mean food intake and adequacy of diets of police women in relation to suggested balanced diets is presented in Table 1. The results indicated a wide variation in consumption of different foods among the police women, thereby indicating a wide range of adequacies. Noticeable among the foods was the intake of fats which was exceptionally more than adequate (170.90 g) with a range of values between 146.00 to 299.45 per cent by the police women. The mean intake of fats was 34.18 g (29.20 to 59.89 g) even the lower value were higher than the suggested balanced diet for women.

Cereals were the next most adequate food (116.67% with a range of 71.48 to 151.85%) with mean consumption of 315 g (193 to 410 g), indicating inadequate consumption level by some women police.

Table 1: Mean food intake and adequacy in diets of women police in relation to suggested balanced diets N=90

Food (g)	Suggested balanced diet (g)	Mean intake (g)	Adequacy (%)
Cereals	270	315.00 (193-410)	116.67 (71.48-151.85)
Pulses	60	43.34 (21.40-56.20)	72.23 (35.67-93.67)
Roots and tubers	200	45.26 (22.50-63.20)	22.63 (11.25-31.60)
Green leafy vegetables	100	42.77 (19.20-55.63)	42.77 (19.20-55.63)
Other vegetables	200	65.63 (37.82-76.50)	32.81 (18.91-38.25)
Fruits	100	49.09 (23.16-58.63)	49.09 (23.16-58.63)
Meat and meat products	60	37.34 (15.30-55.60)	62.23 (25.50-92.67)
Milk and milk products (ml)	300	180.92 (75.00-250.00)	40.07 (25.00-69.44)
Fats	20	34.18 (29.20-59.89)	170.90 (146.00-299.45)
Sugars	20	18.92 (15.60-25.89)	94.60 (78.00-129.45)

Values in parenthesis indicate ranges
Suggested balanced diet, NIN (2011) for sedentary women

The data indicated that consumption of sugar was near to adequate as suggested for sedentary women with mean intake of 18.92 g (15.60 to 25.89 g) and mean adequacy of 94.60 per cent (78.00 to 129.45%). Although the mean values indicated optimum consumption, it is noteworthy that some women consumed more than suggested.

Mean intake of pulses, meat and meat products and milk and milk products were less than the suggested balanced diet. The intake of protective foods such as roots and tubers, green leafy vegetables and other vegetables was less than 50 per cent of suggested balanced diet for sedentary women. Lowest intake of 45.26 g (22.50 to 63.20 g) as compared to suggested intake of 200 g by women was noticed. The maximum adequacy was as low as 22.63 per cent, with a range of 11.25 to 31.60 per cent. Further mean consumption of green leafy vegetables and fruits were 42.77 and 49.09 g, respectively, revealing the mean adequacies of 42.77 and 49.09 per cent, respectively. The range of adequacies for the two foods were 19.20 to 55.63 and 23.16 to 58.63 per cent, respectively, for green leafy vegetables and fruits. Similar levels of consumption for roots and tubers were observed in the diet of police women. The mean intake was 45.26 g as against 200 g suggested balanced diets for women. The range of intake varied from as low as 22.50 to 63.20 g, revealing adequacy levels ranging from 11.25 to 31.60 per cent, with a mean of 22.63 per cent, indicating lowest adequacy among the foods. Further, it was revealed that although the mean consumption of other vegetables was 65.63 g (37.82 to 76.50 g), it was lower than the suggested amount in balanced diet of 200 g. The mean adequacy was 32.81 per cent (18.91 to 38.25%). Thus, it was noticed that the consumption of protective foods was lower than the suggested.

Information on food consumed in daily diet helps to assess the nutritional status of individuals indirectly. Computation of nutrient composition and comparison with RDA revealed salient aspects of nutritional behaviour of women police. It was observed that the nutrient adequacy of women police varied widely. Energy, fat, thiamine and magnesium content of diets were more than the RDA. Mean niacin intake was adequate. Rest of the nutrient contents were lower than the RDA for women.

Table 2 depicts nutrient adequacy of diet consumed by women police. It was observed that among women police, the mean energy (2037 kcal) intake was in the range of 1537-2987 kcal with adequacy of 107.23 per cent, the adequacy ranging approximately between 81 to 157 per cent. Fat intake (52.81 g) was in the range of 21.97-75.92 g. With an adequacy of 264.05 per cent (109.85 to 379.60%), thiamine intake (1.52 mg) was in the range of 0.96-2.11 mg with an adequacy of 152.00 per cent (96.00 to 211.00%). Niacin intake (12.14 mg) was in the range of 7.01-19.3 mg with an adequacy of 101.16 per cent (58.42 to 161.92%). Although the mean intake of these nutrients was more than the recommended dietary allowances, it could be observed that the diets of some women police contained the same nutrients in lower proportion. The lower range of adequacy of these nutrients needs to be considered carefully for women police.

The data on nutrient composition of diets of women police revealed inadequacies with respect to several important nutrients such as protein, calcium, iron, β carotene, riboflavin, vitamins B_6, vitamin C, folate, vitamin B_{12} and zinc. Protein intake (41.01 g) was found in the range of 30.60-52.60 g (74.56% adequate with a range of about 56 to 94%), calcium intake (416.21 mg) was in the range of 278-567 mg (69.36% adequate ranging between 46 to 94%), iron intake (17.12 mg) was in the range of 7.40-19.90 mg (81.52% adequate ranging between about 35 to 95%), β carotene intake (743.48 µg) was in the range of 193.73-1081.08 µg (only 15.49% adequate ranging between 4 to 22%), riboflavin intake (0.94 mg) was in the range of 0.60-1.11 mg (94% adequate ranging between 54 to 100%), vitamin B_6 intake (0.23 mg) was in the range of 0.05-0.27 mg (11.50% adequate ranging between 2 to 13%), vitamin C intake (24 mg) was in the range of 14.91-41.58 mg (60.00% adequate ranging between 37 to 103%), folate intake (136.87 µg) was in the range of 96.26-280.91 µg (68.43% adequate ranging between 48 to 140%), vitamin B_{12} intake (0.83 µg) was in the range of 0.42-1.22 µg (83.00%

Table 2: Nutrient adequacy of diet consumed by women police

Nutrients	Recommended dietary allowances	Mean ± SD	Adequacy (%)
Energy (kcal)	1900	2037 ± 570.30 (1537-2987.65)	107.21 (80.89-157.24)
Protein (g)	55.0	41.01 ± 13.11 (30.60-52.60)	74.56 (55.64-95.64)
Fat (g)	20	52.81 ± 16.83 (21.97-75.92)	264.05 (109.85-379.60)
Calcium (mg)	600	416.21 ± 142.90 (278.00-567.20)	69.36 (46.33-94.53)
Iron (mg)	21	17.12 ± 7.60 (7.40-19.90)	81.52 (35.24-94.76)
β-carotene (µg)	4800	743.48 ± 557.90 (193.73-1081.08)	15.49 (4.03-22.52)
Thiamine (mg)	1.0	1.52 ± 0.42 (0.96-2.11)	152.00 (96.00-211.00)
Riboflavin (mg)	1.1	0.94 ± 0.62 (0.12-1.11)	94.00 (10.90-100.90)
Niacin (mg)	12	12.14 ± 3.63 (7.01-19.43)	101.16 (58.42-161.92)
Vitamin B_6 (mg)	2.0	0.23 ± 0.21 (0.05-0.27)	11.50 (2.50-13.50)
Vitamin C (mg)	40	24.00 ± 9.38 (14.91-41.58)	60.00 (37.28-103.95)
Folate (µg)	200	136.87 ± 55.81 (96.26-280.91)	68.43 (48.13-140.46)
Vitamin B_{12} (µg)	1.0	0.83 ± 0.39 (0.14-1.22)	83.00 (14.00-122.00)
Magnesium (mg)	310	410.00 ± 262.11 (96.75-925.32)	132.25 (31.20-298.49)
Zinc (mg)	10	7.26 ± 1.57 (4.00-12.60)	72.60 (40.00-126.00)

Values in parenthesis indicate ranges

adequate ranging between 4 to 122%) magnesium intake (310.00 mg) was in the range of 96.75-925.32 mg (132.25% adequate ranging between 31 to 298%) and zinc intake (7.26 mg) was in the range of 4.00-12.60 mg (72.60% adequate ranging between 40 to 126%), and were less than the recommended dietary allowances. It is noteworthy that the mean values gave a buffered level of inadequacies, because the lower range of values for inadequacies were very low as in case of calcium (46%) and iron (35%). β carotene and vitamin B_6 of the diets of women police was very low even the upper range of values were lower than 25 per cent adequate for women police. The results of the present investigation were on par with the results of Sharan and Purttaraj (2003) who revealed that cereal consumption was high among canteen food consumers compared to home food consumers in a study on food consumption pattern of executives and non-executives employees of Bharat Electronics Ltd., Bengaluru. Devadarsini *et al.* (2012) also reported that adequacy of cereals and pulses ranged around 80 per cent among shift and day workers of Bhuvaneshawar,

Table 3: Categorization of diet quality of women police N=90

Diet quality	Scores	Frequency (%)
Very good	8.6 and above	04 (4.44)
Good	7.1-8.5	20 (22.22)
Fair	5.6-7.00	49 (54.45)
Poor	5.5 below	17 (18.89)
Total		90

Values in parenthesis indicate percentage
Maximum possible score: 10

Orisssa. Working efficiency output are dependent on the health and physical fitness of individuals. Adequate diets are essential for optimum work output.

A perusal of data in Table 3 reveals that among 90 women police, about 50.00 per cent consumed diet categorized as fair, followed by 22.22 per cent and 18.89 per cent consuming good and poor quality diets, respectively, very few women police (4.44%) consumed diet of very good quality.

The study concludes that optimum nutrition is important for health and maintenance of all body functions. Discrepancies in food behaviour, nutrient intake led to malnutrition among police-women. Empowerment of women with appropriate nutritional knowledge would help police women to follow balanced diet for maintenance of good health.

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Manuscript recived on 22.1.2019

Manuscript accepted for publication on 23.3.2019

Journal of Eco-friendly Agriculture **14**(2): 78-81. 2019

Short Communication

Homoeopathic plant nutrients and plant protectors

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ABSTRACT

It is an universally accepted fact that chemical fertilizers are the cause of increase in pests and discases and use of pesticides is more harmul to human and animals. A solution is found with homeopathy, a dynamic force in the invisible plane, for superior one, than the gross physiological watter (chemicals) but without bad effects of chemicals. New agriculture makes use of many different forces-solar, planetary and terrestrial, in order to return to the soil, what is taken from it by growth and harvest of different products.

Key words: Homeopathy, chemicals, solar, planetary, terrestrial

The need of the time is higher yields to meet the demand of higher population growth.

To have increased yield, hybridization and high yielding varieties, have been developed. The scientists have further found that with the high yields the soils are getting depleted of their nutrients (NPK). Thought the yields have been increased with the chemical fertilizers in the initial stages, pests and diseases have multiplied several hundred fold and started damaging the crops, as a natural follower. To avoid the damage to crops and maintain the average yields level, the pesticides have taken birth. More than the increased pest complexity, the animals and humanity who are dependent on plants are forced to eat the poisonous foods, knowingly.

The humanity is now caught by the multiplication of known diseases and eruption of new health hazards. The average health of human being is in chaos. It is universal truth that organic farming is the only solution to the present problem and is acceptable to all provide similar yields as chemical fertilizers, are possible with the modern farming, if the chemical fertilizers which are detrimental to the soils, plant life and a source of pest and diseases man and animals are to be avoided. At the same time, provided one has an open mind, it should be accepted, that with organic farming a sure method or a formula with which a common farmers can unhesitatingly adopt, in order to have optimum yields, is lacking. Only fragmented broad methods, not easily adaptable and those which can not give a guaranteed optimum yields are available and hence organic farming is being ignored, especially in the presence of "cooked food" _ NPK, through chemicals. It is not easy to turn the minds of the farmers towards organic farming as the "cooked food" is readily at hand, unless organic farming also is as "cooked food".

A study and research had been taken up to replace the "cooked food" since 1973 and a solution is found with "homoeopathy", a dynamic force in the invisible plane, far superior one, than the gross physiological matter (chemicals) but without bad effects of chemicals.

The theory

It is required to explain the homoeopathic theory in order to make one appreciate the superiority and convince himself of its equivalence to organic farming.

Every living being is endowed with a self protecting force to adopt itself to the environment and live well. This is the arrangement of the benefit of the creation. This self protecting force, pervades the whole economy (living being) and makes all adjustments automatically, to see that life is maintained well, as long as life is present, according to law of *Karma*. When there is a disturbance in the rhythm of life, the cause of disturbance is traced in the gross physical body by modern scientist and tries to correct the imbalances. Whereas the ancient seers (not to be called as scientists as they are more than scientists) have realized the one that can protect the life, this self protecting force is not on the physical plane and hence the scientists are blind to it. A force can be tackled by another force only. So, the assisting agency also should be a force unseen the physical plane. The materialistic scientists having capacity to realize the changes in the physical matter concluded that changes in the physical matter should be corrected by **supplementation**, not recognizing the self protecting force. That is why the scientist failed utterly in bringing harmony to the life but drawing conclusions and new theories in successions, condemning their own earlier theories and finally misleading the common man. The correct methods should then be to understand the nature and its arrangements; is it not wise to assist the self protecting force which is capable of maintaining the life

harmoniously, than drawing conclusions and building up our own theories ignorantly to be corrected later.

Hence, local correction and supplementation apparently gives good effect but the system having been addicted to it, craves more and more, i.e., requires the dose every time. But finally those supplements not absorbed the system engrafts in the system, a peculiar condition or a malady, making it more complex. If the system is corrected in a way that it absorbed from the food that is taken all the required nutrition, the system grows naturally and healthy. As the self protecting agency is more intelligent than the human being (who is still trying to understand from its results, in a half knowledge state) the health and order of the system is maintained very well to the required degree of accuracy. For ex. A sick baby after conducting all the known tests is found to be sick because of lack of calcium, the remedy therefore is unhesitatingly suggested, as to feed a baby with additional calcium. But, to the intelligent man, it should suggest that the cause of sickness is that the system is deranged in such a way, as to make the baby not able to take up and assimilate the calcium from the food it takes. The remedy of feeding the baby with additional calcium that is not absorbed and assimilated by the system gets deposited in the physical body. This effect is not seen immediately, but after a loss of some time, is recognized as a separate disease (we have that intelligence in giving any number of names to the result of diseases ignoring the whole, i.e., man) and start treatment for a different disease. There is no end to this viscious circle. Is it not the best, if the system is so corrected by some means, in such a way that it absorbs and assimilates the required calcium so that the baby is restored to health. Homoeopathy does it. The medicinal agent is potentized to the required degree to suit to the degree of affection of the self protecting force of the system and when administrated, being dynamic in nature (not be recognized by the physiologist through his instruments) instead of acting on the physical body, helps to correct the self protecting force itself, so that it can function in an orderly way and carry on its functions to be required degree of perfection.

The same analogy is taken on the vegetable kingdom also, as the plant having life in it, has also at this juncture a nature's arrangement, a self protecting force is present in it and because of its existence only, the adaptability to environment is becoming possible so, it can be seen that instead of feeding the plant with gross NPK minute doses of correcting agency is introduced into the plant through soil and foliar application, so that the plant judiciously absorbs the naturally available nutrients (not only NPK but many more) from the atmosphere and soil and live well. It is not a superior method than feeding the soil with only NPK. Even the scientists, have come to a stage of ignoring many more micro-nutrients that needed (over this the farming community is totally ignorant). Getting allured with immediate effects of only N, have ignored partially P and totally K, at present modern farming is now being carried out mostly with 'N' alone – a fact, one has to be bold to accept, best the possibility of correction, It is required to reproduce the writings of a great botanist, a scientist and a homoeopathist of earlier 20th century – Mrs. Dorothy Shepherd for a better understanding of the science.

About the essentiality of a healthy soil and bad effects of chemical fertilizers

A healthy living soil is the first essential towards raising of the general standards of health and # cosmic radiations introduced through the agency of specially prepared herbs are vital. It is agreed that the inorganic minerals, which are used freely in agriculture, show at first an increased growth of the plants, enormous cabbage, cauliflower, etc., are produced, but in time, diseases become rampant and fertility of the soil, decreased appreciably.

Not only that, the humus of the soil and the radiation through the soil disappear because man is working against and not with the nature. The rhythmic balance of the soil is disturbed and in time one finds it becomes more like fine dust without any life in it at all and sooner or later man has to leave that particular part of the land having extracted all the fertility stored up in the soil for centuries, because it produces less and less for him and for his beasts. He has to move on then repeating the destructive processes elsewhere, until we have reached the stage, where there is no fresh land to be found. We come across vast deserts in various parts of the globe which were fertile once, as a result of wrong agricultural methods.

Employed by lands which were found in North America only a short hundred years ago are now fast becoming vast dust bowls, and the middle north American states suffer frequently from dust storms which blow up the humus and top soil away and burry other parts of surrounding country in time with principal of sand. And gradually we have similar conditions arising here comparable to that of Gobi, Sahara, etc., deserts extending continuously.

This new agriculture makes use of many different forces,

Here also a dispute arises with the scientists, who are totally relying on their instruments, can not realize their existence and many unseen conditions keeping the life in existence harmoniously. An open mind is needed to learn and appreciate many facts unseen and hence unknown to man.

solar, planetary, and terrestrial, in order to return to the soil, what is taken from it by growth and harvest of the different products. Every year compost made by this or similar methods either by bio – dynamic manner or by the "Quick Return" methods, returns humus to the soil, for if humus is not reapplied every year, the soil is in course of composting but only these two are dealt with.

Agriculture is closely inter – woven with astrology and for thousand of years, the ancient men would always sow their crops under the favourable sign of the particular planet and at a particular phase of the moon. Modern man with his superior knowledge of chemical compounds and with the aid of the many mechanized agricultural aids and machines remembers little of the ancient wisdom but he will have to return to it, unless he wishes to loose fertility of the whole of the earth, within the appreciable short of time.

About soils and compost

Though it is an insignificant plant (yarrow) yet investigation, have shown that it is not only the store house of many substances, but it is a channel, as well, for powerful forces for good, I believe, it is metaphysician Rudolf Steiner who first suggested yarrow as an important constituent for increasing the working efficiency of the organic compost, heap, in order to reintroduce new life into the soil. His suggestions were that* the flowers should be placed in to the bladder of a stag after which it is ….. in the sun.*

Dr. Ooliske proved that the higher potencies of the prepared yarrow produced much growth in plants than the lower potencies did. The organic preparations obtained from this plant with other herbal preparations made from yarrow when incorporated in the compost heap in minute quantities brought certain defined cosmic forces into compost heap as well as the various chemical compounds contained in the individual herbs. These forces enliven, potentize and strengthen the action of compost or manure heap, so that we therewith, return great powers and forces by radiation into the soils, when this specially made organic compost is spread on the soil, in a thin layer. This is shown as already referred to by increasing steady growth of the crops, which are not only stronger and sturdier than those crops grown under the influence of inorganic minerals but the particular vegetables, corn and grasses, grown with the aid of these preparations, fermented in the compost heap, by the special methods are much healthier in appearance, more resistant to pests and virus diseases.

A second more practical method was worked out by Miss Maye E. Bruce who suggested using the same herbs as suggested by Dr. Rudolf Steiner, but she added honey, a product of flower essences obtained and worked out by bee, This "Quick Return" (QR) method of activating compost or manure heaps has been experimented with and proved by Miss Bruce and although the herbs may be the same, the application and the methods of preparation are different, she chopped up herbs such as yarrow, etc., and added them to a mixture of lawn moving, weed stand, vegetable matter in a number of glass jars, they were then treated with the herbal solutions in varying strengths. The contents had almost completely broken down long before the other preparations made from more material strengths, thus confirming one of the homoeopathic principal "The higher the dilution, the greater the energy". The divine essence are within the plants and need only to be freed by right method of preparation. These herbs contain between them when used in solution, the chief elements, needed by plant life, though very few plants have been analyzed yet, their chemical and biological constituents vary each year quantitatively and qualitatively. Miss Bruce is convinced that nature provides little extra of the various elements to the plants, so as they decay, disintegrate and return to the soil, balance is established. Elements which have been disturbed by either so that the normal growth of plant life may be maintained. These solar/cosmic radiations and planetary influence which enter into the compost heap through the action and channel of the specifically prepared, yarrow plant, in combination with other herbs, show that yarrow is indeed one of the sacred or holy plants, thus the necessary cosmic influences are introduced and passed through this plant into the soil, in order to procure healthy crops for the use of man. By these methods the health of men, animals and plants will be raised considerably and many diseases die a natural death, because the soil and the plants grown there on become so resistant to disease it can no longer get a foot hold …….. Mrs. Dorothy Shephered.

This is quite in line with my thought (I came in touch with this book in 1984 – 11 years after my first experiment) and rather confirmed my theory. Of course the methods are quite different she suggested the making and use of compost with herbal preparations, but I started these, directly to the plants through root and soil application.

The result

With large scale repeated experimentation on floriculture, horticulture, vegetables and certain by land

*This being impossible to common man it is left incomplete & can be ignored.

crops, the result were satisfactory in the sense that the yields are at – par or even more than chemical fertilizers. In certain cases the bad effects of certain deficiencies are corrected automatically without any special attempts. To make sure, the treated specimen were kept under observation for more than 3 to 10 years and conformed beyond doubt, that there are no side effects due to prolonged use of these nutrients.

This study was then extended to rice crop on my own fields in the year 1977 and the results were satisfactory. Having not been satisfied with what has been realized. I associated myself with scientists of Agricultural Research Action Ragolu (ARAU) and approached APAU for conducting scientific experiment were quite satisfactory, i.e., the yields are either at par or even significant over the chemical fertilizers in 3 consecutive years 1987, 1988 and 1989 but due to some technical flaws, I am deprived of the certificate. Every year, simultaneously I was conducting field trails on bulk in farmer's fields and in my own fields, to know the difference between lab and land. To improvise the formulae CAPART (Ministry of Rural Development) was approached, who were kind enough to sanction a project in the year 1992-1993. The article "Studies on the performance of rice with homoeopathic nutrients" could be read for a proper understanding of the result. The results are more than satisfactory as in certain cases, high significance over chemical fertilizers were realized. In all these 16 years, all the farmers who have used these nutrients were satisfied and there was no single report of dissatisfaction, though it can't be constructed as a mark of success.

In addition, there is a great advantage, with homoeopathy, as certain micro-nutrients can be given to the required degree which are totally ignored by both scientists, who advocate this, and the farmers, as the scientists failed totally to make these available to the farmers, in words, these have not left the lab so far and there appears to be no attempt in this direction.

Plant protection

The Birth: Necessity is the mother of invention – In the year 1986 there was very high incidence of Jaulmidge on rice crop in Palakonda area, Srikakulam and in the subsequent year 1987 Jaulmidge was found in the nursery stage and several thousands of nurseries were affected creating a par icy condition. A farmer near Palaikonda approached me to try something with homoeo, as other methods were ineffective and day by day several hundreds of nurseries were infected. One out of the six treatments, saved the nursery, that is the only one out of thousands in the vicinity, with the same formula, later on the subsequent crop was saved in about 30 acres that year. Several scientists from different parts of the country have made very special efforts to control the gaulmidge those years, but having not been able to, named it as a special "Palakonda Jaulmidge". This gaulmidge had come to stay in this district and the only suggestion for control of Jaulmidge by the scientists is the use of "Forate at the puddling stage (to destroy the whole land and its bacterial including several invisible helpers like earth worms, snails, etc.). With this homoeopathic formula, control of gaulmidge is very effective, i.e., more or less 100 per cent since then, without affecting the healthy conditions of soil, plants, etc.

The thought

This has given birth to homoeopathic plant protection. It appears unbelievable, as to how at all these non-poisonous material can control the pests. Here an example is to be cited. In cases of tetanus resulting from injuries and septicemia, allopathy (material) is doubtful to cure, whereas homoeopathy (force) is effective capable of curing the tetanus condition safely. Is it by killing the dreaded bacterial. If so, allopathy should not fail in cholera, brain fever, dog bite etc., but homoeopathy proved beyond doubt that it is capable of effecting a safe recovery without introducing poison into the system. Because it is potentized, the invisible force in the medicine gives the required assistance to the invisible life force in man keeping the system safe. The same analogy is applied for plant protection too. The correctness of the principle is proved by the effective control of many pests. In certain cases these are better than conventional chemical pesticides, i.e., where chemical pesticides have failed, these plant protectors are saving the crops or plants or trees as the case may be. Though they are found to be effective, there is still a lot to be explored of the possibility of total plant protection with homoeopathy- the simple safe and cheap method.

It is an universally accepted fact that chemical fertilizers are the cause of increased pests and diseases and use of pesticides is more harmful and is the cause of many dangerous diseases to the humanity, animals depending on the fodder and food. Incidentally it has come to light in practice, that these plant protectors in addition to protection, is giving nutritional effect also at the same time, which is of a very great advantage.

Manuscript recived on 28.9.2018
Manuscript accepted for publication 12.12.2019

Journal of Eco-friendly Agriculture **14**(2): 82-83. 2019

Short Communication

Naturally coloured cotton

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ABSTRACT

In recent years, coloured cotton is receiving increasing importance in view of their eco-friendly character. The urge for eco-friendly cotton can only be fulfilled preferably by organically grown coloured cotton, dispensing harmful chemicals in dying and processing. Coloured cotton is not a product of any recent genetic engineering or biotechnology. Several lint colours (brown, black, mahogany red, red, khaki, pink, blue, green, dirty white of course and white) are found in the four species of the genus Gossypium.

Key words: Coloured cotton

Cotton with naturally coloured lint, other than white, is commonly referred as coloured cotton. Evidence for its cultivation in India has been obtained from the remains of the Indus Valley civilization. Naturally coloured cotton has a long history, dating from 3400 to 2300 BC in Mexico, 3100 BC in Peru (with fibres from 12 to 43 mm), 2250 BC in Egypt (19 to 22 mm) and sometimes before 1200 AD in China.

In 1982, Sally Fox, started researching on coloured cotton, introduced first long fibre of naturally coloured cotton with the help of her knowledge and experience in technology. Later, started her company in the name of Natural Cotton Colors, Inc. In 1988, natural colour cotton of Sally Fox succeeded at commercial level.

The vast majority of cotton grown commercially in the world has white lint. However, there are genotypes/species which produce naturally coloured cotton and most of the wild species of cotton have coloured lint or fuzz. Though, historical evidence like the fossils obtained from the excavations at Huca Preita in Northern coastal Peru indicated the usage and cultivation of coloured cotton with lint colour from tan to red shades before 2500 BC, only some exist today. It seems others have been lost, as they have never been described in the botanical literature. The ability of the white cotton to take up any colour to produce a large range of shades and colours in fabrics has led to the popularization of white cotton. The coloured cotton producing nations in the world are Russia, China, Israel, South and Central America, India, Brazil, Peru, Greece, Turkey and Soviet Union.

Unlike naturally coloured cotton, white cotton has to be bleached and processed before imparting colours. Many of the processing chemicals and dyes used in cotton industry are known to cause health hazards. Chlorinated products, bleaching agents, phenols, formaldehydes, which are employed for bleaching and processing, produce skin diseases. Dyes containing traces of heavy elements such as arsenic, lead, cadmium, cobalt, zinc, chromium, are also skin irritants. Children are especially sensitive to these effects. The azo dyes are proven carcinogens. Processing and dyeing are also water intensive. There are two important sources of coloured lint in cotton.

Genetic resources are most vital for improvement of any crop. In India, about 40 coloured genotypes of upland cotton (*G. hirsutum*), mostly of various shades of brown and green, are available in the National Gene Bank of Cotton maintained at the Central Institute for Cotton Research, Nagpur. These genetic stocks are indigenous collections as well as exotic accessions from USA, erstwhile USSR, Israel, Peru, Mexico, Egypt, etc. In Asiatic diploid cottons (*G. arboreum* and *G. herbaceum*), about 10 germplasm lines possessing mostly light brown lint colour are also available. Most of the coloured linted germplasm lines have been evaluated for their economic attributes as well as fibre characteristics.

Wild species are important sources of coloured lint. Many of the wild species of genus Gossypium, including putative donors of present day tetraploid cotton, i.e., *G.herbaceum* race *africanum* and *G.raimondii* have coloured lint. The brown colour in different shades is most common.

Development of lint colour

Lint colour is a genetically controlled character. Accumulation of pigments in the lumen of lint starts before boll bursting. In upland cotton (*G.hirsutum*), the pigmentation in the developing lint starts 32 days after fertilization and takes nearly six days to develop colour. In Asiatic cotton (*G.arboreum*), pigmentation is observed 46-47 days after fertilization and takes 5-6 days for colour development. Complete expression of lint colour takes place only when the boll bursts open and the lint gets exposed to sunlight. It takes about a week for the lint to develop a complete natural colour. However, the intensity and the time taken for

complete development of colour varies with the genetic background of the genotypes. The two commonly occurring lint colours, i.e., brown and green, are briefly discussed below.

Brown colour: Among the coloured cotton, brown is the most common colour. The brown colour is found in different shades which ranges from light brown to intense mahogany red. Depending on the intensity of colour, it is named as light brown, khaki/camel colour, brown, dark brown/ chocolate colour, dirty grey, tan and red. It is found in all the four cultivated as well as many of the wild species. Brown colour is more stable than the green colour. On continuous exposure to sunlight, the brown colour gradually fades at a very slow rate. In India, brown linted varieties of *G.arboreum*, namely, Cocanada-1, Cocanada-2 and red Northerns were under commercial cultivation during first half of the 20th century.

Green colour: Green is the second important commonly occurring lint colour in cotton. Green colour is less common than brown and occurs mainly in two shades, i.e., light green and green. Green colour is more prone to fading, fades faster than the brown colour. Prolonged exposure to sunlight during boll opening leads to rapid fading of green colour and the colour turns to white, off-white or brownish. Portion of lint which is not directly exposed to sunlight retains its original lint colour. Green colour is mostly observed in *G.hirsutum* and probably varieties possessing green lint have not yet been released for commercial cultivation.

Naturally coloured cotton, though short, coarse, weak and amenable only for hand spinning, are 100 per cent organic, safe, eco-friendly, comfortable and also provides protection against harmful UV rays. However, these having small fibre is not suitable for heavy machine spinning. Besides low yield potential, poor fibre quality, availability of limited colours, low market demand and lack of marketing facilities are its limitations.

Coloured cotton in India

Indian scientists did excellent breeding work on cotton in the last century and in this decade, but none could excel the white cotton breeding program. The coloured cotton cultivation and use did not receive encouragement for various reasons and the setback it brought to the textile industry. It has also high environmental influence especially on soil types, nutrition, sunlight and post-boll opening environment. These features affect the lint length development, maturity and strength. Cotton Research Stations at Khandwa (Madhya Pradesh) and University of Agricultural Sciences (UAS), Dharwad, Karnataka, India have done excellent research on coloured cotton including organic production of cotton as well as yarn and cloth, and also made coloured cotton shirting, etc., in cooperation with organic farmers and ginning, spinning, weaving and garment industries without the use of chemicals and other dyes.

It may be environment friendly, aesthetic and fascinating, but its continued patronage will be determined by economic outlook and long-term benefits.Commercial production of colour-linted cotton does not appear to be in the interest of seed and textile industry at present. But biotechnology may have some interest, if there is a future demand. These are being utilized in casual clothing, home fashions and upholstery fabrics.

Problems of commercial cultivation of coloured cotton

1. Low yield (about half that of white cotton) and susceptibility to certain pests.
2. When cultivated in large areas, natural cross-pollination may occur from white linted to colour and *vice-versa*. Hence, isolation distance of the order of 50 meters or more may be required between varieties.
3. Contamination may also occur during harvesting, transportation, ginning, pressing and spinning.
4. Since white cotton is still a major agricultural produce, its contamination with colour lint may have disastrous effects on agricultural economy.

These along with the application of biotechnology and modern farming techniques may give much-needed boost to revive this gift of nature.

Manuscript recived on 16.5.2018
Manuscript accepted for publication on 20.6.2018

Author Index

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FORM IV

(See Rule 8)

1.	Place of Publication	Lucknow – 226 016
2.	Periodicity of its Publication	Biannual
3.	Printer's Name	Dr. R.P. Srivastava
	Nationality	Indian
	Address	Dr. R.P. SrivastavaDoctor's Krishi Evam Bagwani Vikas SansthaA-601, Indira Nagar, Lucknow-226 016
4.	Publisher's Name	Dr. R.P. Srivastava
	Nationality	Indian
	Address	Dr. R.P. SrivastavaDoctor's Krishi Evam Bagwani Vikas SansthaA-601, Indira Nagar, Lucknow - 226 016
5.	Editor's Name	Dr. Jagdish Chandra Former Principal Scientist, CISH, Lucknow
	Nationality	Indian
	Address	K-899, Ashiana Colony, Lucknow
6.	Name and addresses of individual who own the newspapers and partners or shareholders holding more than one per cent of the total capital	Doctor's Krishi Evam Bagwani Vikas Sanstha A-601, Indira Nagar, Lucknow - 226 016 Registration No. 1-131380

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Registered under Society Registration Act 21, 1860
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