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Indian Institute of Horticultural Research
Hessaraghatta Lake P.O., Bengaluru - 560089

Pest Management in Horticultural Ecosystems

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COVER PHOTO : Pupal cocoons and freshly emerged adults of *Chiloloba acuta* (Wied.)
(Courtesy : Dr. K. Sreedevi, IARI, New Delhi)

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FIRST ANNOUNCEMENT

International Symposium on Phytophthora Taxonomy, Genomics, Pathogenecity Resistance and Disease Control

Venue : Hotel Windsor Manor, Bengaluru, Karnataka, India
Date : 9-11 September 2015

Organised by

Association for Advancement of Pest Management in Horticultural Ecosystems (AAPMHE), Bengaluru
Indian Institute of Horticultural Research (IIHR), Bengaluru
Indian Council of Agricultural Research (ICAR), New Delhi

Why this symposium?

The genus *Phytophthora* causes devastating diseases on a wide range of agricultural crops, natural vegetation and forestry worldwide. *Phytophthora infestans*, the causal organisnm of Great Irish potato famine still remains the most devastating pathogens of potato and tomatoes causing annual loss of US $ 6.7 billion. Plant trade between countries led to devastating disease outbreaks including potato late blight which spread by migration of infected tubers and sudden oak death which has moved through nursery plants. These migrations led to rising concern about thr threat posed by *Phytophthora* and need to devise biosecurity mechanisms. In order to take the stock of situation at global level on all aspects related to Phytophthora, this symposium is being organised.

For further details please contact

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Editor's Choice

Bug for a bite!

Entomophagy simply means eating insects. Animals like lizards, spiders, birds and some predatory insects are natural members of this exclusive club. But soon man is going to compete in a big way with these creatures to grab a grub or two. Let me elaborate on this.

While browsing the net to gather information on the forthcoming events in the area of Entomology, I was struck by an announcement of the 'International Conference on Insects to feed the World' to be held in May 2014 in the Netherlands. The fact that it is being jointly organized by the Wageningen University and FAO enhances the value and the relevance of the topic. Though it is not an unfamiliar thing that insects form part of the traditional diet in some parts of the world, what is interesting is the seriousness with which this branch of Entomology is being taken forward. The opening remarks of the brochure of the above mentioned conference certainly provide a food (!) for our thoughts.

"..With a growing world population and increasingly demanding consumers, can we still produce sufficient animal protein in the future? Urgently we need to identify alternative protein sources, and insects have great potential in contributing to global food security."

Further probe into the topic took me to a latest compilation (2013) on edible insects broughtout by FAO. After going through the pages of the publication, it is realized that a lot has already been happening to popularize insects as delicacies and get an industry status to this field. Some basic facts from the publication are provided here to sensitize the taste buds of 'insect lovers'.

> As per the latest inventory by Dr. Yde Jongema of Wageningen University, worldwide, there are 1900 edible insect species.
>
> Globally, the most common insects consumed are beetles (31%) followed by caterpillars (18%), bees, wasps and ants (14%), grasshoppers, locusts and crickets (13%), hemipterans (10%)and termites (3%) with least contribution (2%) from dipterans. Lepidoptera are consumed almost entirely as caterpillars while Hymenoptera are consumed mostly in their larval or pupal stages. Both adults and larvae of the Coleoptera order are eaten, while the Orthoptera, Homoptera, Isoptera and Hemiptera orders are mostly eaten in the mature stage.
>
> The mopane caterpillar (*Imbrasia belina*) is the most popular and economically important caterpillar consumed in Angola, Botswana, Mozambique, Namibia, South Africa, Zambia and Zimbabwe.
>
> Insects are a highly nutritious and healthy food source with high fat, protein, vitamin, fibre and mineral content. For example, the composition of unsaturated omega-3 and six fatty acids in mealworms is comparable with that in fish and the protein, vitamin and mineral content of mealworms is similar to that in fish and meat.

Besides nutritive value, other merits that add weight to insects as food source include their low emissions of GHGs, low requirements for land and the high efficiency at which they can convert feed into food.

So, edible hexapods are all set to compensate for the losses caused by their pestiferous counterparts and contribute to the global food security. Of course, the challenge before this new 'food' industry will be to ensure the cost-effective, reliable and hygienic production of an insect biomass with adequate regulatory frameworks in place. FAO has been actively working on various issues pertaining to insects as food and has also been hosting an exclusive web portal to share the information. Those interested to learn further may direct their cursor to: **www.fao.org/forestry/edibleinsects.**

May be it is not very long before we come across beetle fry and caterpillar curry in menu cards at a nearby restaurant…

P.V. RAMI REDDY
Chief Editor

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REVIEW ARTICLE

Nanotechnology in crop protection: Status and scope

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ABSTRACT: Pests, including insects, mites, nematodes and pathogens, are the major limiting factor in profitable crop production. Frequent application of pesticides has resulted in development of pest and disease resistance, accumulating residues in produce and environmental pollution. So there is a need for alternative approach as to control pests and pathogens. Application of nanotechnology in crop protection holds a significant promise in management of insects and pathogens, by controlled and targeted delivery of agrochemicals and also by providing diagnostic tools for early detection. Nanoparticles are highly stable and are biodegradable; it can be successfully employed in production of nanocapsules for delivery of pesticides, fertilizers, and other agrochemicals. Nanoparticles display slow release of encapsulated functional molecules and reduce its frequent applications. Nanoparticles are smaller in size with more charge and larger surface area with higher stability and solubility, so behave differently from their bulk sized counterparts. The biological agents such as plants and microbes have emerged as cost effective and efficient candidates for the synthesis of nanoparticles by green synthesis approaches. They have advantages over conventional chemical methods which associated with eco toxicity. This review is focused on potential applications of nanomaterials in crop protection for a cleaner and greener agriculture.

Keywords : Crop protection, nanoparticles, nanotechnology, pathogens.

INTRODUCTION

Nanotechnology has developed as one of the most ground-breaking scientific fields in decades. Nanotechnology is the understanding and control of matter at dimensions between approximately 1 to 100 nanometers (nm) where unique phenomena involved novel applications. A nanomaterial is one billionth of a meter. Nanoscale ranges from 1 and 100 nanometers (http://www.nano.gov/node/241). Nanoscale materials show unusual physical, chemical and biological properties, which are completely distinct from their bulk materials and individual molecules (Li *et al*., 2001). These unique properties find its novel applications in all the fields. Nanoparticles have large surface to volume ratio, chemically alterable physical properties, and possess strong affinity to targets such as proteins (Kumar *et al*., 2010). Through the ever growing global food demand due to changing climate, urbanization and environmental issues such as run-off and accumulation of agrochemicals, there is an increasing need to feed an estimated population growth from the 6 billion to 9 billion by 2050 (Chen and Yada, 2011). With the limited natural resources such as land, water and soil fertility, demand for food has increased tremendously. The cost of chemical fertilizers, pesticides and other production inputs has been drastically increased due to limited reserves of natural gas and petroleum (Ditta, 2012). There is a need to overcome these constraints with the help of precision farming practices and effective application of nanotechnology to agriculture.

Nanoscale systems like encapsulation and entrapment of agrochemicals such as fertilizers, pesticides, herbicides, plant growth regulators and other active substances by using polymers, dendrimers, surface ionic attachments and other mechanisms may be used in controlled and slow release of agrochemicals, which allow the slow uptake of active ingredients and in turn reduces the amount of agrochemical application by minimizing the input and waste. Importance of nanoscale delivery system in agriculture is because of its improved solubility and stability to degradation in the environmental factors. The nanoscale delivery vehicles increase effectiveness by binding firmly to the plant surface and reduces the amount of agrochemicals by preventing run-off into the environment (Johnston, 2010; Chen and Yada, 2011). Nanomaterials also play an important role in promoting sustainable agriculture and provide better foods globally (Gruère, 2012). In developing countries, nanotechnology has got important application for enhancing agricultural productivity, along with other emerging technologies such as biotechnology including genetics, plant breeding, disease control, fertilizer technology, precision agriculture, and other allied fields (Sastry *et al*., 2010; Jha *et al*., 2011). Nanotechnology can be used for combating the plant diseases either by

controlled delivery of functional molecules or as diagnostic tool for disease detection. Nanosensors and other field sensing devices can be used in detection and measurement of crop nutrient status, insects, pathogens, weeds, moisture level, soil fertility, temperature, etc. which helps in real time monitoring of the crop growth and provide essential data for precision farming practices leading to minimize agricultural inputs and maximizing resource output and yield (Scott and Chen, 2003). Nanosensors can also provide information about optimal times for planting and harvesting crops and provides useful information for timely application of agrochemicals like fertilizers, pesticides and herbicides.

With the global efforts to reduce generated hazardous waste and the growing demand for synthesis of safe nanomaterials, researchers adopted "green" synthesis methods. The synthesis of nanoparticles from the plant extracts and microbes is a boon for advance research in nanotechnology. Synthesis of extracellular nanoparticles with variety of locally available biological agents is a novel and economical concept for bioprospecting. It provides new avenues to exploit wide variety of biological species for product development (Sharma *et al*., 2010). Green synthesis practice helps in reducing generation of hazardous waste by using environmentally safe solvents and nontoxic chemicals. The effective use of nanoparticle synthesized by green synthesis is very important, as several pathogenic bacteria have developed resistance against various antibiotics. Because of the introduction of new populations and sexual recombination within the species, pathogens have become resistant to systemic bactericides and fungicides. For instance, *Staphylococcus aureus* has developed resistance to methicillin, *Candida albicans* is resistant to fluconazole and *Phytophthora infestans* developed resistance against metalaxyl (Schaller *et al*., 2003; Chowdappa *et al*., 2013). Among the recent advancement in agricultural sciences, nanomaterials play a significant role in crop protection because of its unique physical and chemical properties. Nanoparticles remain bound to the cell wall of pathogen and causes deformity due to high energy transfer leading to its death. Nanotechnological application in plant pathology targets specific agricultural problems in plant–pathogen interactions and provide new perceptions for crop protection. Nanocomposites fulfil the two most important criteria in disease management: efficacy with minimal ecological impact and less toxicity on humans. While there is no much evidence of harm to people or the environment at present, nanotechnology is a new and evolving area of study where the research and developments are still at bench-top scale. Social and ethical implications of nanotechnology applications in agriculture have to be addressed and toxicity of nanomaterials has to be clearly understood before its commercialization and field application. The potential application of nanomaterials in different agricultural applications needs further research investigation with respect to synthesis, toxicology and its effective application at field level. In the field of agriculture, there are still many possibilities to explore with new nano products and techniques.

Here, we have highlighted some of the most promising and potential applications of nanotechnology in crop protection including plant growth enhancement, pest and pathogen control with biopolymer and metallic nanoparticles, post-harvest disease management, green synthesis of nanomaterials and we also focused on limitations and current issues of applying nanomaterials in agriculture. This review examines whether nanotechnology can provide the science and technological boost for the improvement of agriculture in a cost effective, and environmentally friendly greener way.

Nanoparticles in plant growth enhancement

With the goal to promote its use for agricultural applications, nano materials (NM) can be effectively used in plant germination and growth. Khodakovskaya *et al*. (2012) reported the application of carbon nanotubes as regulators of seed germination and plant growth. They have shown that multiwalled carbon nanotubes (MWCNTs) have the ability to enhance the growth of tobacco cell culture by 55-64% when compared to control at a wide range of concentrations from 5-500 μg/ml. At low concentrations, activated carbon enhanced cell growth while at higher concentrations it intensely inhibited the cellular growth. They demonstrated the correlation between the activation of cell growth exposed to MWCNTs and the up regulation of genes like aquaporin NtPIP1, CycB and NtLRX1involved in cell division/cell wall formation and water transport, compared to control cells. Penetration of nanomaterials into the seed plays a key role in increasing seed germination rate. Carbon nanotubes (CNTs) can regulate cell division and plant growth by a unique molecular mechanism that is related to the activation of water channels (aquaporins) and major gene regulators of cell division and extension. They have highlighted the novel positive effects of MWCNTs at the cellular level and have provided an understanding of the complex mechanism underlying the enhancement of plant growth. However, to consider the possible use of carbon nanoparticles in agriculture, the consequences of the introduction of carbon nanotubes

into the environment have to be thoroughly investigated. Some of the recent studies have shown enhanced germination rate by the use of NM in the seeds. Recently, Khodakovskaya *et al*. (2009) have shown that use of MWCNTs in tomato seeds resulted in 90% increase in the rate of seed germination (compare to control which showed only 71%). CNTs penetrate into the hard coat of germinating tomato seeds and enhanced growth due to increased water uptake. MWCNTs could find an application in delivering desired molecules into the seeds during germination, which in turn protect the seeds from diseases. Because of their growth promoting effect, they will not pose any toxic or adverse effect on the plant. Authors also insist on additional studies evaluating the resistance to pests by tomato plants germinated through CNTs. Canas *et al*. (2008) studied the effects of functionalized (poly-3-aminobenzenesulfonic acid) and non-functionalized single-walled carbon nanotubes on root elongation of six commercially important crop species such as cabbage, carrot, cucumber, lettuce, onion, and tomato (regularly used in phytotoxicity studies). Root growth measurement at different time intervals, concurred that the non -functionalized carbon nanotubes affected root length more than functionalized nanotubes. Scanning electron microscopy (SEM) images displayed the presence of nanotube sheets on the root surfaces with no visible uptake of nanotubes by plant.

Zheng *et al*. (2005) studied the effect of nano and non-nano TiO_2 on the growth of spinach seeds. They reported that plants produced by seed treatment of nano-TiO_2 had 73% more dry weight, increase in chlorophyll-a formation and three folds higher photosynthetic rate, compared to the control. Photo sterilization and photo generation of superoxide and hydroxide anions by nanoTiO_2 play an important role in increasing seed germination and growth of spinach seeds. Particle size plays an important role in behaviour, toxicity and reactivity of nanomaterials. Germination increases with the decrease in size of the nanomaterials. Recently Mahmoodzadeh *et al*. (2013) investigated effects of nano titanium dioxide particles on plant growth and development. They chose Canola seeds as the model system for their investigation and treated them with different concentrations of nanoscale titanium dioxide and observed their effect on seed germination and seedling vigor. The authors reported that the treatment of nanoscale TiO_2 (~20 nm) at 2000 mg/L concentration promoted both seed germination and seedling vigor when compared to control. Both positive and negative effects of nanoparticles were observed in living plants.

In view of the potential influence of nanoscale zinc oxide (ZnO) particles on growth and development of peanut, Prasad *et al*. (2012) reported the effects of zinc oxide nanoparticles (25 nm) at 1000 ppm concentration promoted the seed germination, seedling vigor and growth of peanut plant. Their study also focused on determination of early establishment of flowering, higher leaf chlorophyll content, increasing stem and root growth in treated plants. Particles were very effective in increasing pod yield and root growth of peanut. Field experiment on foliar application of ZnO nanoparticles showed determinable effect on pod yield at 15 times lower dose compared to control. Nanomaterials can be effectively employed in growth and germination of plant when used in controlled conditions. Investigation of the adverse effect of nanomaterials on the seed and plant properties increases the effective applicability of nanomaterials in crop production.

Nanotechnology: scope in pathogen control

A wide variety of bacterial and fungal pathogens spoils vegetables. Among them the most common bacterial agents are *Erwinia carotovora, Pseudomonas spp., Corynebacterium* and *Xanthomonas campestris* which attack most vegetables. Fungal pathogens causing spoilage of vegetables are species belonging to genera *Alternaria, Aspergillus, Cladosporium, Colletotrichum, Phomopsis, Fusarium, Penicillium, Phoma, Phytophthora, Pythium, Rhizopus* spp., *Botrytis cinerea, Ceratocystis fimbriata, Rhizoctonia solani, Sclerotinia sclerotiorum,* and some mildews. Some of these organisms are host specific whereas others affect a wide variety of vegetables causing huge economic losses. Some pathogens produce toxic metabolites and adversely affect human health. Many of these agents enter the plant tissue over mechanical or chilling injuries, and cause overwhelming losses (Tournas, 2005). With the estimated doubling in global food demand in next 50 years huge challenges have been posed in food production. In the year 2000 the pesticide production was about three million tons of active ingredients worldwide (Tilman *et al*., 2002). It is reported that very small amount (less than 0.1 %) of pesticide reaches the sites of action, due to loss of pesticide in air during application and as run-off, spray drift, off-target deposition and photodegradation affecting both the environment and application costs (Pimentel, 1995; Castro *et al*., 2013). With the growing demand of pesticide worldwide to control the pathogens and pests, there is an urgent need to tackle the excessive usage of pesticides and fertilizers by finding alternatives. Table 1 shows some of the nanomaterials used for control of plant pathogens.

Potential applications of nanotechnology in crop protection include controlled release of encapsulated pesticide, fertilizer and other agrochemicals in protection against pests and pathogens, early detection of plant disease and pollutants including pesticide residues by using nanosensors (Ghormade *et al.*, 2011). The potential applications of nanomaterials in crop protection, helps in the development of efficient and potential approaches for the management of plant pathogens. As an efficient and potential modern approach, nanotechnological research studies carried out related to management of agriculturally important pathogens are reviewed here.

Nanoparticles in disease management

Various nanoparticles employed in plant disease management are listed in Table 1.

Biopolymer nanoparticles: Development of nanoformulation for field application of agrochemicals requires the use of readily biodegradable, nontoxic, environment friendly, safe and low-cost materials. So, use of biopolymers produced by natural sources with good physical and chemical properties is a fascinating approach to prevent the use of petrochemical and toxic chemical substances in production of nanomaterial.

Chitosan: Chitosan nanoparticles have got various applications in biology due to its biodegradable and nontoxic properties. In acidic condition the free amino groups of chitosan protonates and contributes to its positive charge (Phaechamud and Ritthidej, 2008). The inhibition mode of chitosan against fungi is defined by the following three mechanisms.

i) The positive charge of chitosan interacts with negatively charged phospholipid components of fungi membrane, which in turn alter cell permeability of plasma membrane and causes the leakage of cellular contents, which consequently leads to death of the cell (García-Rincón *et al.*, 2010).

ii) Chitosan chelates with metal ions, which has been implicated as a possible mode of antimicrobial action (Rabea *et al.*, 2003). On binding to trace elements, it interrupts normal growth of fungi by making the essential nutrients unavailable for its development (Roller and Covill, 1999).

iii) It is suggested that chitosan could penetrate fungal cell wall and bind to its DNA and inhibit the synthesis of mRNA and, in turn, affect the production of essential proteins and enzymes (Sudarshan *et al.*, 1992; Kong *et al.*, 2010).

In search of natural antimicrobials to avoid harmful synthetic chemicals, chitosan and chitosan nanoparticles are found to be more effective against plant pathogens like *Fusarium solani*. Inhibitory effect was also influenced by particle size and zeta potential of chitosan nanoparticles. The chitosan therefore could be formulated and applied as a natural antifungal agent in nanoparticles form to enhance its antifungal activity (Ing *et al.*, 2012). Chen *et al.* (2010) studied antibacterial activities of low molecular weight chitosan products (molecular weight ranges from 6.9 kDa to 22.4 kDa) with average nanoparticle sizes of 117 nm to 965 nm. They emphasised that antimicrobial activity of chitosan nanoparticles depends on its zeta potential, which plays a significant role in binding with negatively charged microbial membrane. Antimicrobial activity of chitosan, chitosan derivatives bound metal ions and nanoparticles are well studied (Sanpui *et al.*, 2008; Jagadish *et al.*, 2012; Kaur *et al.*, 2012).

Metallic nanoparticles: Metallic nanoparticles possess unique chemical and physical properties, small size, huge surface to volume ratio, structural stability and strong affinity to their targets (Kumar *et al.*, 2010). Metal nanoparticles can be used as new antimicrobial agents and an alternative to synthetic fungicide to delay or inhibit the growth of many pathogens species because of its multiple mode of inhibition.

Silver nanoparticle: Due to emerging plant diseases, agricultural production is reduced worldwide. Every year millions of dollars have been spent to control plant diseases. Various natural and artificial control measures have been used for plant protection. Use of pesticides is the most prevalent method for disease control at present. Scientists are searching for alternative measures against pesticide application, due to its environmental hazards and residual problem. As an alternative to chemical pesticides, use of silver nanoparticles as antimicrobial agents has become more common (Jo *et al.*, 2009; Kim *et al.*, 2012). Silver has been used as an antimicrobial agent since ancient civilizations; it has been used extensively due to its broad-spectrum and multiple modes of antimicrobial activity (Wei *et al.*, 2009). Its specific antimicrobial mechanisms are still unclear. Silver exhibits higher toxicity to microorganism and lower toxicity to mammalian cells. The application of silver nanoparticles as antimicrobial agents is because of its economical production and multiple modes of inhibitory action to microorganisms (Clement and Jarrett, 1994). Silver nanoparticles are the most studied and utilized nano particles in bio-system

because of its strong inhibitory and antimicrobial activities. Kim *et al*. (2008) evaluated the antifungal efficacy of colloidal nano silver solution, against rose powdery mildew. Nano silver colloid is more adhesive on bacterial and fungal cell surface; hence act as better fungicide because of its well dispersed and stabilized silver nanoparticles solution. Nano silver is classified as pesticide (Baier, 2009). Since silver acts as an excellent antimicrobial agent it is now an accepted agrochemical replacement. It acts as plant-growth stimulator and reduces unwanted microorganisms in soils and hydroponics systems (Sharma *et al*., 2012).

Silver in ionic or nanoparticle forms has a high antimicrobial activity and is therefore widely used for various sterilization purposes including materials of medical devices and water sanitization. Relatively few studies were reported on the applicability of silver in controlling various plant pathogens in a relatively safer way compared to synthetic fungicides (Park *et al*., 2006). Since nanoparticles efficiently penetrate into microbial cells, lower concentrations of silver nanoparticles are sufficient for microbial control. This would be effective, especially for those organisms that are less sensitive to antibiotics because of poor penetration of some antibiotics into microbial cells (Samuel and Guggenbichler, 2004). Lamsal *et al*. (2011a) showed the effective usage of silver nanoparticles instead of commercial fungicides. They evaluated the effect of silver nanoparticles against six *Colletotrichum* species associated with pepper anthracnose under different culture conditions and found that, application of 100 ppm concentration of silver nanoparticles inhibited the growth of fungal hyphae as well as conidial germination *in vitro* when compared to the control. Silver nanoparticles showed significantly high inhibition of fungi in field conditions when applied on the plants before disease outbreak. Recently, Aguilar-Méndez *et al*. (2011) studied the dose-dependent fungistatic activity of the silver nanoparticles on *Colletotrichum gloesporioides*. Jo *et al*. (2009) tested various forms of silver ions and nanoparticles to examine their antifungal activity on two plant-pathogenic fungi, *Bipolaris sorokiniana* and *Magnaporthe grisea*. The *in vitro* and *in planta* evaluations of silver showed that both silver ions and nanoparticles effect colony formation of spores and disease progress of fungi. Kim *et al*. (2012) reported the inhibitory effect of three different silver nanoparticles (WA-CV-WA13B, WA-AT-WB13R, and WA-PR-WB13R) against eighteen different commercially important plant pathogenic fungi on potato dextrose agar (PDA), malt extract agar, and corn meal agar. They found that inhibition of fungal pathogens with silver nanoparticles is concentration dependent and also on type of silver nanoparticles used. Most fungi showed a good inhibitory effect at 100 ppm concentration of silver nanoparticles on PDA, compared with others. WA-CV-WA13B showed the highest inhibition effect compared to other silver nanoparticles. Effect of silver nanoparticles on the growth of sclerotium-forming species *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor*, revealed that silver nanoparticles effectively inhibit the hyphal growth in a dose-dependent manner. Further, the microscopic observation of hyphae exposed to silver nanoparticles showed severe damage and resulted in the separation of layers of hyphal wall and collapse of fungal hyphae (Min *et al*., 2009). A recent study on *in vitro* and *in vivo* efficacy of silver nanoparticles against powdery mildew before and after disease outbreak in plants under different cultivation conditions, showed maximum inhibition of fungal hyphae and conidial germination with less concentration of nanoparticle on cucumbers and pumpkins (Lamsal *et al*., 2011b).

Silica nanoparticle*:* Silicon (Si) increases disease resistance and stress resistance in plants (Brecht *et al*., 2004). It also stimulates the physiological activity and growth of plants (Carver *et al*., 1998). Table 2 reports some of the recent applications of nanomaterials in agrochemical and genetic material delivery to plants. Torney *et al*. (2007) used honeycomb mesoporous silica nanoparticle (MSN) system with 3nm pores to deliver DNA and chemicals into plant cells and intact leaves. They loaded the gene and its chemical inducer into the MSN system and capped the ends with gold nanoparticles and studied the release pattern of chemicals and induction of gene expression in the plants under controlled-release conditions. Their study showed an application of silica nanoparticles in target-specific delivery of proteins, nucleotides and chemicals in plant biotechnology.

Copper nanoparticle*:* Copper-based fungicides produce highly reactive hydroxyl radicals which can damage lipids, proteins, DNA, and other biomolecules. It plays an important role in disease prevention and treatment of large variety of plants (Borkow and Gabbay, 2005). Complexation of copper with chitosan nanogels was shown to have strong synergistic effect between chitosan and copper in inhibiting the growth of phytopathogenic fungus *Fusarium graminearum*. Because of its bio-compatibility, these nanohydrogels are included as a new generation of copper-based bio-pesticides and it could also be developed into an efficient delivery system for copper based fungicides for plant protection (Brunel *et al*., 2013). Low melting point soda-lime glass powder

containing copper nanoparticles showed efficient antimicrobial activity against gram-positive, gram-negative bacteria, yeast and fungi, key reason for the increased antimicrobial activity is because of inhibitory synergistic effect of the Ca^{2+} lixiviated from the glass (Esteban-Tejeda *et al*., 2009).

Zinc nanoparticle*:* Mechanism of action of zinc nitrate derived nano-ZnO on important fungal pathogen *Aspergillus fumigatus* showed hydroxyl and superoxide radicals mediated fungal cell wall deformity and death due to high energy transfer (Prasun Patra. and Goswami, 2012). Zinc oxide nanoparticles (ZnO NPs) could be used as an effective fungicide in agricultural and food safety applications. Recent study by He *et al*. (2011) showed significant inhibition of two postharvest pathogenic fungi *Botrytis cinerea* and *Penicillium expansum* with ZnO NPs with sizes of approximately 70 nm at less concentration, their mode of action was confirmed by SEM and Raman spectroscopy. ZnO nanoparticles cause deformation of fungal hyphae and prevent the conidiophores and conidial development which ultimately leads to the death of fungal hyphae.

Nano composites*:* Silver has been studied as an antibacterial agent and less as an antifungal agent. Pinto *et al*. (2013) described preparation and antifungal activity of composite films of pullulan and Ag nanoparticles (NP) against *Aspergillus niger* as a model system. They found that these composite films show strong inhibitory action on fungal sporulation, which was confirmed by disruption of the spores cells when observed under SEM. Silver in an ionic state exhibits high antimicrobial activity (Thomas and McCubbin, 2003). Park *et al*. (2006) developed a new nano-sized Silica-Silver composite for control of various plant diseases. Composite showed good antifungal activity where pathogens disappeared from the infected leaves within three days of spraying and the plants remained healthy thereafter. They also attempted to determine the effective concentration of composites and also used it effectively for suppression of growth of many pathogens. Nano composites showed 100% growth inhibition of *Pythium ultimum*, *Magnaporthe grisea*, *Colletotrichum gloeosporioides*, *Botrytis cinerea* and, *Rhyzoctonia solani* at 10 ppm concentration. Whereas, *Bacillus subtilis, Azotobacter chrococuum, Rhizobium tropici, Pseudomonas syringae* and *Xanthomonas compestris pv. vesicatoria* showed 100% growth inhibition at 100 ppm concentration.

In our recent study, we have used chitosan–silver nanoparticle (chitosan -Ag Np) composite (size distribution from 10 nm to 15 nm) for inhibition of conidial germination in *Colletotrichum gloeosporioides*. We observed complete inhibition of spore at 100µg/ml concentration of the composite, but chitosan alone did not show significant inhibition at the same concentration. We also found the shrinking of spores and other morphological changes in the spores treated with CS-Ag nanocomposite (Fig.1) (Chowdappa *et al*., unpublished data).

Nanotechnology in pest management

Globally insect pests cause a huge crop loss of 14% and plant pathogens cause an estimated loss up to 13% with a value of US $2,000 billion per year (Pimentel, 2009). Nano materials are used efficiently for safe administration of pesticides, herbicides, and fertilizers at lower doses (Kuzma and VerHage, 2006). Pesticides cause adverse effects on human health and on pollinating insects. So, nanomaterials play an important role in decreasing toxicity and in turn help in increasing the efficacy of pesticides (Mousavi and Rezaei, 2011). Nano pesticide formulations increase the solubility of poorly soluble active ingredient and helps in releasing the active ingredient slowly. The bioavailability of poorly water-soluble agrochemicals can be increased through the use

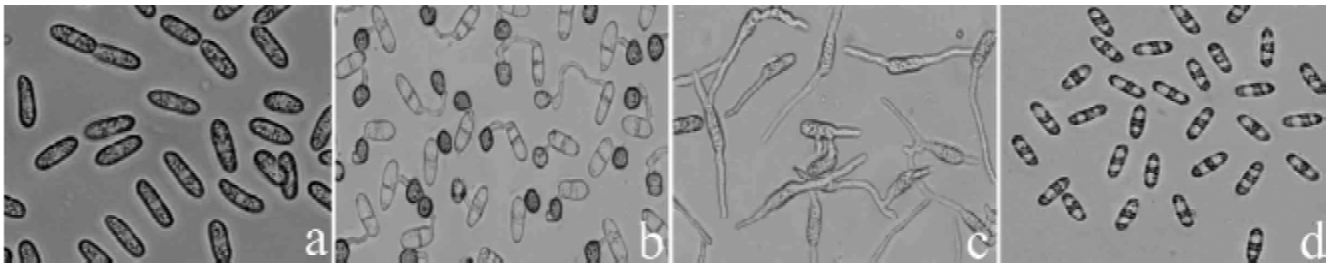

Fig 1. Effect of chitosan-AgNP composite on conidial germination of *Colletotrichum gloeosporioides*a) Normal conidia b) Water control with 0.1% (v/v) acetic acid (appressoria formation) c) Conidial germination in chitosan (100µg/ml) with 0.1% (v/v) acetic acid d) Complete inhibition of conidial germination by chitosan-AgNP composite at concentration of 100 µg/ ml.

of additives or by nanoparticulate formation of agrochemicals (Kah *et al.*, 2012). Nanoparticles are loaded with pesticides and released slowly based on environmental trigger (Lauterwasser, 2005). Because of their high reactivity at nanoscale compared to their bulk counterparts, lesser quantity of nanocides show enhanced effect in crop protection (Debnath *et al.*, 2011). Silva *et al*. (2011) synthesized chitosan alginate nanoparticles (635 nm) for delivering herbicide -paraquat. They show the association of paraquat with nanoparticles and also noted significant differences between the release profiles of free paraquat and the loaded alginate/chitosan nanoparticles. Loading of paraquat into nanoparticle has greater significance in reducing negative impact caused by paraquat. Insects synthesize some natural nano-structures which possess temperature dependent ferromagnetic resonance. In insects, magnetic material is present in the head, thorax and abdomen region. In social insects these magnetic nanoparticles act as geomagnetic sensors (Esquivel, 2007). Nanoencapsulation of chemical such as an insecticide and pesticide for slow and efficient release to a particular host plant for insect pest control allows the chemical to be properly adsorbed by plants (Scrinis and Lyons, 2007).

Rotenone, a water-insoluble botanical insecticide used to control aphids, trips, acari from decades, however its effective utilization has limited due to its poor water solubility, stability, degradation and isomerization when exposed to sunlight. Lao *et al*. (2010) successfully synthesized nanoparticles by embedding octadecanol-1-glycidyl ether to amino groups and sulphate to hydroxyl groups with novel amphiphilic chitosan derivatives N-(octadecanol-1-glycidyl ether)-O sulphate chitosan (NOSCS), with particle sizes of 167.7 to 214.0 nm and zeta potential of -45.0 to -51.9 mV. They found that loading of insecticide rotenone into a nanoparticles was increased up to 13000 times compared to free rotenone in water and solubility in NOSCS micelles aqueous solution was increased, their study showed a way to encapsulate and slow release of water-insoluble agrochemicals in pant protection. As a cheap and reliable alternative for control of insect pests, nanostructured alumina was successfully employed in controlling two major stored insect pests *Sitophilus oryzae L*. and *Rhyzopertha dominica (F.),* on wheat (Stadler *et al.*, 2010). Debnath *et al*. (2011) attempted to determine the efficacy of surface functionalized silica nanoparticle against rice weevil *Sitophilus oryzae*. They found that silica nanoparticle treated stored rice was not affected by pest even after 2 months of treatment, they also concurred that amorphous form of nanoparticles were found to be highly effective in controlling this insect pest. Additionally Kah *et al*. (2012) have reviewed application of various nanoformulations used in enhancing the effect of pesticides such as microemulsion, nanoemulsion, nanodispersion, polymer-based nanoparticle, solid lipid nanoparticle, clay, porous hollow silica nanoparticles, layered double hydroxides and metal based nanoparticles for plant protection. Encapsulation of biocides into liposomes may prolong the action of these agents on plants. Liposomes may prolong the action of active biocides in plants by reducing the fast washing from the plant surface and can be effectively used to deliver essential nutrients and biocides to plant (Lasic, 1993). Wang *et al*. (2007) demonstrated the potential use of water-insoluble pesticide, β-cypermethrin (β -CP), into the nano and microemulsion system to increase the stability of sprayed solution compare with commercial β–CP. This system is found to be very effective in reduction of active pesticidal ingredients in water-insoluble pesticide delivery. Barik *et al*. (2008) summarized the recent developments, application and mechanism of action of nanosilica as nanopesticide. Goswami *et al*. (2010) studied the effects of oxides nanoparticles like aluminium oxide, zinc oxide, titanium dioxide and silver nanoparticles against insect pests and pathogens. Nanosilica showed 100% mortality against insect pests whereas nanosulfur inhibited the sporulation and growth of fungi. For the first time Stadler *et al*. (2010), reported the insecticidal effect of nanostructured alumina on two globally important stored insect pests *Sitophilus oryzae* L. and *Rhyzopertha dominica* (F.). They showed significant mortality rate on treated wheat after 3 days of continuous exposure. They also suggested that inorganic nanostructured alumina can be used as a cheap and reliable alternative for pest management. Pesticides can be effectively loaded into nanoparticles and can be slowly released related to an environmental trigger, they have uniform and extremely small droplet sizes (Forgiarini *et al.*, 2001). Nanomaterials have low viscosity, high kinetic stability and optical transparency which make them smart and efficient delivery systems for many industrial applications (Lee and Tadros, 1982). The use of nanoparticles was found to be a viable alternative to conventional pesticides in combating pests which have developed pesticide resistance.

Nanoparticles in post-harvest disease management

Ever increase in human population, depleting natural resources and emergence of new resistant pathogens has made the supply of sufficient and healthy food a daunting

task. This problem might be magnified several folds in near future. Now, there is a need to increase production efficiency and decrease post-harvest wastage with application of emerging technologies like biotechnology and nanotechnology in post-harvest products. Nanotechnology has been effectively applied in agricultural and horticultural products by increasing shelf life, controlling growth of microorganisms by nanofilms and coatings, controlling influence of gases and the harmful rays (UV), using Nano biosensors for detection of quality and spoilage (Yadollahi *et al.*, 2009). Nanotechnology can be applied in postharvest operations such as drying, storage and preservation of agricultural products. Chitosan, a deacetylated derivative of chitin, is found to be very effective in reducing postharvest decay of fruit and vegetables (Liu *et al.*, 2007). Chitosan at a concentration of 1 g /L has found to be very effective in reducing the growth of several phytopathogenic fungi causing post-harvest spoilage of fruit and vegetables (Hirano, 1997). Yu *et al*. (2012) investigated the effect of 1% chitosan film with 0.04% nano-silicon dioxide on the qualitative properties of harvested jujube after 32 d of storage under ambient temperature, they studied the related defence enzymes in fruits and found that coated sample showed lower red indices, decay incidence, respiration rate, and weight loss. Shi *et al*. (2013) studied the novel chitosan/nanosilica hybrid film and its effect on preservation quality of longan fruits under ambient temperature. Coating extended shelf life, reduced browning index, retarded weight loss and inhibited the increase of malondialdehyde amount and polyphenoloxidase activity in fresh longan fruit.

Liu *et al*. (2009) investigated the effects of nano silver (with 2–5 nm diameters) on post-harvest shelf life of cut gerbera (*Gerbera jamesonii*) cv. Ruikou flowers. They observed that, pulsing for 24 h with 5 mg/L nano solution extended vase life and inhibited the bacteria growth in vase solution for initial 2 days when observed *in vitro* under microscope. A postharvest treatment of nano silver was extensively studied. Nano silver was found to be very significant in prolong the vase life by inhibiting the growth of bacteria on several cut flowers with different cultivar variety, including Rose, Gerbera and *Acacia holosericea*, (Lü *et al*., 2010; Li *et al*., 2012; Liu *et al*., 2012; Mohsen Kazemi., 2012; Nazemi Rafi and Ramezanian, 2013). In our recent study, we have evaluated the applicability of the CS-Ag Np composite as a fruit coating material to inhibit the growth of *C. gloeosporioides* associated with mango anthracnose. We found that nanocomposites showed a significant effect in reducing the percentage of rotting fruit tissue (71.28% at 1% concentration). The addition of 0.1% non-ionic surfactant tween 80 enhanced the wettability and adhesion property of coating solution and exhibited significant disease reduction compared to control (84.55% at 1% concentration). Thus, these nanocomposites can be utilized as coating material in preventing quiescent infections of *C. gloeosporioides* on mango to prevent post-harvest losses (Chowdappa *et al*., unpublished data). Nanomaterial has important implication in management of postharvest diseases. Research findings showed the better applicability and advantages of nano-packing materials over conventional normal packing material on physicochemical and physiological quality of stored fruits, vegetables and other horticultural crops.

Going green with nanomaterials

With the potential adverse effects of agro-chemicals on human health and ecosystem the use of green technology to prevent the environmental damage has become major concern by research community. Green synthesis reduces the use of hazardous substances during the synthesis and protects the environment (Table.3). Nanotechnology is an important contribution to green chemistry; it helps in development of microscopic and submicroscopic devices with less cost and provides huge savings in materials (Badami, 2008). A group of researchers have successfully used, photosynthesis protein units (PSI) isolated from leafy vegetables and plants to form a biohybrid photoelectrochemical cell that converts light energy into electrical energy, it was interesting to note that this biohybrid devices display remarkable stability at ambient conditions up to one year (Ciesielski *et al.*, 2010). Nanotechnology has a revolutionary impact on agriculture including pest management in future. Nanoencapsulation ensures promise in crop protection against insect pests and pathogens. nanotechnology plays an important role in green revolution (Bhattacharyya *et al*., 2010). With the effective application of emerging technologies like microemulsions, liposomes and nanoemulsions in agrochemicals formulations, use of petrochemical solvents can be reduced in effective delivery of herbicides, fungicides and pesticides (Castro *et al.*, 2013). Recently, Tokarek *et al*. (2013) reported one-step green synthesis of chitosan-stabilized copper nanoparticles.

Application of silver nanoparticles in agriculture has made researchers to focus on synthesis of silver nanoparticles by chemical, electrochemical,

Table 1. Nanomaterials for control of plant pathogens

Nanomaterial	Application	Functions	Reference
Nano silver	Antibacterial activity of nano-silver against *Xanthomonas campestris* pv.campestris towards the control of cabbage black rot in the pot.	Dose dependent study of nanosilver on the *X.campestris* pv.campestris showed significant reduction of cabbage black rot in the pot experiment.	Gan *et al.* (2010)
Validamycin loaded nano sized calcium carbonate	Controlled release of validamycin loaded nano sized calcium carbonate (50 to 200 nm) against *Rhizoctonia solani.*	The formulation showed better germicidal efficacy Tagainst *Rhizoctonia solani* compared to conventional technical validamycin when studied after7 days. The nanoformulation showed the release of validamycin upto 2 weeks.	Qian *et al.* (2011)
Thiamine di-lauryl sulfate (TDS) nanoparticles	Antifungal activity of TDS nanoparticles (258.6 nm) against *C. gloeosporioides* associated with pepper anthracnose.	Nanoparticles at 100 ppm concentration showed 80% growth inhibition of *C. gloeosporioides* compared to the control. TDS nanopaticles penetrated inside the hyphal cell membrane and destructed the cells.	Seo *et al.* (2011)
Chitosan nanoparticles (CS NPs)	Efficacy of CS NPs on fungal growth and chilli seed quality.	CS NPs at a concentration of 0.6% (w/v) significantly delayed mycelial growth of *Rhizopus* sp. *Colletotrichum capsici*, *C. gloeosporioides*, and *Aspergillus niger* when compared with control.	Chookhongkha *et al.* (2012)
Nanocopper	Antibacterial activity against *Xanthomonas axonopodis* pv. punicae, the causative of pomegranate bacterial blight	Nanocopper inhibited the growth of *Xanthomonas axonopodis* at 0.2 ppm, i.e., >10,000 times lower than that usually recommended for Cu-oxychloride.	Mondal and Mani (2012)
Light activated nanoscale formulations of TiO_2	Nanoscale formulations of TiO_2 with Ag and Zn on *Xanthomonas perforans* towards the control of bacterial spot of tomato	Compared to control, TiO_2/Ag and TiO_2/Zn had high photocatalytic activity against *X. perforans* and the combination significantly reduced bacterial spot severity without causing any adverse effects on tomato yield.	Paret *et al.* (2012)
Copper nanoparticles	Cu-based NPs (11–55 nm) were tested on tomato (*Lycopersicon esculentum*) against *Phytophthora infestans* for their antifungal activity.	The synthesized Cu-based NPs were more effective than the commercial agrochemicals at lower concentrations and drastically reduced the active ingredient rate. The particles were not showed phytotoxicity on treated plants.	Giannousi *et al.* (2013)

DNA directed silver nanoparticles on graphene oxide	Antibacterial activity of nanoparticles against *Xanthomonas perforans*	Nanoparticles at 100 ppm concentration severely reduced the bacterial spot disease compared to untreated tomato transplants in greenhouse condition without causing phytotoxicity.	Ocsoy *et al.* (2013)
Light-activated nanoparticle formulation of Titanium dioxide with zinc	Nanocomposite for management of bacterial leaf spot on Rosa 'Noare'	Field applications of TiO_2/Zn nanoformulation at H'500 to 800 ppm on Rosa 'Noare' significantly reduced bacterial spot severity compared with the untreated control and other commercial bactericides.	Paret *et al.* (2013)
Chitosan based nanoparticles (chitosan, chitosan-saponin and Cu-chitosan nanoparticles)	*In vitro* evaluation of Chitosan based nanoparticles against *Alternaria alternata*, *Macrophomina phaseolina* and *Rhizoctonia solani*.	Cu-chitosan nanoparticles were most effective at 0.1% concentration and showed 89.5, 63.0 and 60.1% growth inhibition of *A. alternata*, *M. phaseolina* and *R. solani*, respectively. Nanoparticles were very effective in inhibiting spore germination.	Saharan *et al.* (2013)

Table 2. Application of nanomaterials as delivery vehicles in plants

Nanomaterial	Application	Functions	Reference
Honeycomb Mesoporous silica nanoparticles (MSN)	Delivery of DNA and chemicals into plants	MSN system (3nm pores) loaded with gene and its chemical inducer can be successfully delivered to plant cells and triggered gene expression in the plants under controlled release conditions.	Torney *et al.* (2007)
Fluorescence starch-nanoparticle coated with poly-*L*-lysine	As plant transgenic vehicle	DNA-nanoparticle (50–100 nm) complexes bind and transport genes across the cell wall of plant cells by inducing instantaneous pore channels in cell wall, cell membrane and nuclear membrane with the help of ultrasound treatment.	Liu *et al.* (2008)
Single-walled carbon nanotubes (SWNT)	Molecular transporters for walled plant cells	SWNT penetrated the cell wall and cell membrane of intact plant cells and successfully delivered different cargoes into different cell organelles.	Liu *et al.* (2009)

Magnetic carbon-coated nanoparticles	Absorption and translocation of nanoparticles through the root of different crop plants	Nanoparticles were penetrated through the root of pea, sunflower, tomato and wheat. Particles reach the vascular cylinder, through transpiration and spread through the aerial part of the plants in less than 24 hours	Cifuentes *et al.* (2010)
Ultra small Anatase TiO_2 Alizarin Red S Nanoconjugates	Study of uptake and distribution of nanoconjugates in the plant model system- *Arabidopsis thaliana*	Nanoconjugates traversed cell walls, entered into plant cells, and accumulated in specific subcellular locations such as cell vacuoles and nuclei.	Kurepa *et al.* (2010)
ZnS nanoparticles modified with positively charged poly-L-lysine(PLL)	Delivering DNA into plant cell	Successfully delivered β-glucuronidase encoding plasmid DNA into tobacco cells by means of ultrasound assisted method.	FU *et al.* (2012)
Gold functionalized mesoporous silica nanoparticle (Au-MSN)	Protein and plasmid DNA codelivery to plant cells Via- biolistic method	Protein-loaded Au-MSN (10 nm pores) was coated with plasmid DNA and delivered into plant tissues through particle bombardment. The delivery and release of protein and plasmid DNA was detected in the same plant cells.	Martin Ortigosa *et al.* (2012)
Functionalized mesoporous silica nanoparticles	Gene delivery	Foreign DNA is delivered into intact *Arabidopsis thaliana* roots without applying any mechanical force. Gene expression was observed in the epidermal layer and in the endodermal root tissues, conformed by both fluorescence and antibody labelling.	Chang *et al.* (2013)
Mesoporous silica nanoparticles functionalised with amine cross-linked fluorescein isothiocyanate.	Delivery of biomolecule into plants	The uptake and distribution of nanoparticles (20 nm) were observed in wheat, lupin and *Arabidopsis* during seed germination, in roots of plants grown in a hydroponic system and in whole leaves of plants via vacuum infiltration.	Hussain *et al.* (2013)
Plant Bio-transformable HMG-CoA reductase gene loaded calcium phosphate nanoparticle (Cap)	Encapsulation of plasmid DNA into nanoparticle to enhance the stability and frequency of the genetic transformation in plants	DNA releasing in acidic media showed, initial slow release followed by fast release of Cap nanoparticles. It was stable at ambient temperature and humidity. It significantly enhanced the transformation efficiency and antioxidant activity of the biotransformed plants compared to normal plant	Sohadi *et al.* (2013)

Table 3. Green synthesis of metallic nanomaterials by microorganisms

Nanoparticle	Microrganism	Reference
Cadmium sulfide nanoparticles	*Schizosaccharomyces pombe*	Kowshik *et al*. (2002)
	E. coli	Sweeney *et al*. (2004)
	Rhodopseudomonas palustris	Bai *et al*. (2009)
Gold nanoparticles	*Verticillium*	Mukherjee *et al*. (2001)
	Fusarium oxysporum	Mukherjee *et al*. (2002)
	Thermomonospora	Ahmad *et al*. (2003)
	Trichothecium	Ahmad *et al*. (2005)
	Rhodopseudomonas capsulata	He *et al*. (2007)
	Pseudomonas aeruginosa	Husseiny *et al*. (2007)
	Bacillus licheniformis	Kalishwaralal *et al*. (2009)
	Penicillium	Du *et al*. (2011)
	Shewanella oneidensis	Suresh *et al*. (2011)
Gold and Silver Nanoparticles	*Brevibacterium casei*	Kalishwaralal *et al*. (2010)
	Bacillus Subtilis	Reddy *et al*. (2010)
	Chrysosporium tropicum	Soni and Prakash (2012)
Magnetite nanoparticles	*Actinobacter*	Bharde *et al*. (2005)
	Theroanaerobacter ethanolicus and *Shewanella*	Roh *et al*. (2006)
Selenium nanoparticles	*Klebsiella pneumoniae*	Fesharaki *et al*. (2010)
Silver	*Fusarium semitectum*	Basavaraja *et al*. (2008)
	Alternaria alternata	Gajbhiye *et al*. (2009)
	Staphylococcus aureus	Nanda and Saravanan (2009)
	Escherichia coli and Aspergillus niger	Kathiresan *et al*. (2010)
	Aspergillus clavatus	Verma *et al*. (2010)
	Phytophthora infestans	Thirumurugan *et al*. (2011)
	Penicillium	Nameirakpam *et al*. (2012)
	Trichoderma	Devi *et al*. (2013)
TiO_2 nanoparticles	*Lactobacillus* and *Sachharomycescerevisae*	Jha *et al*. (2009)
	Aspergillus flavus	Rajakumar *et al*. (2012)
Titanium nanoparticles	*Lactobacillus*	Prasad *et al*. (2007)
Zinc oxide nanoparticles	*Aspergillus aeneus*	Jain *et al*. (2013)

photochemical, and now via green synthesis route. Chemical methods of synthesis are simpler than physical methods, but they leave toxic residual agents in the final product and cause environmental toxicity. As a convenient and cleaner approach, green synthesis has a potential impact in safe delivery of agrochemicals for crop protection. Recently, Hettiarachchi and Wickramarachchi (2011) reported the synthesis of chitosan stabilized silver nanoparticles using gamma ray irradiation. Singh *et al*. (2010) demonstrated the cost effective and environment friendly technique for green synthesis of silver nano particles (30nm) by conversion of silver nitrate solution into silver nanoparticles with weed leaf extract of *Argemone maxicana* as reducing and capping agent. The antimicrobial activity of synthesized nanoparticles was commendable. They also found that, reduction of silver ions into silver nanoparticles was due to the participation of leaf proteins and metabolites. Stable silver nanoparticles can also be synthesized with the leaf extract of *Eucalyptus hybrid* and *Cycas*, (Dubey *et al*., 2009; Jha and Prasad, 2010). MubarakAli *et al*. (2011) reported the plant extract mediated synthesis of silver and gold nanoparticles. They also studied its antibacterial activity against clinically isolated pathogens *Staphylococcus aureus* and *Escherichia coli*. Apart from plant extracts, microorganisms can also be effectively used in synthesis of silver nanoparticles in a cost effective, eco-friendly, economic and efficient way. Bacteria and fungi can be effectively used to control the synthesis of metallic nanoparticles (Mandal *et al*., 2006). Pathogenic organisms such as *Cryphonectria* and *Phytophthora infestans* were exploited for the synthesis of silver nanoparticles (Thirumurugan *et al*., 2009; Dar *et al*., 2013). Sharma *et al*. (2009) reviewed the green synthesis of silver nanoparticles with different synthesis procedures such as mixed-valence polyoxometallates, polysaccharide, tollens, irradiation, and biological method. They also discussed the mechanism of silver nanoparticles bactericidal activity and their interaction with bacterial cell membranes. Table 3 shows some of the recent advancements in application of green synthesis methods to synthesize metallic nanomaterials by different microorganisms. Metal oxide semiconductor nanostructures help in degradation of organic pesticides and industrial pollutants into nontoxic and useful components through photo catalysis in a greener way. It offers great dividends in detection and removal of contaminants from water and soil sources (Baruah and Dutta, 2009).

The synthesis of stable metal based nanoparticles with environment friendly and convenient green route by using biopolymers, plant extracts and microorganisms has attracted the researchers to adopt green nanotechnology, because of its nontoxicity, cost effective eco-friendly synthesis at large-scale and can be used very effectively against phytopathogenic microorganisms. It acts as a viable alternative for traditional chemical synthesis procedures.

Limitations and issues

Nanotechnology has a significant role to play in agriculture, food processing, food packaging, food security and water purification. But it may pose negative effects on the environment, ecosystem, and humans. The potential risks associated with releasing nanomaterials into the environment (soil and water organisms) are still unclear by scientists. Recent findings showed the potential harmful effects of nanomaterials on the digestive systems of a beneficial soil organism -earthworm (Ruitenberg, 2013). Xu *et al*. (2010) summarized the increased safety concerns over application of nanomaterials in food and agriculture. They emphasized their study on main exposure routes and determinants of nano toxicities involving particle size, surface, structure, chemical composition, and dosage. Griffitt *et al*. (2008) reported the toxic effect of metallic nanomaterials on aquatic organisms. They have taken zebrafish, daphnids, and an algal species as a model organism and exposed them to silver, copper, aluminum, nickel, and cobalt in both soluble salts and nanoparticle form. They noted that, nanosilver and nanocopper cause toxicity in all tested organisms at median lethal concentrations. With the technological advancement, large amount of engineered nanomaterials are reaching environment through consumer and commercial products. Colman *et al*. (2013) studied the adverse impact of nano-silver on plants and microorganisms. They found that nanosilver treatment led to changes in microbial community composition, biomass, extracellular enzyme activity, and affected some ground plant species. All nanostructures will not cause the same toxicity risks. Biopolymers, liposomes and natural organic compounds based nanocapsules such as lipids and chitin are potentially less hazardous than heavy metals nanoparticles (Perez-de-Luque and Rubiales, 2009). Current important limitation in application of nanocarriers to agriculture is the production scale and cost. Large scale manufacturing of nanomaterials and its effective application to agriculture will bring down the cost to a drastic range.

There are many gaps and unresolved problems and new challenges concerning the biological effects of nanoparticles. Monica and Cremonini (2009) reported

both positive and negative effects of nanoparticles on higher plants. With the rapid growth in nanotechnology, there is an urgent need for right regulation over the indiscriminate use of nanomaterials and their effective disposal. With the increasing nanomaterial production and their subsequent release into the environment, a concern has been increased on their effect on ecosystem health. Many commercial nanomaterials pose greater toxicity risks than the same materials in their larger particle form, if a substance has been approved in bulkform; it remains legal to sell it in nano form. The potential application of nanomaterials in different agricultural applications needs further research investigation with respect to synthesis, toxicology and its effective application at field level. Nanotechnology has got applicability in various fields, but research and development is still at bench-top scale. Great efforts are required in commercialization of nanomaterials for agricultural applications, which requires proper protection needs, testing priorities, risk assessment and regulatory guidance at global level (Chen and Yada, 2011).

CONCLUSION

Nanotechnology holds the promise of controlled delivery of agrochemicals to improve disease resistance, plant growth enhancement and nutrient utilization. Nanoencapsulation shows the benefit of more efficient and targeted use of pesticides, herbicides and insecticides in environment friendly greener way. Research and development in post-harvest nanotechnology can help in preservation of the freshness and quality and prevent diseases in a relatively safer way. With the advancement of nanotechnology, application of green chemistry in synthesis of nanomaterials by using plant extracts and living cells has reduced the use of toxic solvents and guarantees ecoprotection. Nanotechnology in conjunction with biotechnology has significantly extended the applicability of nanomaterials in crop protection and production. Even though the toxicity of nanomaterials has not yet clearly understood, it plays a significant role in crop protection because of its unique physical and chemical properties. The application of nanomaterials is relatively new in the field of agriculture and it needs further research investigations. Barring the miniscule limitations, nanomaterials have a tremendous potential in making crop protection methodologies cost effective and environmental friendly.

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Evaluation of citrus germplasm for resistance to Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera:Psyllidae)

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ABSTRACT: Thirty two germplasm collections of *Citrus* spp. were screened for their relative resistance to Asian Citrus Psyllid (ACP), *Diaphorina citri* Kuwayama under field conditions at National Research Centre for Citrus, Nagpur during 2008-10. A significant variation was observed among the citrus germplasm in their response to infestation of ACP. Results revealed that Willits citrange, Trifolia, Rich 16-6, Flying dragon, Trifoliate Orange (Chethali, Gonicoppal), Carizzo Citrange (Chethali), Troyer Citrange (Gonicoppal) showed resistance to ACP. The identified resistance germplasm forms a basis for further studies on morphological and biochemical basis of resistance to understand the host plant resistance mechanism to the pest.

Keywords: Asian Citrus Psyllid, Citrus, *Diaphorina citri*, germplasm, host plant resistance

INTRODUCTION

Citrus is one of the important fruit crops in India with the production of 74.64 lakh tons from 8.46 lakh hectares with the productivity of 8.8 tons/ha at national level as compared to 25-30 tons/ha in advanced citrus producing countries (NHB, 2011). The productivity and quality of citrus is severely affected by several factors; insect pests being one of them. *Citrus* spp. is attacked by more than 823 and 250 insects in the world (Ebeling, 1959) and India (Nair, 1975, Srivastava and Butani, 1999), respectively right from nursery to harvesting stage. Among the insect pests attacking citrus, Asian Citrus Psyllid (ACP), *Diaphorina citri* Kuwayama, is one of the major insect pests of citrus cultivars attacking the new flush in all the three seasons *viz.* spring, summer and winter with its peak activity in spring season across India and is also known to transmit the Greening disease, which accelerates 'citrus decline' syndrome (Bove, 2006). Chemical control has been widely used to contain the pest, but the deleterious effects associated with its indiscriminate use results in insecticide resistance, pest resurgence. Among the safer and viable methods to combat insect pest attack, host plant resistance plays an important role in Integrated Pest Management. Evaluation of available indigenous/exotic citrus germplasm to ACP results in identification of resistant germplasm which further helps in the development of insect resistant rootstocks/scions which culminates in to development of rational pest management strategy for ACP. Keeping this in view, screening studies were carried out to know the relative resistance level among 32 germplasm collections of citrus against ACP.

MATERIALS AND METHODS

Field screening of citrus germplasm (32 Nos.) against ACP was conducted during 2008-2010 at Experimental Farm of National Research Centre for Citrus, Nagpur. The exotic gemplasm collections (21 Nos.) used for the study were Sunlix beneck S.C., Kusaic Rangpur, Shekwasha x Rough lemon, X-639, Benton citrange, Sun-chu-sha mandarin, Rangpur lime X Troyers, Willits citrange, Florida Rough lemon, Norneo Rangpur, Trifoliate hybrid, Trifolia, Rich 16-6, Flying Dragon, Schaub Rough lemon, Alemow, C-32 citrange, Smooth Flat Seuilli, (SFS), Dr. Knoor, Chase Rough lemon and Argentina Trifoliate orange. Indigenous gemplasm (11 Nos.) included Cleoptra mandarin (Tirupati), Cleopatra mandarin (Gonicoppal), Rough lemon (Tirupati), Rough lemon (Rahuri), Sour orange (Tirupati), Trifoliate orange (Chethali), Trifoliate orange (Gonicoppal), Troyer citrange (Gonicoppal), Troyer citrange (Chethali), Carizzo citrange (Chethali) and Rangpur lime (Gonicoppal). All the agronomic practices were carried out regularly and no insecticidal spray was carried out during the study period.

Observations on incidence of ACP (no. of nymphs/ 5cm twig) were taken during spring season. Four trees of each germplasm were selected for recording data. Nymphal count / 5cm twig covering four directions of each tree was taken. The data were transformed to square root values and subjected to ANOVA to test the significance of differences. Citrus germplasm were classified as resistant (< 5 nymphs / 5cm twig), moderately resistant (5.1 - 10 nymphs / 5cm twig), susceptible (10.1-25 nymphs / 5cm twig) and highly

Table 1. Infestation of Asian Citrus Psyllid, *Diaphorina citri* in different exotic and indigenous citrus germplasm during 2008, 2009 and 2010.

Citrus germplasm		Psylla population (nymphs/5 cm twig)			
		2008	2009	2010	Pooled Mean
Exotic					
1.	Sunlix beneck S.C.	7.4(2.72)^{def}	7.3 (2.70)^{ef}	8.6(2.93)^{cdefg}	7.7(2.78)
2.	Kusaic Rangpur	14.6(3.82)^{hi}	22.5 (4.74)^{mn}	21.7(4.66)^{j}	19.6(4.40)
3.	Shekwasha x Rough lemon	5.2(2.28)^{de}	6.8 (2.61)^{ef}	8.2(2.86)^{cdef}	6.7(2.58)
4.	X – 639	5.4(2.32)^{de}	7.0 (2.64)^{ef}	72(2.68)^{cd}	6.5(2.54)
5.	Benton Citrange	16.8(4.10)^{ij}	22.1 (4.70)^{lmn}	21.0(4.58)^{ij}	19.9(2.46)
6.	Sun-Chu-Sha Mandarin	5.4(2.32)^{de}	7.1 (2.66)^{ef}	7.6(2.76)^{cd}	6.7(2.58)
7.	Rangpur lime X Troyers	5.1(2.26)^{cd}	6.4 (2.53)^{def}	NR	5.7(1.59)
8.	Willits citrange	1.2(1.10)^{a}	0.2 (0.45)^{a}	NR	0.7(0.51)
9.	Florida Rough lemon	24.9(4.99)^{k}	25.8 (5.08)^{n}	12.6(3.55)^{defgh}	21.1(4.54)
10.	Norneo Rangpur	9.2(3.03)^{fg}	8.8 (2.97)^{fg}	7.5(2.74)^{cd}	8.5(2.91)
11.	Trifoliate Hybrid	8.8(2.97)^{fg}	9.4 (3.07)^{fgh}	7.9(2.81)^{cd}	8.7(2.95)
12.	Trifolia	2.4(1.55)^{ab}	2.2 (1.48)^{bc}	NR	2.3(1.01)
13.	Rich 16-6	2.5(1.58)^{ab}	0.8 (0.89)^{ab}	0.9(0.95)^{a}	1.4(1.15)
14.	Flying Dragon	2.4(1.55)^{ab}	1.5 (1.22)^{bc}	1.2(1.10)^{a}	1.7(1.29)
15.	Schaub Rough lemon	18.0(4.24)^{j}	20.4 (4.52)^{klmn}	3.9(3.73)^{fghi}	14.1(4.16)
16.	Alemow (*C. macrophylla*)	18.3(4.28)^{j}	24.3 (4.93)^{n}	13.7(3.70)^{efghi}	18.7(4.30)
17.	C – 32 Citrange	5.5(2.35)^{de}	6.5 (2.55)^{ef}	8.0(2.83)^{cde}	6.6(2.57)
18.	Smooth Flat Seuilli (SFS)*	13.7(3.65)^{hi}	16.4 (4.05)^{ijk}	16.3(4.04)^{hij}	15.5(3.91)
19.	Dr. Knoor*	13.3(3.65)^{hi}	16.8 (4.10)^{jkl}	18.9(4.35)^{hij}	16.3(4.03)
20.	Chase Rough lemon*	11.8(3.44)^{fgh}	13.1(3.62)^{hij}	14.4(3.79)^{ghij}	13.1(3.61)
21.	Argintina Trifoliate Orange*	5.4(2.32)^{de}	6.8 (2.61)^{ef}	5.7(2.39)^{bc}	6.0(2.42)
Indigenous					
22.	Cleoptra Mandarin (Tirupati)	5.1(2.26)^{cd}	6.9 (2.63)^{ef}	7.9(2.81)^{cd}	6.6(2.56)
23.	Cleoptra Mandarin (Gonicoppal)	5.0(2.24)^{cd}	7.2 (2.68)^{ef}	7.1(2.66)^{c}	6.4(2.52)
24.	Rough Lemon (Tirupati)	12.9(3.59)^{h}	17.0 (4.12)^{jklm}	15.8(3.97)^{hij}	15.2(3.89)
25.	Rough Lemon (Rahuri)	8.0(2.83)^{f}	12.3(3.51)^{ghi}	14.2(3.77)^{ghi}	11.5(3.37)
26.	Sour Orange (Tirupati)	2.0(1.41)^{a}	5.1 (2.26)^{de}	8.6(2.93)^{cdefg}	5.2(2.2)
27.	Trifoliate Orange (Chethali)	2.8(1.67)^{b}	2.0 (1.41)^{bc}	2.5(1.58)^{ab}	2.4(1.55)
28.	Trifoliate Orange (Gonicoppal)	3.2(1.79)^{bc}	2.5 (1.58)^{c}	2.6(1.61)^{ab}	2.8(1.66)
29.	Troyer Citrange (Gonicoppal)	2.4(1.55)^{ab}	3.0 (1.73)^{cd}	NR	2.7(1.09)
30.	Troyer Citrange (Chethali)	7.1(2.66)^{def}	7.9 (2.81)^{ef}	8.9(2.98)^{cdefg}	8.0(2.81)
31.	Carizzo Citrange (Chethali)	2.2(1.48)^{ab}	3.2 (1.79)^{cd}	2.2(1.48)^{a}	2.5(1.58)
32.	Rangpur lime (Gonicoppal)	7.6(2.76)^{ef}	13.0 3.61)^{hij}	NR	10.3(2.12)
	SED ±	0.234	0.310	0.432	—
	CD (5%)	0.48	0.63	0.88	—

Figures in parentheses are square root transformed values; NR = Not recorded
Values followed by same letter in a column are not significantly different (P=0.05).

Table 2. Response of different Citrus germplasm collections against Asian Citrus Psyllid during 2008, 2009 and 2010

Resistance category	Exotic	Indigenous
Resistant (< 5 nymph /5 cm twig)	Willitus Citrange, Trifoliate, Rich 16-6 Flying dragon	Trifoliate orange(Chethali) Trifoliate orange(Gonicoppal) Troyer citrange (Gonicoppal) Carizzo citrange (Chethali)
Moderately resistant (< 10 nymph/5 cm twig)	Sunlix beneck S.C. Shekwasha x Rough lemon X-639, Sun-cun-sha mandarin, Argentine Trifoliate, Rangpur lime x Troyers Norneo Rangpur Trifoliate Hybrid C-32 Citrange	Cleopatra mandarin (Tirupati) Cleopatra mandarin(Gonicoppal) Troyer Citrange (Chettalli) Sour Orange (Tirupati)
Susceptible (< 25 nymph/5 cm twig)	Kusaic Rangpur, Smooth Flat Seuilli (SFS) Benton citrange , Dr. Knoor Florida Rough lemon Alemow (*C. macrophylla*) Schaub Rough lemon, Chase Rough Lemon	Rough lemon (Tirupati) Rough lemon (Rahuri) Rangpur Lime (Gonicoppal)
Highly Susceptible (> 25 nymph/5 cm twig)	Nil	Nil

susceptible (>25 nymphs/ 5cm twig) based on the extent of damage was adopted.

RESULTS AND DISCUSSION

Among the 32 citrus germplasm collections, Willits Citrange (1.2 nymphs/ 5 cm twig) and Sour Orange (Tirupati) (2.0 nymphs/ 5 cm twig) recorded significantly low ACP incidence but were at par with Carrizo citrange (Chethali) (2.2 nymphs/ 5 cm twig), Trifeola (2.4 nymphs/ 5 cm twig), Flying dragon (2.4 nymphs/ 5 cm twig), Troyer citrange (Gonicoppal) (2.4 nymphs/ 5 cm twig) and Rich 16-6 (2.5 nymphs/ 5 cm twig) during 2008. Similarly in 2009, Willits citrange (0.2 nymphs/ 5 cm twig) recorded significantly low ACP incidence but was at par with Rich 16-6 (0.8 nymphs/ 5 cm twig). In 2010, Rich 16-6 (0.9 nymphs/ 5 cm twig), Flying dragon (1.2 nymphs/ 5 cm twig) and Carizzo Citrange (Chethali) (2.2 nymphs/ 5 cm twig) recorded significantly low ACP incidence but were at par with Trifoliate orange (Chethali) (2.5 nymphs/ 5 cm twig) and Trifoliate orange (Gonicoppal) (2.6 nymphs/ 5 cm twig) (Table 1).

According to germplasm classification based on three year mean data, Willits citrange, Trifolia, Rich 16-16, flying dragon in case of exotic germplasm and Trifoliate Orange (Chethali, Gonicopal), Carizzo Citrange (Chethali), Troyer Citrange (Gonicopal) in case indigenous germplasm were found resistant to ACP (Table 2).

The foregone results showed that a total of eight, thirteen and eleven germplasms were found resistance, moderately resistant and susceptible to ACP. Of the 32 citrus germplasm screened, none were found highly susceptible to ACP (Table 2). Bhagat and Nehru (2005) and Batra *et al.* (1970) reported the variation in different citrus cultivars/germplasm regarding their susceptibility to ACP.

The variation in resistance levels to ACP in different citrus germplasm may be due to time of availability of tender flushes and seasonal fluctuations in the populations of the ACP, ovipositional preferences and host suitability for growth and development of the ACP and presence of allelochemicals. Further, the presence of aromatic aminoacids in the flowers also plays a role in nutritional ecology (Broadbeck *et al.,* 2001). The identified germplasm resistance to ACP can be considered as potential resistance source in future citrus improvement programmes through breeding/ hybridization programmes to suit best in IPM strategies. The identified resistance germplasm may be further studied to know the mechanism of resistance for ACP.

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Models to predict infestation of *Bactrocera dorsalis* (Hendel) in guava (*Psidium guajava* L.)

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ABSTARCT: Guava, *Psidium guajava* L. is an important fruit crop of India. Being one of the preferred hosts of the fruit fly, *Bactrocera dorsalis* (Hendel), it is at a high risk of loss. Though the pest is serious, its attack varies with season and region. For need based interventions, models are required. Studies were conducted in 2009 and 2010 at Indian Institute of Horticultural Research, Bangalore. In both the years the infestation in second harvest could explain the variability in total infestation to the extent of 86% and 73% respectively. IPM strategies can be decided from the second harvest infestation, thus obviating loss by > 95% in the subsequent 3-12 harvests.

Keywords: *Bactrocera dorsalis*, IPM, prediction models, *Psidium guajava*

INTRODUCTION

Guava, *Psidium guajava* Linnaeus, is a native of tropical America but is now pan tropical in distribution. The fruit is very rich in vitamin C and A and also calcium as well. In India, total area under guava is 2, 05,000 hectares with annual production of 24, 62,000 metric tons. It occupies 3.2% of the total area under fruits contributing 3.3% of the total fruit production in the country and productivity is 12 metric tons / hectare (Indian Horticulture Database, 2011). About 80 species of insects have so far been recorded on guava (Butani, 1979; Tandon and Verghese, 1987; Singh *et al.*, 2003) affecting yield and quality of fruits. The Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) is the most important and destructive pest associated with mango and guava (Verghese and Sudhadevi, 1998 and Rajitha and Viraktamath, 2005) and the incidence ranges from 10-41 per cent in various varieties of guava (Reddy and Vasugi, 2002).

In India, total economic loss of 2,558 and 26,902 million rupees was estimated due to fruit flies with and without control measures respectively (Stonehouse, 2001). Fruit fly infestation being the major obstacle in the production and marketing of the guava, successful cultivation and export are highly dependent on sound control of fruit fly. Fly attacks have led to the virtual abandonment of guava cultivation in Sind (Singh, 1988). Clarke *et. al.*, (2001) found that *Terminalia catappa* along with *P. guajava* constituted the major hosts for *B. dorsalis* in a survey of Thailand and Malayasia. Quarantine regulations imposed by an importing country can either deny the fruits of producing country or force the producer to carry out expensive disinfestation treatment. This work is aimed at studying the initial yield loss of guava due to *B. dorsalis* so that appropriate intervention can be taken up at the right time to prevent loss due to infestation in subsequent harvest.

In guava, diseased and infested fruits prematurely drop and rot. Such fruits form the breeding source for subsequent infestation. Therefore, sanitation by collecting and destroying fallen, rotten and semi ripe fruits is recommended (Verghese and Jayanthi, 2001). Integrated pest management for orchard fruit fly was developed between 2002 and 2005 which consisted of male annihilation technique (MAT), bait application technique (BAT) and cultural controls (Stonehouse *et al.*, 2005). The MAT was subsequently modified (Verghese *et al.*, 2011) to facilitate commercialization. Guava is a table fruit and cover sprays with insecticides are fraught with residue-problems, hence this cannot be recommended and also they are not economical compared to IPM interventions. At the Indian Institute of Horticultural Research 10 traps/acre weekly bait application (10% jaggery with a toxicant) and weekly sanitation, for guava have been standardized (Anonymous, 2011). The fruit fly infestation varies with region and season, in winter the infestation is low (Verghese and Sudha Devi, 1998; Verghese *et al.*, 2006b). It has been found in field surveys that under low infestations even sanitation alone ensures fruit fly free orchards. If guava acreage is contiguous as is the sanitation, reinforced with MAT are sufficient even if serious (Anonymous, 2011). So there is a need to anticipate infestation with sound models for need-based IPM intervention. It was hypothesized that if early infestation had a bearing on total infestation, then a prediction of infestation could be possible. This study

was conducted to develop forecast models that would help predict expected infestation.

MATERIALS AND METHODS

A study was undertaken in a guava orchard (cv. Lucknow 49) at the Indian Institute of Horticultural Research, Bengaluru (12°.58 N, 77° 35 E), India during 2009-2010. The trees were maintained following regular agronomic practices but no insecticides were sprayed. Of hundred trees, twenty were selected at random for the study. Fruits were harvested once a week at colour-break from dark to light green. From the harvested fruits (n=25-50), 20% were brought to the laboratory, allowed to ripen and percentage infestation was calculated. Thus the extent of infestation for every week was obtained from September to December from which the total percent infestation was calculated.

The infestation of each week was correlated with the total infestation with coefficient 'r' (at p=0.05) as the test criterion. If a significant relationship was found at an early week's relationship with the total infestation of the season they were regressed to obtain a model using scatter plot of excel (MS Office 2003) with R^2 (coefficient of determination) as a test criterion (Little and Hills, 1979). Based on a high R^2 models were selected to calculate a predicted infestation then compared with observed infestation using't' test (P=0.05).

From every harvest the percent infestation calculated was subjected to regression analysis to arrive at appropriate conclusions. The percent infestation in harvested fruits at weekly interval is a good index of total loss as guava is harvested periodically through the season (September- December) which varies with region and variety.

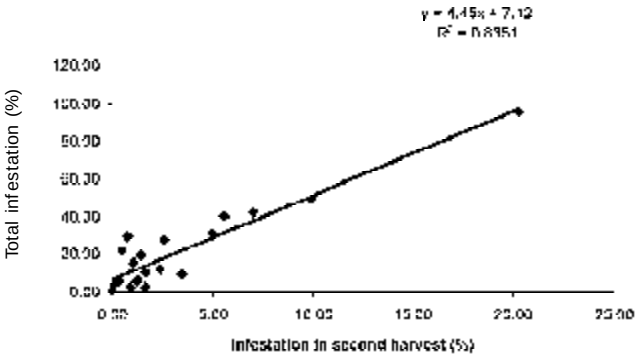

Fig 1. Relationship between infestation in second harvest and total infestation (2009)

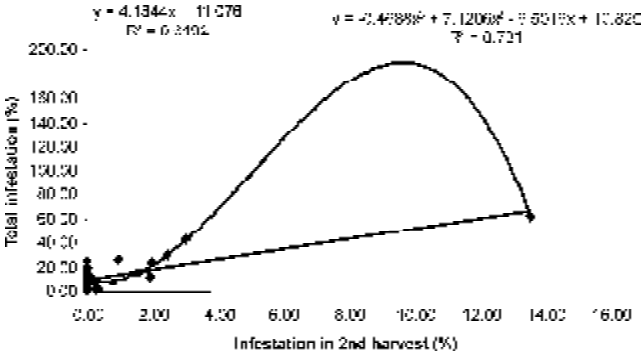

Fig 2. Relationship between infestation in second harvest and total infestation (2010)

RESULTS AND DISCUSSION

The correlation analysis revealed infestation level was positively significant between second harvest and total infestation in both the years *viz* 2009 (r=0.93), 2010 (r=0.79) respectively. When the data were subjected to regression analysis in the first year the infestation in second harvest could explain the variability in total infestation to the extent of 86% (R^2=0.86) (fig.1). The second year's (2010) model obtained explained the variability in total infestation to the extent of 73% (R^2=0.73) through polynomial order (3) and to the tune of 61% through linear equation (fig.2). The models which fitted the relationship between total yield (y) and each week's infestation (x), appropriated to second week's infestation to be the earliest harvest related to total infestation. Thus the second week's harvest (x) was a useful predictor of total infestation (y) to take appropriate early interventions (by second week) to curb or reduce further loss of yield. The models were found to be reliable as predicted and actual infestation was not very significant (NS; *P=0.5*). The mean infestation in the year 2009 was found to be almost same (22.16 and 22.15) for observed and predicted respectively with slight variation in 2010 as seen in table 1 i.e 14.69 and 12.18 observed and predicted respectively.

Table 1. Actual and predicted levels of fruit fly infestation

	Mean total infestation (%)	
	2009	2010
Actual	22.16	14.69
Predicted	22.15	12.18
't' test (p=0.5)	NS	NS

The IPM interventions (Stonehouse, 2005) should be practiced based on the second week's infestation in

harvested fruits to lower the yield loss. Many studies have indicated that an integrated management of fruit flies, in which more than one component combine to suppress population, is the most effective in farm situation and farmers (Singh, 1997; Verghese *et al*., 2004). Combined as an integrated package atleast two methods one- collection and destruction of fallen fruits, two- raking or ploughing of the soil to disrupt pupae, three- MAT, four- bait sprays and five- early harvest can definitely reduce the yield loss by 90% (Verghese, 2004). At the Indian Institute of Horticultural Research 10 traps / acre; weekly bait application (10% jaggery with a toxicant) and weekly sanitation, for guava have been standardized which has reduced infestation from 54 to 18 percent (Anon, 2011). As MAT, BAT and cultural methods uses meager amount of chemicals or insecticides for the control of fruit fly and away from the fruits, the quantity of chemicals the fruits are exposed to is nil when compared to cover sprays. Clean sanitation in orchard including removal of fallen fruits regularly and methyl eugenol traps can keep fruit flies under check (Verghese and Jayanthi, 2001).

The knowledge of yield-loss relationship between a crop and its associated pests is an important aspect to any IPM program also it is very important for determining IPM strategies in agriculture in order to reduce yield-loss (Pitan and Ejoka, 2011). The basis of good pest control programme is prevention than cure. Further the standardized IPM makes prevention an environment friendly, without cover sprays of insecticides. The prediction model developed here will help farmers to take decision on need-based IPM intervention. If infestation is low, high cost interventions (Male annihilation, pheromone trap and bait) can be avoided to save on input cost and more returns to farmers. The models help to decide the need for intervention as well as avoid delay in IPM intervention if needed to save the crop in subsequent harvests.

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Biology of sapota midrib folder, *Banisia myrsusalis elearalis* Walker (Thyrididae: Lepidoptera) infesting sapota under hill zone of Karnataka

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ABSTRACT: Biology and morphometrics of sapota midrib folder, *Banisia myrsusalis elearalis* Walker, which has been recorded for the first time under hill zone of Karnataka, were studied at College of Horticulture, Mudigere, under laboratory conditions (16.5 ^{0}C to 23.5 ^{0}C, 72.06 % to 77.2 % RH). The incubation period of egg was 5.23 ± 0.59 days. There were four distinct larval instars and duration of each instar was 6.75 ± 0.78, 4.10 ± 0.91, 3.70 ± 0.47 and 5.15 ± 0.81 days, respectively. The length and breadth of first instar larva was 0.62 ± 0.01 mm and 0.40 ± 0.02 mm and that of fourth instar larvae were 14.49 ± 1.44 mm and 2.48 ± 0.38 mm, respectively. The fecundity of adult female moth was 39.58 ± 10.75 eggs with an oviposition period of 2.75 ± 0.82 days. The adult male and female moths had longevity of 5.5 ± 0.62 and 6.85 ± 0.91 days, respectively. Total life cycle of male and female was completed in 40.8 ± 3.97 and 45.35 ± 4.08 days, respectively.

Key words: *Banisia myrsusalis elearalis*, biology, Sapota

INTRODUCTION

Sapota or Chicku tree is attacked by more than 25 insect pests which includes bud borer, chiku moth, midrib folder, leaf miner, fruit flies and sucking pests (Deshmukh, 2001 and Shukla, 2011). Among these sapota midrib folder, *Banisia myrsusalis elearalis* Walker relatively a new pest, is becoming serious under hill zone of Karnataka. The sapota midrib folder was recorded for the first time at ZHRS, Mudigere under hill zone of Karnataka. So far, the biology of *B. myrsusalis elearalis* was not studied in climatic conditions of Karnataka. Hence, we studied biology and morphometrics of *B. myrsusalis elearalis* under laboratory conditions which will be useful to evolve appropriate control measures under field conditions.

MATERIALS AND METHODS

The biology of midrib folder on sapota was studied in the laboratory conditions, Department of Entomology, Mudigere, UHS, Bagalkot during 2012-2013. Weather parameters *viz.*, temperature and relative humidity recorded during the study period (December-2012 to January-2013) were 16.5 ^{0}C to 23.5 ^{0}C and 72.06 % to 77.2 % RH respectively. Late instar larvae of midrib folder were collected from sapota orchard of ZHRS (Zonal Horticultural Research Station), Mudigere for rearing on sapota leaves in laboratory. One week after pupation, pupae were examined under microscope for identification as male and female. In male pupa, lesser distance between genital and anal slit than that of female pupa (Patel, 1990). The male and female pupae were kept in separate wooden cages (35 x 30 x 35 cm) having mesh on lateral sides and transparent glass on top. The newly emerged moths ten pairs (one pair in each cage) were released in an oviposition cage (35 x 30 x 35 cm). Before confinement of adults, an excised fresh sapota terminal shoot having tender leaves and buds was dipped in conical flask containing water and placed in the cage for oviposition. Every day, fresh shoot was replaced for egg laying. A cotton wad dipped in 10 per cent honey solution was placed on lid of a petriplate to provide food for moths. Every day eggs laid on shoot as well as on walls of the cage were collected by using camel hair brush and stored in petridish under laboratory condition. Observations were made on colour, shape and size of eggs. To determine the number and duration of different larval instars, newly emerged larvae were transferred to the petriplates (20 cm x 5 cm) individually and reared on the tender leaves. Everyday each individual (10 per replication) was observed for change in size, colour and moulting behaviour. The length and breadth of first and second instar larvae was measured under microscope on a graph sheet where as third and fourth instar larvae were measured by using graph sheet itself. The changes in colour, length and breadth, duration of pre-pupal, pupal stages and adult, longevity of male and female moths and fecundity of female were also recorded.

RESULTS AND DISCUSSION

The adult male possesses dark almond colour head, thorax and abdomen, while light colored head, thorax and abdomen in female. A white irregular spot in middle of

each forewing was visible in few adults but not in all. The tip of abdomen was blunt in female whereas pointed in male. The mean sex ratio of male: female in laboratory was 1: 1.38 ± 1: 0.35 during different period of observation. The pre-oviposition, oviposition and post-oviposition period were 2.58 ± 0.51, 2.75 ± 0.82 and 1.33± 0.84 days, respectively (Table 1). The female moth (*B. myrsusalis elearalis*) within four to five days of oviposition period laid eggs in three to four batches (7-14 eggs per batch) in a group. The eggs were firmly glued on lower surface of tender leaves as well as matured leaves near to the midrib. The freshly laid egg was oval with vertical ribs, pale yellow with orange-red circular band at middle as well as at operculum region (Plate 1). At the time of hatching the colour of the eggs were changed to grey. The length and breadth of eggs varied from of 0.58 ± 0.01 mm and 0.41± 0.02 mm respectively. The incubation period was 5.23 ± 0.59 days (Table 1).

The neonate larva moulted four times with five distinct larval instars, similar observation was reported by Patel *et al.* (1993). All the four instars possessed reddish brown colour head and brown colour prothoracic shield with three thoracic and ten abdominal segments. Three pairs of brown circular spots were observed on the dorsal side of each segment and also fine hairs on all over the body surface. Five pairs of slender pale white prolegs were observed from third to six abdominal segments. The neonate larva was dark yellow possessing reddish brown head and light brown prothoracic shield (Plate 1). The length and breadth of first instar larvae were 0.62 ± 0.01 mm and 0.40 ± 0.02 mm respectively. The width of head capsule was 0.32 ± 0.04 mm and the duration of first instar was 6.75 ± 0.78 days (Table 1).

The second instar larva was pale yellow with brown head and brown prothoracic shield (Plate 1). The length and breadth of second instar larvae were 4.32 ± 0.04 mm and 0.75 ± 0.05 mm respectively. The width of head capsule was 0.69 ± 0.07 mm and the duration of second instar was 4.10 ± 0.91 days (Table 1). The third instar larva was light yellowish green, possessed reddish brown head and light brown prothoracic shield (Plate 1). The length and breadth of third instar larvae were 9.23 ± 1.36 mm and 1.39 ± 0.15 mm respectively. The width of head capsule was 0.97 ± 0.09 mm and the duration of third instar was 3.7 ± 0.47 days (Table 1). The fourth instar larva was pale green with reddish brown head and light brown prothoracic shield (Plate 1). The length and

Table 1. Biology and morphometrics of sapota mid rib folder, *Banisia myrsusalis elearalis* Walker under laboratory conditions

	Stage	Duration (Days) (Mean ± S.D)	Length (mm) (Mean ± S.D)	Breadth (mm) (Mean ± S.D)	Head capsule width (mm)	Distance b/w GP & A.P
Egg		5.23 ± 0.59	0.58 ± 0.01	0.41 ± 0.02		
	First instar	6.75 ± 0.78	0.62 ± 0.01	0.40 ± 0.01	0.32 ± 0.04	
	Second instar	4.10 ± 0.91	4.32 ± 0.40	0.75 ± 0.05	0.69 ± 0.07	
Larva	Third instar	3.70 ± 0.47	9.23 ± 1.36	1.39 ± 0.15	0.97 ± 0.09	
	Fourth instar	5.15 ± 0.81	14.49 ± 1.44	2.48 ± 0.38	1.91 ± 0.03	
Total larval period		19.70 ± 2.20	-	-	-	
Pre-pupa		2.70 ± 0.73	13.18 ± 0.23	2.82 ± 0.50	-	
Pupa	Male	8.90 ± 0.91	11.10 ± 0.24	3.03 ± 0.13	-	0.14 ± 0.02
	Female	11.5 ± 1.0	11.47 ± 0.33	3.39 ± 0.25	-	0.25 ± 0.04
Adult	Male	5.5 ± 0.62	9.45 ± 0.35	25.37 ± 1.07**	-	
	Female	6.85 ± 0.91	11.53 ± 0.31	33.91 ± 0.94**	-	
Total developmental period	Male	40.8 ± 3.97 -	-	-		
	Female	45.35 ± 4.08	-		-	

n = 10 Breadth with wing expansion
G. P= Genital pore; A. P= Anal pore

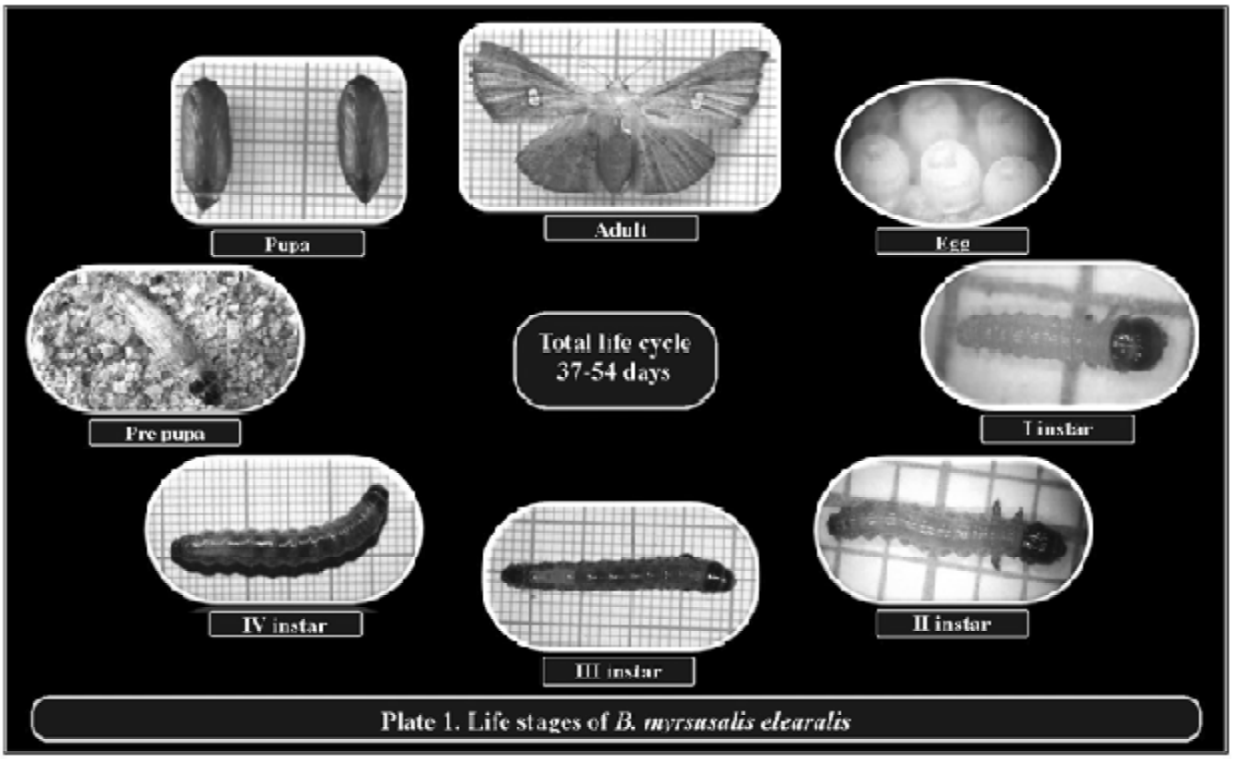

Plate 1. Life stages of *B. myrsusalis elearalis*

breadth of fourth instar larvae were 14.49 ± 1.44 mm and 2.48 ± 0.38 mm, respectively. The width of head capsule was 1.91 ± 0.03 mm and the duration of fourth instar was 5.15 ± 0.81 days (Table 1).

The total larval period varied from 19.70 ± 2.20 days. These observations are more or less similar to Silva *et al*. (2003). But according to Patel *et al*. (1993) the total larval period was less (13.52 days) this might be due to the variation in climatic condition in that area. The larvae of all stages were found feeding the tender leaves of sapota. Initially, the newly hatched larva scrapes the green matter of tender leaves. Later on the larva bound two edges of tender leaf making it like a green pea pod. It remains inside the folded leaf and feed on chlorophyll content. Later instars bind 3 or 4 leaves together and feed from inside the bounded leaves. From the study it was noticed that, in the field the infested leaves on terminal shoots dries up with an emergence hole which is clearly visible from a distance. These observations are similar with the findings of Patel (1990).

Final instar larva before pupation turned from pale green to creamy white, stopped feeding, sluggish and shrunken body (Plate 1). The larva pupates in soil by forming earthen cocoon. However, the larvae could also able to pupate between leaves or anywhere in the rearing boxes. The newly formed (obtect) pupa was reddish brown and turned to black before adult emergence. The pre-pupal period was 2.70 ± 0.75 days. The distance between anal and genital pore was more in female than that of male. This was taken to identify sex during pupal stage. Pupal period was with a mean of 11.50 ± 1.0 days in female and 8.9 ± 0.91days in case of male. The length and breadth of female pupa was with a mean of 11.47 ± 0.33 mm and 3.39 ± .025 mm, respectively while, male pupa was 11.10 ± 0.24 mm and 3.03 ± 0.13 mm, respectively (Table 1). The findings are comparable with those of Patel (1990) and Patel *et al*. (1993).

The adult male possessed dark almond colour head, thorax and abdomen, while light coloured head, thorax and abdomen in female (Plate 1). The length of female 11.53 ± 0.31 mm and the breadth with wing expansion was 33.91 ± 0.94 mm. Whereas, in case of male, the length was 9.45 ± 0.35 mm, while the breadth with wing expansion was 25.37 ± 1.07 mm. The mean longevity of male and female was 5.50 ± 0.62 and 6.85 ± 0.91 days respectively. The fecundity of each female individual was 39.58 ± 10.75 eggs. The total life cycle from eggs to the death of adult in male and female was 40.8 ± 3.97 and 45.35 ± 4.08 days, respectively (Table 1). These findings are in close agreement with those of Patel (1990)

and Patel *et al*. (1993). Since midrib folder is an emerging pest, our studies established the biology which would form basis to develop management strategies.

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Varietal evaluation of sapota for resistance to Oriental fruit fly, *Bactrocera dorsalis* (Hendel)

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ABSTRACT : Field and laboratory experiments were conducted during 2010-2011 at Fruit Research Station, Navsari Agricultural University, Gandevi, Gujarat, India to investigate the reaction of sapota varieties for their resistance/susceptibility to fruit fly, *Bactrocera dorsalis*. The genotypes *viz.*, PKM -1, PKM-2, DHS-1, DHS-2, Bhuripatti, Pilipatti and Singapore were categorized as least susceptible to *B. dorsalis*. However, Zumakhiya, CO-2 and Kirthibarthi were found to be moderately susceptible. Likewise Kalipatti, Cricket Ball, Paria Collection, Murabba and Mohangoote were recorded as highly susceptible to *B. dorsalis*. Studies on effect of chemical constituents of all 15 sapota germplasm collections against fruit fly infestation revealed that the fruit fly infestation had significant positive correlation with total soluble solids (TSS) and total sugars whereas, it had negative correlation with acidity. Physical characters *viz.*, fruit shape and skin pulp ratio had no significant impact on fruit fly incidence. Sapota germplasms with thin fruit skin were susceptible to fruit fly damage as compared to those with thick fruit skin.

Keywords : *Bactrocera dorsalis*, fruit fly, germplasm, sapota

INTRODUCTION

Sapota or chiku or sapodilla [*Manilkara achras* (Mill) Fosberg] synonym (*Achras sapota* Linn.) is an evergreen fruit tree, native of tropical America especially Southern Mexico in Central America and spread to other countries like Philippines, Malaysia, USA, Sri Lanka and India, where it adopted well (Chadha, 1992). India is considered to be the largest producer of sapota in the world. The major sapota producing states in India are Karnataka, Maharashtra, Gujarat, Andhra Pradesh and Tamil Nadu. Sapota is a hardy fruit crop but various factors affect the yield and economic value of fruit. Among them, damage by various insect pests and mites is a major constrain. As many as 25 insect pests have been reported attacking sapota trees in India (Butani, 1979) whereas, in Gujarat 16 insect pests and mites were found damaging sapota (Patel, 2001). In Gujarat, insect pests were reported to damage sapota throughout the year and the fruit fly, *Bactrocera dorsalis* (Hendelis) is one among them (Patel, 1981). Fruit flies deposit their eggs on the host fruit, when they are physiologically ripe. After incubation period, maggots feed on fruit pulp which results in softened and discoloration of fruit tissue. The affected fruit drops prematurely and get distorted and loose market value. A total economic loss of 29,460 million rupees per annum in mango, guava, sapota and citrus fruit crop is recorded in India (Mumford, 2001); whereas, in south Gujarat its damage has been reported as 4 to 52 per cent in sapota. Though there are various management strategies, none is fool proof and hence exploring host plant resistance deserves attention. The present study was carried out to evaluate 15 varieties/ germplasm collections for their reaction to fruit fly infestation in relation to morphological and biochemical characteristics of fruits.

MATERIALS AND METHODS

Field and laboratory experiments were carried out at Fruit Research Station, Navsari Agricultural University, Gandevi, Gujarat, India during 2010-2011 to evaluate the response of sapota germplasm against fruit fly, *B. dorsalis*. Fifteen accessions used for evaluation were Kalipatti, Cricket Ball, Zumakhiya, PKM-1, PKM-2, Kirthibarthi, Murabba, CO-2, Bhuripatti, Mohangootee, DHS-1, DHS-2, Paria collection, Singapore and Pilipatti. The response was evaluated on the basis of per cent fruit infestation as well as number of maggots in the infested fruit. Thirty vulnerable fruits of each germplasms were collected three times at four days interval from sapota germplasms of varying maturity, to undertaken the present investigation. Collected fruits were dissected to confirm the fruit fly damage and record the per cent infestation in each variety. Ten vulnerable fruits from each germplasms were selected, cut and record the numbers

*Part of M.Sc. (Ag) Thesis

of maggots per fruits. Thus, obtained data was analyzed using completely randomized design. The following rating system for fruit fly infestation has been utilized to estimate relative preference (Anonymous, 2003) *i.e.* No damage (pest free), Less than 10 per cent(Least susceptible), between 11 per cent to 20 per cent (moderate susceptible) and more than 20 per cent (highly susceptible).

Effect of physiochemical parameters of sapota on fruit fly infestation

To study the effect of biochemical constituents, 100 physiologically matured fruits of each genotype were collected to record the level of infestation. Three fruits were used for analysis. The data obtained on level of different chemical constituents for each germplasms were correlated with respective level of infestation. The observation on some endogenous metabolites *viz.*, total soluble solids (TSS), total sugar (%) and acidity (%) were recorded by using standard methods as suggested by Ranganna (1979).

Effect of morphological characters on fruit fly infestation: The following morphological characters were recorded.

Shape and surface of fruits: Fruit were categorised as round, oval, ablong and egg shape by visual method as well as having thick or thin surface by palpation method.

Skin pulpratio: For calculating skin pulp ratio, five fruits of marketable size of each germplasms were randomly taken and dissected separately. The pulp of fruit was separated by removing the fruit skin. The seeds were also separated. The weight of fresh pulp and skin was recorded separately. The skin to pulp ratio was calculated by simple arithmetical calculation.

RESULTS AND DISCUSSION

Reaction of sapota accessions to fruit fly, *B. dorsalis*

Table 1. Varietal reaction of sapota against fruit fly, *B. dorsalis*

Variety/Germplasm	Per cent fruit infestation	No. of maggots/fruit
Bhuripatti	14.62 (6.66)	2.63 (7.24)
Co- 2	25.72 (18.89)	4.21 (19.17)
Cricket Ball	35.82 (34.44)	5.57 (31.57)
DHS- 2	16.78 (8.89)	2.97 (9.27)
DHS- 1	18.26 (10.00)	2.88 (8.75)
Kalipatti	33.67 (31.11)*	3.26 (29.07)**
Kirthibarthi	21.30 (13.33)	3.59 (14.08)
Mohangootee	28.23 (23.33)	4.65 (24.02)
Murabba	29.45 (24.44)	5.15 (27.29)
Paria Collection	27.23 (21.11)	4.60 (23.92)
Pilipatti	1 8.25 (9.99)	3.33 (11.15)
PKM - 2	15.62.00 (7.77)	2.71 (8.58)
PKM -1	13.14.00 (5.55)	2.37 (6.01)
Singapore	16.51 (8.88)	2.82 (8.52)
Zumakhiya	24.90 (17.77)	3.75 (15.96)
S.Em ±	0.713	2.88
CD at 5 %	08.32	2.05
CV at 5 %	22.07	32.75

*Figures in parenthesis are original values and those outside are arc sign “percentage transformed values
** Figures in parenthesis are original value and those outside are “X + 1 transformed values

Per cent Fruit infestation: The results revealed that the cultivar Cricket Ball showed significantly higher per cent fruit infestation (34.44%), which was followed by Kalipatti (31.11%), Murabba (24.44%), Mohangootee (23.33%) and Paria Collection (21.11%). Looking to the level of infestation, significantly moderate per cent infestation were observed in germplasms *viz.*, CO-2 (18.89 %), Zumakhiya (17.77%) and Kirtibarthi (13.33%). Furthermore, it was observed that the accessions *viz.*, PKM -1 (5.55%), Bhuripatti (6.66%), PKM-2 (7.77%), Singapore (8.88 %), DHS-2 (8.89 %), Pilipatti (9.99 %) and DHS-1 (10.00%) established its superiority by imparting lower per cent fruit infestation (Table-1). The present findings corroborate with the work of Bansode (2009) in mango.

Number of maggots per fruit : The accessions were evaluated for their resistance on the basis of numbers of maggots per fruit. A significant difference was observed in number of maggots per fruit among the various germplasm collections. Significantly higher number of maggots were observed in highly susceptible germplasms *viz.*, Cricket Ball (31.57/fruit), Kalipatti (29.92),Murabba (27.29), Mohangootee (24.02), Paria collection (23.92).It is revealed from the studies that germplasms *viz.*, CO-2, Zumakhiya, Kirthibarthi showed moderate susceptibility with 19.17, 15.96 and 14.08 mean number of maggots per fruit, respectively. The descending order of number of maggots per fruit in different collections of sapota was PKM–1 (6.01), Bhuripatti (7.24), PKM – 2 (8.58), Singapore (8.52), DHS -2 (8.58), DHS–1(8.75), Pilipatti (11.15). These germplasms were recorded as less susceptible to fruit fly damage (Table 1). Earlier, Bansode (2009) reported that, in the resistance varieties of mango lower number of maggots per fruit was found. Verghese *et al.,* (2002) revealed that the screening of mango varieties by counting mean number of maggots per hundred fruits. In most susceptible varieties, Banganpalli and Totapuri recorded higher number of maggots as compared to tolerant cultivars *viz;* Alphonso, Suvarnarekha and Bombay Green.

Effect of biochemical constituent of sapota on fruit fly infestation:

Total soluble solids: The per cent fruit infestation exhibited significantly positive correlation with the total soluble solids (TSS). The data presented in Table-2 clearly indicated that the collections PKM 1 (17.4 %), PKM 2 (20.8%), Bhuripatti (20.00 %), Singapore (18.2%), DHS-1 (17.6%) and DHS-2 (20.1%) had low total soluble solids with less fruit infestation (6 to 11 %). Significantly, higher TSS contents exhibited in the germplasms *viz.*, Cricket ball, Kalipatti, Paria Collection, Mohangootee, and Murabba had TSS levels of 24.8, 23.26, 24.09, 23.52 and 23.01 per cent, respectively suffers more to fruit fly infestation ranged from 23 to 30 per cent. Moreover, CO-2 (22.26 %), Zumakhiya (21.25%) and Kirthibhrati (21.40%) had significantly moderate amount of TSS exhibited moderate fruit damage (14 to 16 %).

Total Sugars: During the present investigation the total sugar content in sapota fruits had significantly positive effect on the fruit infestation (Table 2). The results revealed that, PKM- 1 (11.70%), PKM 2 (9.40%), Bhuripatti (11.00 %), Singapore (10.50%), Pilipatti (10.30%), DHS-1 (11.90%) and DHS-2 (12.20 %) had lowest fruit fly infestation (6 to 11%) contained lower total sugars as compared to others whereas Cricket Ball, Kalipatti, Paria Collection, Mohangootee and Murabba had significantly higher amount of total sugars *viz.*, 15.05, 13.50, 14.50, 15.20, 13.60 per cent, respectively exhibited significantly more fruit damage. While, germplasms, CO-2, Zumakhiya and Kirthibarthi were categorized as moderate susceptible contained moderate per cent of total sugars (11.00 to 12.60%).

Acidity: There was non significant negative correlation between fruit fly infestation and per cent acidity of the fruits. There were no apparent differences in acidity (0.050 to 0.088%) in all 15 collections of sapota during the present investigation.

The data presented in Table-2 revealed that, in all the germplasms of sapota, the fruit fly infestation showed significantly positive correlation with total soluble solid and total sugar, this implied that the fruit fly infestation increased with increasing the above said biochemicals in sapota. Correlation between fruit fly infestation and per cent acidity was non significant, indicating that these chemical constituents do not influence the fruit fly infestation. The present results are in agreement with that of Kumar *et al.* (1994) who reported positive correlation of fruit fly infestation with total sugar in mango. Similarly, high total soluble solids and total sugar content in guava showed susceptibility to fruit fly (*B. dorsalis*) as reported by Arora *et al.*(2000). Bansode (2009) also found that, total soluble solids and total sugars had significantly positive correlation between per cent fruit infestation of *B. dorsalis* in mango fruits and per cent acidity have not any impact on incidence of fruit fly.

Evaluation of physical characters of sapota fruits for resistance

Fruit shape: The shape of fruit was round in Cricket ball, Zumakhiya, DHS – 1, CO – 2, whereas, PKM – 1, Bhuripatti, Pilipatti and Paria collection having oval shape. Moreover, Kalipatti, DHS – 2, Murabba and Singapore having ablong shape and germplasm PKM-2 having ablong to oval shape. Egg shaped fruit was observed in germplasms, Mohangootee and Kirthibarthi. The shape of sapota fruits had no clear cut impact on preference of fruit fly (Table-2).

Fruit surface: The surface of sapota fruit categorized thin and thick in different screened germplasms. Germplasms *viz.*, Kalipatti, Cricket ball, Zumakhiya, CO -2, Murabba, Kirthibarthi, Paria collection and Mohangootee were found in thin fruit surface group and showed high degree (14 to 30 %) of fruit infestation. Whereas, germplasms PKM -1, PKM -2, DHS -1, DHS – 2, Bhuripatti, Pilipatti and Singapore having thick surface suffered lower fruit infestation which ranged from 6 to 11 per cent (Table-2).

Skin pulp ratio: The data presented in Table-3 indicated that skin pulp ratio had not any significant impact to resist the fruit fly infestation. The observed data ranged between 1:9 to 1:11.1. Present findings partially corroborated with that of Reddy and Vasugi (2002) who reported that, the shape of fruits in guava varieties had no clear cut impact on preference of fruit damage, however, fruits with rough skin had less damage as compared to those with smooth skin fruits. Smooth skin fruits of guava were severely attacked by fruit fly than those fruits having rough and wrinkled skin as reported by Sandu *et al.* (1979) supports the present findings.

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Table 2. Infestation of *B. dorsalis* in relation to certain morphological and biochemical characters of different germplasm collections of sapota

Variety/Germplasm	Fruit Shape	Fruit Surface	Pulp/Skin ratio	Total Soluble Solid (%)	Total Sugar (%)	Acidity (%)
Bhuripatti	Oval	Thick	9.0	20.0	11.00	0.078
Co -2	Round	Thin	11.1	22.26	12.60	0.065
Cricket ball	Round	Thin	10.2	24.80	15.05	0.050
DHS -2	Oblong	Thick	9.3	20.1	12.20	0.058
DHS - 1	Round	Thick	9.6	17.6	11.90	0.066
Kalipatti	Oblong	Thin	11.0	23.26	13.50	0.075
Kirthibarthi	Egg Shape	Thin	10.3	21.4	11.00	0.085
Mohangootee	Egg Shape	Thin	9.5	23.52	15.20	0.055
Murabba	Oblong	Thin	10.6	23.1	13.60	0.088
Paria collection	Round	Thin	10.1	24.9	14.50	0.060
Pilipatti	Oval	Thick	10.3	19.5	10.30	0.083
PKM -2	Oblong to oval	Thick	9.4	17.4	11.70	0.062
PKM -1	Oval	Thick	10.9	20.8	9.40	0.088
Singapore	Oblong	Thick	9.4	18.2	10.50	0.080
Zumakhiya	Round	Thin	9.8	21.25	12.40	0.067
Correlation coefficient between character and infestation			0.890 *	0.875 *	- 0.295	

*(r valuessignificant at p = 0.05)

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Effect of natural hosts and alternate food sources on the biological parameters of acarophagous predator, *Stethorus rani* Kapur

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ABSTRACT: Effect of different food sources *viz.*, red spider mites, aphids, pollen, honey and extra floral nectary on the biological parameters of acarophagous predator, *Stethorus rani* (Coccinellidae : Coleoptera) was studied. Adult coccinellids fed upon red spider mites, aphids and pollen recorded highest longevity, mean fecundity, egg hatchability and adult emergence rate. The survival rate of larvae and pupae on the natural hosts and honey was statistically superior from the diet consisting of extra floral nectaries and pollen. The study concluded that alternate food containing honey (20 ml in 100 ml distilled water) and pollen (5g) can be used to maintain immature and adult stages of *S. rani* under *in vitro* conditions under sub optimal or lesser availability of natural hosts.

Keywords: Food source, *Stethorus rani*, predator

INTRODUCTION

Red Spider mite, *Tetranychus urticae* Koch, a polyphagous pest, and is a major sucking pest of grapes causing serious threat to grapevine cultivation in India. The nymphs and adults of the mites suck the sap from the lower surface of the leaves resulting in the development of pale chlorotic spots. The feeding activity of mites causes severe discoloration, removal of chlorophyll thereby affecting the photosynthetic ability of the leaves. Infested leaves presents a burnt up appearance finally withers and dry off. Severe infestation of mites cause delay in the ripening of the berries and reduce the sugar content of the berries (Kulkarni *et al.*, 2008). Several coccinellid species belonging to the genus *Stethorus* have been documented as the natural enemies of red spider mites (James *et al.*, 2001; Ragkou *et al.*, 2004; Roy *et al.*, 2005; Latifian, 2012). Among them *S. rani* Kapur is an active native predator of the red spider mites infesting grapes in Maharashtra.

Maintenance of laboratory stock culture of predatory insects is a pre-requisite to study their predatory potential against the target mite hosts and to conduct compatability studies with recommended chemicals. The survival and fecundity of acarophagous predator are the important factors to be considered while rearing to assure the continuous supply of the bio-agents for the *in-vitro* studies. Coccinellid predators feed on a wide variety of non-prey foods like fruits, fungi, pollen, seeds, honeydew (Lundgren, 2009). Adriaens *et al.* (2008) reported that coccinnellid predator, *Harmonia axyridis* survived in a wide range of habitats and variety of non-prey foods. In this study, an attempt was made to explore the suitability of different natural hosts and alternate foods to rear *S. rani* and their impact of predator biology so as to standardize the dietary inputs for the *in vitro* rearing of *S. rani* in the absence of appropriate mite hosts.

MATERIALS AND METHODS

The present study was undertaken in the Entomology Laboratory of National Research Centre for Grapes, Pune, Maharashtra. The initial stock culture of the *S. rani* was established by collecting the beetles foraging on the mite colonies in the grape field. The beetles were brought to the laboratory (27-28 °C and 65-70% RH) and maintained in plastic boxes (5x17cm) covered with fine mesh to provide sufficient ventilation to the insects. The experiment was conducted in a Completely Randomized Design (CRD) with five replication of each diet. The treatments included two natural insect hosts *viz.*, red spider mites, *Tetranychus urticae* Koch, and aphids, *Aphis craccivora* Fab. and three alternate food sources *viz.*, pollen (5g), honey (20ml in 100 ml distilled water), and extra floral nectaries of bottle gourd (*Lagenaria siceraria*). Both food sources and water were replaced every day regularly. To obtain eggs, one day old adult beetles (10 female: 10 male) were collected from the stock culture and allowed to mate in a small glass petri dish (9 cm diameter) for 24h provided with the selected five different diets. After 24h, female beetles were separated and kept for oviposition. The eggs were collected daily and kept over sterilized slightly moist filter paper maintained in glass petri dish so as to prevent dehydration of eggs.

Biological parameters of *S. rani*

The number of eggs per replicate was counted for a period of 20 days. The hatching percentage of the eggs was worked out by taking the ratio of the number of hatched eggs by the total number of eggs laid by the adults. The grubs hatching out from the eggs were collected instantaneously using fine brush taken in petri dishes twice a day to avoid cannibalism. Five replicates of predatory larvae (20 larvae per replicate) were taken in petri dishes provided with five different diets. The predatory larvae were observed regularly for the change in instars and formation of pupae. The behavior of pupation, number of survived larvae and pupae were recorded. The survival rate of larvae, pupae and adult emergence rate was worked out. The predator was reared for two generations using the natural and alternate foods.

Statistical Analysis

General Linear Model (GLM) procedures were used to perform the analysis of variance (ANOVA) and the treatments means were compared using Tukey's Honest Significance Difference (HSD) at 5% level of significance.

RESULTS AND DISCUSSION

The effect of different food sources over the survival and development of immature stages of *S. rani* was presented in Table 1. The mean fecundity of the adults supplied with mites (91.20) was statistically on par with the pollen ($F_{(4, 16)} = 76.22$; $P<0.0001$). The number of eggs laid by the adults supplied with aphids was found higher (69.60). Beetles fed with extra floral nectaries recorded lowest mean fecundity of the beetles which was statistically on par with that of honey. The hatching percentage of eggs was found highest in the adults supplied with mites (81.81 per cent) which was on par with the aphids (66.90 per cent) and pollen (65.59 per cent). Beetles supplied with extra floral nectaries recorded higher hatching percentage of 56.92 which differed significantly different from that of honey ($F_{(4,16)} = 24.04$; $P<0.0001$).

Survival rate of larvae and pupae of *S. rani* was found highest in the natural hosts which was on par with that of honey. Grubs supplied with extra floral nectaries and pollen recorded comparatively lower survival rate of the immature stages of the predatory beetle ($F_{(4,16)} = 50.48$; $P<0.0001$). There was significant difference in the longevity of adult beetles supplied with different foods ($F_{(4,16)} = 44.18$; $P<0.0001$) (Table 2). The longevity of the adults was found highest in the insects supplied with mites followed by pollen and aphids and they were statistically on par with each other. The adult beetles supplied with the extra floral nectaries survived 15.60 days followed by the honey (7.20 days). The adult emergence per cent was found maximum in the beetles supplied with natural hosts *viz.*, mites followed by aphids, honey and these three treatments were statistically on par with each other. Extra floral nectaries recorded lower adult emergence rate of 16.67 per cent.

Natural hosts like mites, aphids and artificial food consisting of pollen recorded positive influence over the growth, development and reproductive parameters of the adults. The results are in conformity with the observations made by Sarwar and Saqib (2010) who reported that the predatory coccinellid, *Coccinella septempunctata* L. reared on aphids provided consistent results during the rearing period. The pollen substitute was claimed to be a well-defined nutritive resource rich in protein and amino acids for development and

Table 1. Effect of dietary constituents over the immature stages of *S. rani*

Food Source	Mean Fecundity (days)	Hatching Percentage of Eggs	Larval survival Percentage	Percent Pupation
Red Spider Mites	91.20±8.95^{a}	81.81±4.10^{a}	83.74±5.70^{a}	76.99±6.81^{a}
Aphids	69.60±9.24^{b}	66.90±14.46^{ab}	61.00±11.94^{b}	64.48±13.16^{a}
Pollen	79.40±9.26^{ab}	65.59±15.32^{ab}	10.00±7.91^{d}	0.00±0.00^{c}
Honey	14.00±4.64^{c}	16.45±7.11^{c}	75.00±7.91^{ab}	62.30±7.51^{a}
Extra Floral Nectary	29.40±9.56^{c}	56.92±10.98^{b}	31.00±12.94^{c}	22.38±13.44^{b}
F value (4,16)	76.22P<0.0001	24.04P<0.0001	50.48P<0.0001	58.00P<0.0001

Means followed by same letter do not vary significantly from each other by HSD (0.05)

Table 2. Effect of dietary constituents on the adults of *S. rani*

Food Source	Adult Emergence rate (%)	Longevity of Adults (days)
Red Spider Mites	81.74±6.74[a]	24.0±2.36[a]
Aphids	55.38±2.67[a]	20.6±1.14[a]
Pollen	0.0±0.00[b]	22.40±1.95[a]
Honey	66.06±5.67[a]	7.20±2.86[c]
Extra Floral Nectary	16.67±2.37[b]	15.60±2.70[b]
F value (4,16)	25.74P<0.0001	44.18P<0.0001

Means followed by same letter do not vary significantly from each other by HSD (0.05)

reproduction of insects (Roulston and Buchman, 2000; Carter *et al.*, 2006; Van der Steen, 2007). The sustained development and reproduction of native coccinellid beetle, *Harmonia axyridis* (Pallas) provided with pollen diet was reported by Berkvens *et al.* (2008).

Honey source recorded significant survival rate of immature stages of the beetle. Honey being a rich source of monosaccharides and dissacharides served as vital energy reserve (Pemberton and Vandenberg, 1993) thereby provided better survival rate of immature stages of the predator. Honey and water diet as a convenient food source for survival of coccinellid beetle, *Delphastus catalinae* was reported by Simmons *et al.*, 2012. Extra floral nectaries recorded better growth and development of immature and adult stages of the predator. Extra floral nectaries as an alternate option to favour the survival and increased fitness of coccinellid predators was reported by Almeida *et al*. (2011). Predacious coccinellid, *Chilocoris kuwunae* Silv. foraging upon the pollen and extra floral nectaries under field conditions has been reported by Nalepa *et al.*(1992). Alternative foods like honey and pollen can be used as a food source that supports the immature stage development and reproduction of adult coccinellid predators. Use of pollen and other plant foods at times when the host insect population is scarce, provides a competitive advantage to the survival of native coccinellid predators of insects. From the present study it can be concluded that, rearing of *S. rani in vitro* is possible using artificial food sources consisting of honey for the larval stages and pollen for the adults of the predator during the period of scarce availability of natural host insect.

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Persistence of iprodione residues in/on mango fruits after post-harvest treatment and effect of washing on the residue levels

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ABSTRACT : Post-harvest loss of mango fruits due to anthracnose and stem end rot is very high. Iprodione is a contact fungicide which gives good control of both the diseases. Its residue persistence was studied on matured mango fruits after post-harvest treatment @ 2 and 4 g/L. The effect of washing with detergent (0.1%) to disperse residue deposit of iprodione on mango fruits was also studied. Residue levels of iprodione immediately after post-harvest treatment were 7.53 and 12.27 mg/kg. Washing of the fruits 1 day later with water reduced the residue levels by 67%. Major portion of the iprodione residues remained on the fruit surface with minimal movement to the pulp. Analysis of mango pulp of unwashed fruits or fruits washed only with water did not give any conclusive results. But washing the fruits with detergent followed by peeling more than 93% residue deposits could be removed. Matured mango fruits where the peel is discarded can be washed with detergent solution and peeled to remove iprodione residues, but for immature mango fruits where the entire fruit is consumed it should be washed with water to avoid the possibility of detergent residues remaining on the fruits.

Keywords : Iprodione, metabolite, post-harvest, residues, washing.

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important export oriented horticultural crops grown under tropical and subtropical climatic conditions of India. Although India accounts for 50% of the global mango production, its dominance does not translate into international trade due to the spoilage of fruits by various disorders and diseases after harvest. Contamination can be initiated in pre-harvest stage by parasitic presence at the time of picking/ harvesting and after harvest where parasites and spores settle on post-harvested produce. Pre-harvest sprays with chemical fungicides have been shown to reduce post-harvest diseases but the effects have not always been consistent. Post-harvest losses in mango are mainly due to anthracnose (*C. gloeosporioides)* and stem end rot (*Botryodiplodia theobromae*). Anthracnose usually occurs at fruit ripening stage, damaging the fruit production by 20-30%.

Iprodione (3-(3,5-dichlorophenyl)-N-(1-methylethyl) 2,4-dioxo-1-imidazoline-carboximide) is a dicarboximide contact fungicide used for control of a wide variety of crop diseases and in particular for control of stem end rot as well as anthracnose of mango. In a study conducted to evaluate the efficacy of nine fungicides and three botanicals against *Alternaria iprodine* recorded the highest mycelial growth inhibition (Kumar *et al.*, 2006). Mancozeb and iprodione gave excellent control of stem end rot infections of mango and the treated fruits were 100% marketable (Meath, 2000). Dip treatment of mango fruits in iprodione solution for 3-5 min gave control of stem end rot diseases (Meijiao *et al.*, 2005). In the complex post-harvest handling of mango, where control of diseases is necessary to preserve the quality of the produce, the use of safer synthetic fungicides (Kobiler *et al.*, 2001) and physical treatments are integrated (Prusky *et al.*, 2001). Though a lot of information is available on the efficacy of this fungicide to control post-harvest diseases of mango fruits, no information to our knowledge is available on its residue persistence on mango fruits or its decontamination after post-harvest treatment. This study was therefore carried out to evaluate residue persistence of iprodione and its metabolite 3-(3,5-dichlorophenyl) ureido acetic acid in/ on mango fruits after its post-harvest treatment. In addition, effect of washing to remove iprodione residue deposit on mango fruits after the post-harvest treatment was also studied.

MATERIALS AND METHODS

Reference standard of iprodione (purity 97.5%), its metabolite 3-(3,5-dichlorophenyl) ureido acetic acid (purity 99.6%) and the formulated product, Rovral 50 WP were obtained from Bayer crop science Ltd, India. Standard solution of iprodione (1000 µg/mL) was prepared with analytical grade acetone and suitably diluted

to obtain the working standards. All reagents and solvents used were of analytical grade and were purchased locally.

Matured green mango fruits, variety Totapuri (60 kg), were dipped in aqueous solution of the formulated product of iprodione (Rovral 50 WP) for 5 min at the recommended and double the recommended dose of 2 and 4 g/L. Untreated fruits were used as control. The treated fruits were stored in the laboratory at room temperature (25±2^{0}C). Analysis of mango whole fruits (peel + pulp) and pulp (after peeling up off the fruit peel) samples were carried out for residues of iprodione and its metabolite on 0 (2 hr), 1, 5, 10, 15 and 20 days i.e. until the fruits could be stored at room temperature. The fruits were analyzed without washing and after washing with water. Mango fruits were also analyzed after washing with detergent solution and removing the inedible peel.

On every sampling day about 10 kg of treated mango fruits were removed and divided into 3 parts. One part of the fruits was processed without washing as whole fruit and pulp sample. The second part was washed under running water for 2 minutes and analyzed as whole fruit and pulp. The third part was washed with 0.1% detergent solution, peeled and the fruit pulp was analyzed. Extraction and clean up of mango samples was carried out as per Datta and Gopal (1999). The whole fruit and pulp samples were cut into small pieces mixed in a Waring blender and a representative 25 g sample (3 replications) was taken for analysis. The samples were homogenized with 50 mL acetone in a Waring blender and filtered under vacuum through a Buchner funnel. The container and the filter cakes were washed twice with 50 mL acetone and the combined extracts were collected in a 500 mL flask. Acetone was concentrated under reduced pressure in a rotary vacuum evaporator. The aqueous extracts were transferred into a 1 litre separatory funnel and diluted with 40 mL of distilled water. The aqueous phase was partitioned thrice with 30 mL dichloromethane after adding 25 mL saturated sodium chloride solution and dried over anhydrous sodium sulphate. The combined dichloromethane fraction was concentrated to 10 mL and passed through column with florisil as the adsorbent. A glass column (50 cm × 1.5 cm) was packed with 5 g of florisil in between 1 inch layer of sodium sulphate. Iprodione residues were eluted with 100 mL of 95:5 (v/v) dichloromethane + ethyl acetate. The elute was concentrated to dryness and redissolved in 5 mL (10 mL for unwashed whole fruit sample) of toluene for analysis by gas chromatograph (GC).

Recovery experiments were performed to evaluate the accuracy and precision of the analytical procedure used in this study. A 25 g portion of blended untreated mango whole fruit and pulp samples in 5 replicates were spiked with analytical grade iprodione and its metabolite 3-(3,5-dichlorophenyl) ureido acetic acid @ 0.01, 0.1 and 1.0 mg/kg level and processed as per the methodology described above. Residues of iprodione and its metabolite were estimated by using a gas chromatograph (GC), Varian CP 3800 equipped with electron capture detector (ECD) and fitted with a capillary column (fused silica 30 m x 0.25m i.d). The column temperature was initially maintained at 60^{0}C with a hold time of 1 min and programmed @ 15^{0}C/min to 120^{0}C with hold time of 1 min and @ 20^{0}C/min to 280^{0}C with hold time of 16 min. The injector and detector temperatures were maintained at 280 and 300^{0}C, respectively. The carrier gas, ultra pure nitrogen flow rate was 1 mL/min. Under the above parameters, the retention time of iprodione and its metabolite was 14.9 and 7.6 min, respectively. The limit of detection was 0.003 ppm for both the analytes.

Table 1. Recovery of iprodione and its metabolite 3-(3,5-dichlorophenyl) ureido acetic acid on mango whole fruit and pulp at various spiked levels

Spiked level (mg/kg)	Mean recovery (%) ± SD[a]			
	Iprodione		Metabolite	
	Whole fruit	Pulp	Whole fruit	Pulp
0.01	90.36 ± 2.81	89.55 ± 3.68	85.65 ± 2.92	83.38 ± 4.53
0.05	90.54 ± 3.66	90.36 ± 4.11	86.74 ± 3.83	83.77 ± 3.62
0.10	91.44 ± 3.85	90.38 ± 3.27	87.36 ± 2.55	84.24 ± 3.58

[a]Average of five replicates analyses ± SD

Table 2. Residues of iprodione in/on unwashed mango whole fruit after post-harvest treatment and after washing with water

Days after treatment	Untreated control	Residues of iprodione ± SD[a] (mg/kg)			
		Treatment @ 2 g/L		Treatment @ 4 g/L	
		Unwashed	Washed with water	Unwashed	Washed with water
0 (1 hr)	ND	7.54 ± 0.26	-	12.27 ± 0.48	-
1	ND	6.46 ± 0.31	2.12 ± 0.14 (67.1)	11.16 ± 0.44	3.78 ± 0.26 (66.0)
5	ND	4.89 ± 0.15	1.80 ± 0.08 (63)	9.28 ± 0.31	3.29 ± 0.17 (64.5)
10	ND	3.68 ± 0.20	1.51 ± 0.06 (59)	7.33 ± 0.36	2.87 ± 0.15 (60.8)
15	ND	2.92 ± 0.18	1.20 ± 0.04 (58.9)	5.14 ± 0.8	2.06 ± 0.04 (59.7)
20	ND	1.84 ± 0.07	0.78 ± 0.02 57.6)	4.08 ± 0.21	1.71 ± 0.01 (58)

[a]Average residues ± SD from analyses of triplicate laboratory samples taken from a composite field sample
ND – Not detected
The values in the parenthesis are the percent loss of iprodione residues due to washing

RESULTS AND DISCUSSION

Recovery study carried out by spiking mango whole fruit and pulp samples with iprodione and its metabolite 3-(3,5-dichlorophenyl) ureido acetic acid gave the following results. The recovery of iprodione residues from mango whole fruit was in the range of 90.36-91.44% and from pulp it was 89.55 - 90.38%. Recovery of metabolite from whole mango fruit was in the range of 85.65-87.36% and from pulp it was 83.38-84.24% (Table 1). Limit of quantification (LOQ) of iprodione and its metabolite in mango whole fruit and pulp was 0.01 mg/kg.

As indicated in Table 2, initial residue deposits of iprodione on unwashed whole mango fruits immediately after the treatment were 7.54 and 12.27 mg/kg, respectively from treatments @ 2 and 4 g/L. Dissipation of iprodione residues from mango whole fruits was a very slow process. After 20 days of treatment 1.84 and 4.08 mg/kg iprodione residues still remained in/on the fruits. The residue data was subjected to statistical analysis as per Hoskins (1961) to compute the residual half-life ($t_{½}$) and safe pre-harvest interval. The residues dissipated at the half-life of 10 and 12.7 days, respectively. Maximum residue limit (MRL) of iprodione on mango is not notified either by Food Safety and Standards Act (FSSA) 2006, India or by Codex Alimentarius Commission, FAO/WHO. However a provisional MRL value of 10 mg/kg is notified by The Japan Food Chemical Research Foundation (Anonymous, 2007). Based on this value post-harvest interval of 5 days is recommended for consumption of whole mango fruits after giving post-harvest dip treatment of iprodione @ 4 g/L, whereas if treated with the recommended dose of 2 g/L, 1 day post-harvest interval is required. Peeling up of mango fruits without washing did not give any conclusive results. The residue level in the peeled fruits (pulp) was in the range of 10-20% of the residue level on the whole fruit. Considering iprodione is non-systemic in nature it is unlikely that the fruit pulp will have such high residue content. It was concluded that while peeling unwashed fruits there was transfer of residues to the pulp by hand contact. Therefore those results are not presented here. Residues of the metabolite 3-(3,5-dichlorophenyl) ureido acetic acid was not detected in any mango samples.

Washing of the iprodione treated mango fruits was found to be an effective way to reduce its residue deposits. On the 1st day 6.46 and 11.06 mg/kg iprodione was recovered from unwashed fruits, 2.12 and 3.78 mg/

Table 3. Residues of iprodione in mango pulp after washing with detergent and peeling

Days after treatment	Untreated control	Residues of iprodione ± SD[a] (mg/kg)	
		Treatment @ 2 g/L	Treatment @ 4 g/L
1	ND	0.08 ± 0.001 (98.8)	0.12 ± 0.01 (98.9)
5	ND	0.13 ± 0.001 (97.3)	0.21 ±0.02 (97.7)
10	ND	0.22 ± 0.02 (94.0)	0.41 ± 0.03 (94.4)
15	ND	0.16 ± 0.01 (94.5)	0.30 ± 0.01 (94.1)
20	ND	0.12 0.01 (93.5)	0.23 ± 0.02 (94.3)

[a]Average of three replicates analysis ± SD
The values in the parenthesis are the percent loss of iprodione residues due to washing and peeling
ND – Not detected

kg from water washed fruits from treatment @ 2 and 4 g/L, respectively (Table 2). It was also observed that percent reduction of residues due to washing was lesser with time. Reduction of iprodione residues due to water washing 1 day after treatment was in the range of 66-67% while after 20 days it was about 58%. Iprodione residues from the recommended dose of treatment (2 g/L) decreased from 1.84 mg/kg to 0.78 mg/kg (Table 2) after washing with water on the 20th day. Dissipation of iprodione residues with time and effect of water wash to remove its residues is given in Fig. 1. Mango fruits were peeled after washing with water and detergent solution. Since the matured mango fruits are consumed without peel the chances of detergent residues on the edible portion of the fruit was eliminated. Pulp analysis

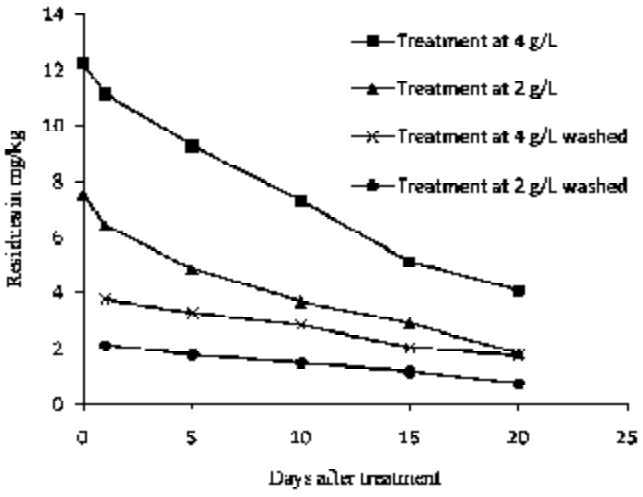

Fig 1. Dissipation of iprodione residues with time and effect of water wash to remove its residues

from water washed fruits gave erratic results. It was observed that the residue deposits on the fruits were reducing with time, but the same was not the case with fruit pulp. The results did not follow any particular pattern and it was not possible to correlate it with other results. Therefore those results were omitted from this presentation.

By removing the fruit peel after washing with detergent solution about 98% of iprodione residues could be removed 1 day after the treatment. With increase in storage period residue loss due to washing and peeling only reduced slightly (to about 93%). On the 1st day 0.08 and 0.12 mg/kg of iprodione was found in detergent washed mango pulp, which increased to 0.22 and 0.41 mg/kg by the 10th day and decreased thereafter (Table 3). Detergent washing might have been effective to remove significant amount of surface deposits from mango whole fruits, peeling up off such washed fruits reduced the chances of the fruit pulp being contaminated with iprodione residues. As in case of unwashed fruits the metabolite 3-(3,5-dichlorophenyl) ureido acetic acid was not detected in washed whole fruit or pulp samples.

In the present study, residue level of iprodione on mango fruits from treatment at the recommended dose of 2 g/L was within the MRL value of 10 mg/kg. When the treatment was doubled the residues exceeded the prescribed MRL. Information on the residue level of iprodione on mango fruits is not available to our knowledge. However information is available for other fruits and some vegetables. Earlier studies on dissipation of iprodione on tomato, the residue level was always within the MRL value (5 mg/kg) and residue level was more during post-harvest storage compared to freshly

harvested ones (Omirou *et al*., 2009). In the surveys carried out on the residue level of iprodione on pears, it was found to be always within the limits allowed by the legislation of European Union (Lopez and Riba, 1999). In the present study it was also observed that the parent compound was only detected in mango fruits and the metabolite was always absent. In studies carried out with (U-^{14}C) phenyl-labelled iprodione spray application on peaches almost all (95%) of the total radioactive residues were found in/on mature peaches was identified as unchanged iprodione (Anonymous, 1994). Also it showed that the chemical is not systemic by foliar application which is the normal method of treatment. Peeling has been found to be an effective way to remove iprodione residues from other fruits besides mango. Peeling could remove 82.5-95% of the residues from post-harvest treated peaches (Lentza, 1995). Post-harvest treatment of banana with iprodione resulted in the residue deposit of 4-7 mg/kg in the peel, 1.7-3.4 mg/kg in the whole fruit, whereas in the pulp the residues were always less than 0.1 mg/kg (Anonymous, 1994).

The results of the present study showed that residue deposits of iprodione from treatments at the recommended and double the recommended dose of 2 and 4 g/L may be every high, but washing is a very effective method to reduce the residue deposit. Washing with water for 5 min could remove 60% iprodione residue deposit on prune (Cabras, 1998) and 57% of iprodione residues on grapes (Cabras, 1998a). In the present study washing of iprodione treated mango fruits with water could remove 57-67% of iprodione residues. Peeling the fruits after washing with detergent solution removed most of the residue deposits, but same was not the case when the fruits were peeled washing only with water. Water wash removes only the pesticides deposited on the dust settled on the surface, whereas detergent wash could dissipate the pesticides entrapped in epicuticular wax while not removing those in the cuticle (Angioni, 2004). Therefore washing with only water may be the reason for water washed fruits to retain sufficient amount of iprodione on the fruit surface. Peeling up off such fruits might have transferred some of the surface deposit to the pulp. Therefore such results did not follow any pattern and it was not possible to arrive at any conclusion. Effectiveness of washing in removing residues depends upon several factors. Surface residues are amenable to simple washing and the effectiveness may be further improved by detergent (Holland, 1994). Mango being a hardy fruit removal of residues by washing using a mild liquid detergent may be an easy option to remove iprodione residues.

These results show that residue deposits of iprodione in/on mango fruits from post-harvest treatment may be very high, but movement of residues from the surface of the fruits to the pulp was minimal. But once it moved to the pulp it remained there for a longer period. By washing the treated fruit with water a major part of the residues can be removed. But when the fruit peel is not consumed washing the fruits with a mild liquid detergent may be considered an option.

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Natural enemies of oil palm defoliators and their impact on pest population

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ABSTRACT: Oil palm, *Elaeis guineensis* Jacq. is an important oil crop introduced to meet the vegetable oil needs of the country. Among the lepidopteran pests that cause defoliation of oil palm, psychid, *Metisa plana*, leaf web worm, *Acria* sp. and slug caterpillar, *Darna catenatus* were observed as major ones. Severe infestation of leaf eating caterpillars in Krishna district of Andhra Pradesh caused 29% yield reduction during first year and 31% in second year after the pest attack. Mortality of the pests due to their natural enemies was recorded at moderate level in all the gardens of Krishna, West and East Godavari districts of Andhra Pradesh. Parasitoids were recorded as major and important ones on both psychid and leaf web worm while virus was predominant on slug caterpillar. *Goriphous bunoh, Brachymeria* spp. and *Dolichogenidea metesae* are the main parasitoids recorded on psychids causing 39.2% parasitism with a range of 15.83 to 65.23%. The former is a pupal parasitoid while the later were recorded on larval stages. *Elasmus brevicornis, Apanteles hyposidrae* and *Brachymeria* spp. were recorded on leaf web worm causing moderate mortality in late stages of the pest. NPV and Granulosis virus were however recorded as the possible bioagents to check the slug caterpillar.

Keywords: Defoliators, granulosis virus, natural enemies, oil palm, parasitoids

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is an important vegetable oil producing crop which can yield an average of 4-6 tonnes of oil per ha per year. To mitigate the gap between demand and supply of vegetable oil in the country, the crop has been introduced in different states of India by providing irrigation which is unique in the entire world. Though the crop has been projected to have less pest problems unlike other oil seeds crops *viz.* groundnut, sunflower, castor etc., the recent reports indicate the incidence of various defoliators *viz.*, bag worms or psychids, leaf web worm and slug caterpillars causing yield losses in the subsequent years to the tune of 33% in the first year and 22% in the second year (Kalidas, 2002). Natural occurrence of various parasitoids feeding on the pest species is reported in all the oil palm gardens (Kalidas, 2012). Intermingling of leaves of adjacent palms and excess irrigation leading to high humid conditions are found to be the few reasons for pest multiplication and out breaks (Kalidas *et al.*, 2002). Leaf web worm (*Acria* sp.) is a new pest on oil palm causing severe incidence during winter periods. Its incidence was reported for the first time in India on oil palm during 1995-96 (Kalidas and Rethinam, 1998 and Saravanan *et al.*, 2012). Pest incidence is seen with the onset of winter period and lasts till the onset of summer and there after vanishes. Incidence was observed on alternate years in the 90s while in the last one decade it is observed every year with the pest becoming endemic to many areas. Slug caterpillar, *Darna catenatus* Snellen is a limacodid lepidopteran pest causing defoliation. Unlike the above mentioned, its incidence is observed during the summer months. Heavy incidence leads to complete defoliation making the palms burnt appearance in a short period (Anon., 2002; Kochu Babu and Kalidas, 2004). Genty *et al.*, (1975) reported leaf eating Lepidoptera are the most numerous pests on oil palm in Latin America and are often most serious. In Indonesia, leaf eating nettle caterpillar *Setothosea asigna* was attacking indifferently on the young and old plantations of oil palm with heavy infestation causing yield losses by 70% in the first year after defoliation and 90% when the attack was continued in the second year (Sipayung *et al.*, 1989 and Sudharto *et al.*, 1998). The present study was carried out to document different natural enemies feeding on defoliating lepidopteran pests of oil palm and their impact on the suppression of pest population.

MATERIALS AND METHODS

A study was carried out in the oil palm plantations of West and East Godavari and Krishna districts of Andhra Pradesh during 2010-2012 to document the natural enemy complex of oil palm defoliators. Observations on the pest incidence and their natural enemies were recorded at monthly intervals using random sampling method. Psychids were collected from the surveyed plantations and kept in the laboratory for the emergence of natural enemies. The emerged natural enemies were identified from NHM, London and other

institutions like NBAII and UAS, Bangalore. In the other study, psychids (bag worms) were collected from the plantations that were fixed to take monthly observations (fixed plot survey) in different areas and age group of palms. The per cent parasitism was calculated using the simple mathematical formula.

RESULTS AND DISCUSSION

Incidence and natural enemies of bagworm/psychid (*Metisa plana* L.)

The pest was observed endemic in most of the gardens with the presence of all the stages at any date of observation. The incidence was observed during July to March ranging from 0.39 to 8.8 in different age groups of palms (Table 1). Young gardens of less than 5 years recorded less incidence compared to middle aged ones. Lack of penetration of sunlight into the aged gardens due to overlapping / intermingling of leaves of adjacent palms was observed as the main reason for the increased incidence compared to young gardens where such conditions were lacking. Cocoa (*Theobroma cacao)*, Coconut (*Cocos nucifera*), Areca nut (*Areca catechu*) and Areca palm (*Dypsis lutescens*) were observed as collateral hosts for the pest.

Three species of parasitoids *viz.*, *Goriphous bunoh* (Fig.1-3) infesting pupal stages and *Brachymeria* spp. and *Dolichogenidea metesae* parasitising larval stages of the pest were recorded in majority of the plantations. The parasitism due to natural enemies was observed with a prominent hole at the base of the bag (Fig. 4). Impact of parasitism on the pest population was observed more in the later stages of the pest. *Brachymeria* spp. was observed as the main parasitoid causing more than 40% parasitism to larval stages during 2012-13. This was observed as predominant one in the surveyed areas. The average per cent parasitism was observed at 33.15% with maximum parasitisation (65.23%) during the month of

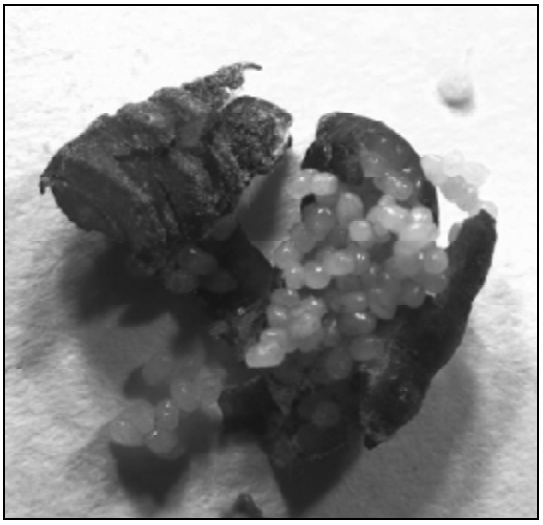

Fig 1. Psychid eggs in the pupal cocoons

Fig 2. Pupal parasitoid, *Goriphous bunoh*

Fig 3. Larval parasitoid, *Brachymeria* spp.

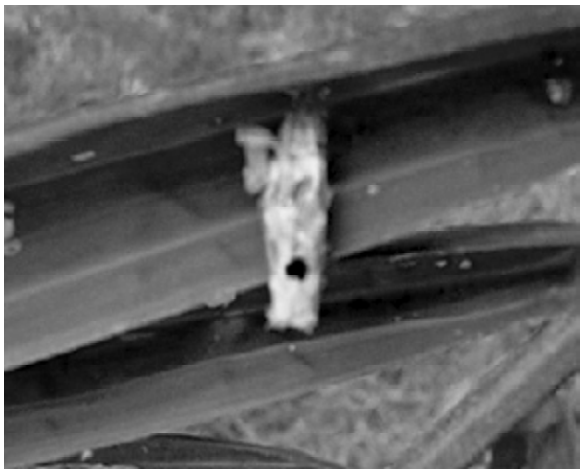

Fig 4. Parasitized bag worm with prominent hole at the base of the bag

Table 1. Incidence of bagworm and extent of natural parasitisation in oil palm plots

Village and age of the plantation	Psychid incidence and extent of parasitisation in different months during 2011-12 (200 fronds= 50 palms, 4 fronds per palm)												
	Apr	**May**	**June**	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**March**	**Average**
Katur (>15 years)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	12.0 (12.6)	12.0 (12.0)	16.6 (16.6)	0.0 (0.0)	4.0 (0.0)	7.0 (4.0)	5.0 (0.0)	7.0 (0.0)	5.30(3.72)
Telaprolu (>15 years)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	05 (7.0)	2.0 (2.6)	0.0 (0.00)	0.0 (0.0)	0.0 (0.0)	5.5 (0.0)	4.5 (0.0)	2.0 (0.0)	1.0 (0.0)	1.29 (0.80)
Chodimella (<15 years)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	6.6 (6.0)	16.2 (16.20)	9.0 (0.0)	3.0 (6.6)	6.0 (8.8)	12.0 (0.0)	25.0 (0.0)	29.0 (2.2)	8.8 (3.30)
Jangareddygudem (<15 years)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.0 (14.4)	4.5 (4.5)	35.1 (35.1)	2.5 (33.0)	6.0 (13.0)	2.0 (8.8)	8.0 (1.0)	2.0 (2.0)	0.0 (0.0)	5.09 (9.30)
Rajahmundry(<15 years)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	14.0 (15.5)	0.0 (0.0)	0.6 (0.0)	12.0 (0.0)	5.5 (8.0)	0.0 (0.0)	2.67 (1.96)
Suryaraopet(<15 years)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	4.7 (4.70)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.39 (090)
DOPR(<15 years)	0.0 (0.0)	0.02 (100.0)	31.1 (13.1)	11.1 (111)	13.3 (13.3)	4.5 (4.5)	1.5 (17.5)	1.0 (0.0)	2.5 (12.0)	2.4 (9.5)	0.0 (0.0)	0.0 (0.0)	5.61 (7.2)

Values in parentheses indicate the per cent parasitisation

Table 2. Parasitization of psychid bags recorded in random surveys during 2011-12

Month	psychid bags		
	Total	parasitized	Parasitization (%)
September	330	70	21.21
October	233	78	33.47
November	768	501	65.23
December	240	38	15.83
January	50	15	30.00
Total	1621	702	43.30
Mean	324.2	140.4	43.15

Table 3. Parasitization of leaf web worm during 2011-12

Month	Parasitization (%)		
	Elasmus brevicornis	*Apanteles hyposidrae*	*Brachymeria* sp
December	17.24	33.70	0.0
January	27.67	45.22	79.75
Average	22.45	39.46	39.87

November while minimum (15.83%) was observed during December (Table 3). During 2012-13 the pest incidence was found very less mainly due to high temperatures and low humidity that were existed during summer months followed by heavy rains. The average annual parasitism during this period was observed varying between 0.8 to 9.3% only (Table 2). The low pest population might be the reason for the poor parasitism. During this period also the parasite activity was found more during July to December and no parasitism was observed during summer and winter months. Oil palm plantations that were not received any insecticide application recorded more parasitism (9.3%) compared to treated plots (0.8%). Ricardo *et al.* (2012) reported the heavy occurrence of *Brachymeria* spp. on causing heavy parasitisation of the pupae of Nymphalidae and Hersperidae families feeding on oil palms in Brazil. However in India, its incidence was found restricted to Psychidae and Xylorectinae families but not on *Darna catenatus*. Although the pest is responding well with the microorganisms like *Beauveria bassiana* and

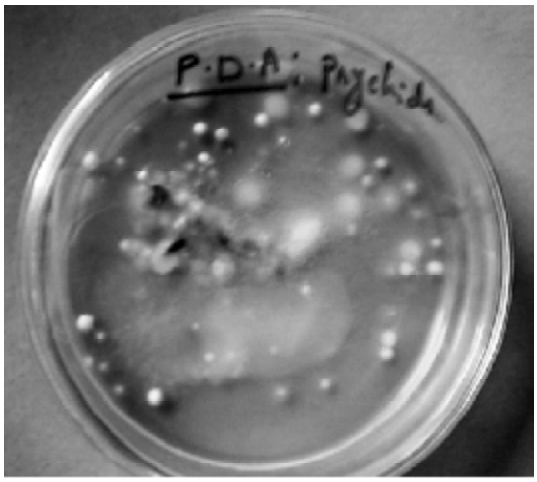

Fig 5. *Beauveria bassiana* infection on psychid.

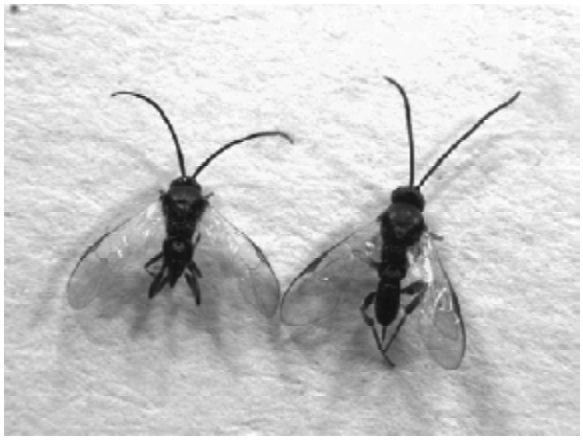

Fig 6. Larval parasitoid of leaf web worm, *Apanteles hyposidrae*

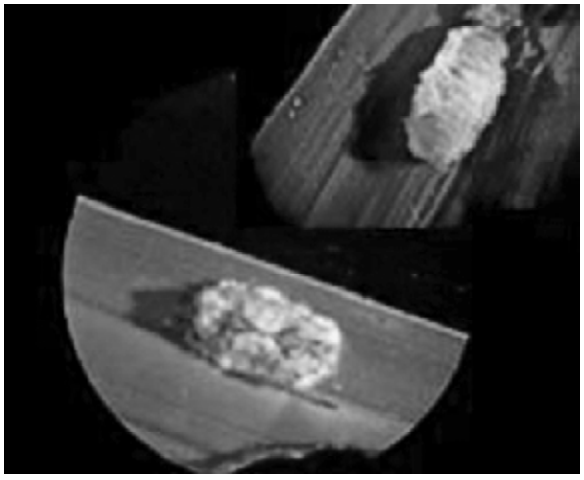

Fig 8. *Beauveria bassiana* infected slug caterpillar

Lecanicillium lecanii that were found to cause white muscardine disease, natural occurrence was however not found in the surveyed areas. The reinoculation work that was carried out using the dead larva recorded profuse

growth of the fungus there by confirms the mortality is due to microorganism (Fig. 7).

Leaf web worm, *Acria* sp.

The pest was first recorded during the winter months of 1995-96 (Kalidas and Rethinam, 1998). The occurrence was observed erratic for a decade confining to a few gardens in alternate years. Since 2005 onwards, it has become a regular pest occurring every year in Krishna, East and West Godavari districts of Andhra Pradesh. The infestation observed was in the range of 80-100% in some orchards. The pest has become endemic in some of the areas where the palms attained tallness and the leaves of adjacent palms are intermingled creating congenial climate for the pest development. The infestation was further aggravated in those orchards where the palms were given basin as well as flood irrigation with excess quantity than the required. A yield loss of up to 34 % was reported for leaf web worm, *Acria* sp. (Kalidas, 2004).

Larvae remain inside a web on the underside of the leaf and in severe incidences, the pest was observed even on upper side of the leaves. They were found feeding on the old leaves causing heavy defoliation. Pupation takes place in the leaf folds. Three species of natural enemies were observed feeding on the larvae and pupae causing 21.21 to 79.75 per cent parasitism (Table 3). The extent of larval parasitism by Elasmids and Braconids was in the range of 21.41 to 36.58% while 79.75% parasitism of pupae was observed due to Chalcid parasitoids. In Thailand, the pest was effectively checked naturally by parasitoids of Braconidae, Chalcidae and Ichneumonidaer besides a tachinid fly and a Clerid beetle predator (Banpot, 2011). Pest was effectively controlled with the application of the microbial organisms namely *Beauveria bassiana, Metarhizium anisopliae* and *Lecanicillium lecanii*. Of these *Beauveria bassiana* caused 98.3% reduction in the pest population after 21 days of application. The microbes on application developed puffy white mat of mycelia on the cuticle causing white muscardine disease. However the natural occurrence of microbe was not found in any of the areas surveyed. Inundative releases of egg parasitoid, *Trichogramma embryophagum* on the management of the pest, were however not found effective in reducing the pest incidence in the released year. But in the subsequent year, the pest was completely vanished from the treated garden which could be correlated with the abiotic and biotic factors like high temperatures and the released parasitoids.

Slug caterpillar, *D. catenatus*

Incidence was first reported on oil palm in 2002 in a few plantations of West Godavari and Krishna districts of Andhra Pradesh. Caterpillars feed on the leaf lamina causing heavy defoliation leaving only midribs. The incidence is very heavy on the lower whorl leaves making them completely dried.

Natural suppression of the pest was observed in a few plantations due to virus infections. The pest was also observed feeding on the adjacent coconut plants but at low levels and probably migrated to the oil palm due to the availability of lush green leaves. High summer temperatures were however not found affecting the pest build up but high relative humidity was found to be important factor for natural suppression. Viral infection of the caterpillars arresting the moulting process was observed in severely infested gardens. The infected caterpillars became a semisolid portion attached to the leaves. Application of microorganism, *Beauveria bassiana* (10^{-1}) that caused white muscardine disease (Fig.8) to the caterpillar proved effective but time taking compared to chemicals insecticides. Mariau (1999) reported that these species are accompanied by large number of parasitoids of families Eulophidae, Chalcididae, Bracinidae, Tachinidae, and Bombyliidae and predators of Pentatomidae families regulating the host populations. He also reported a wide variety of these species are effectively controlled by various viral diseases with the pathogens belong to different virus families namely Baculovirus, Densovirus, Picornavirus, bnudaurelia etc. This is the first report in India on oil palm lepidopteran pests. Present results are in accordance with the observations reported by Sankaran and Syed (1972), Sipayung *et al*., (1979), Syed and Saleh (1998), Sudharto *et al*., (1998) and Charles (2011). The results of our study clearly indicate the presence of a wide range of natural enemies which, if conserved properly, could effectively check the populations of major defoliating pests.

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Diagnostic characters of immature stages of flower chafer beetle, *Chiloloba acuta* (Wiedemann) (Coleoptera: Scarabaeidae: Cetoniinae): Taxonomic importance

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ABSTRACT : The flower chafer beetle, *Chiloloba acuta* (Wiedemann) (Scarabaeidae: Cetoniinae), a pest of cereals and millets is widely distributed in India. The adults are defoliators while larvae are non-pestiferous. The morphological description of immature stages *viz*., egg, larva and pupa of *C. acuta* was carried out at Insect Systmatics laboratory, Division of Entomology, Indian Agricultural Research Institute, New Delhi, India during 2012-13. Eggs were elliptical and creamish in colour with L/W ratio of 1.31. The Scanning electron microscopy studies revealed no visible chorionic ultrastructures but showed distinct aeropyles. The larva was dull white in colour exhibiting 'U' shaped palidia in raster portion with reduced legs. The mandibles, legs and raster of larvae showed distinct characters of species identity. The pupation took place in tough earthen cocoon with larval skin intact. The potential of immature stage characters as taxonomic keys is discussed.

Keywords : Cetonid, egg chorion, larval morphology, raster, Scarabaeidae

INTRODUCTION

The green chafer beetle, *Chiloloba acuta* (Wiedemann), widely distributed in the Indian subcontinent belongs to Pleurosticti Scarabaeidae that comprises of phytophagous scarab clade. This is the only species in genus and can easily be distinguished from other genera of Cetoniinae with the presence of keeled clypeus with produced angles. The adult beetles are shiny metallic green and clothed with yellow hairs, long and decumbent upon the sternum and sides of abdomen, short and erect upon the rest of the body (Arrow, 1910). It is an occasional pest of cereals and millets where the adults feed on the pollen and damage the grains, hence it is also called pollen beetle. In the process of feeding the pollen, the adults may also contribute to the pollination of crops and reported effecting pollination in okra, cucumber, radish and litchi (Thapa, 2006). It is also reported that *C. acuta* adults feed on upland rice in Uttar Pradesh (Garg, 1986).The larvae feed on the decaying organic matter and are non-pestiferous.

Larval morphology yields more diagnostic characters that are informative at different taxonomic levels especially to distinguish phytophagous from non phytophagous species at field level. Some of larval characters offer reliable diagnostic keys because of the consistency of character states. The immature stage descriptions of Scarabaeoidea were provided by Gardner (1935) and within this group tribe Cetoniini (Sipek and Kral, 2012) was widely studied. Larval descriptions of North American species of Cetoniinae were given by several workers, largely being Hayes in 1928, 1929 and Ritcher in 1966. Similarly the larval characters of various genera of other regions were also described by many workers but the literature regarding the larval characters of *C. acuta* is very limited.

On contrary the descriptions of egg stage of cetonids were limited to very few species. One species each from Neotropical, Palaearctic and Oriental and Australian regions was described. *Gymnetis flavomarginata sallei* Schaum, 1849 described as *Paragymnetis flavomarginata sallei* Schaum, 1849 by Arce–Peréz and Morón (1999) of Neotropical distribution; *Valgus hemipterus* (Linnaeus, 1758) by Blanchard (1868) from Palaearctic region; *Protaetia* (*Heteroprotaetia*) *fusca* (Herbst, 1790) by Simpson (1990) of Oriental and Australian distribution. Egg descriptions of two species *viz.*, *Cotinis mutabilis* (Gory & Percheron, 1833) and *Neoscelis dohrni* (Westwood, 1855) from Nearctic region was given by Stone (1982) and Nogueira *et al*. (2004), respectively. Donaldson and van Todner (1992) have described nearly 12 species of Cetoniinae belonging to Afrotropical region. Since there are no studies on egg morphology and very limited studies in the direction of field level identification of *C. acuta*, present study has been taken up to characterize the immature stages to form a basis for further in depth studies. In this paper, certain morphological characters of egg, larva and pupa of *C. acuta*, which could be potential diagnostic keys are described.

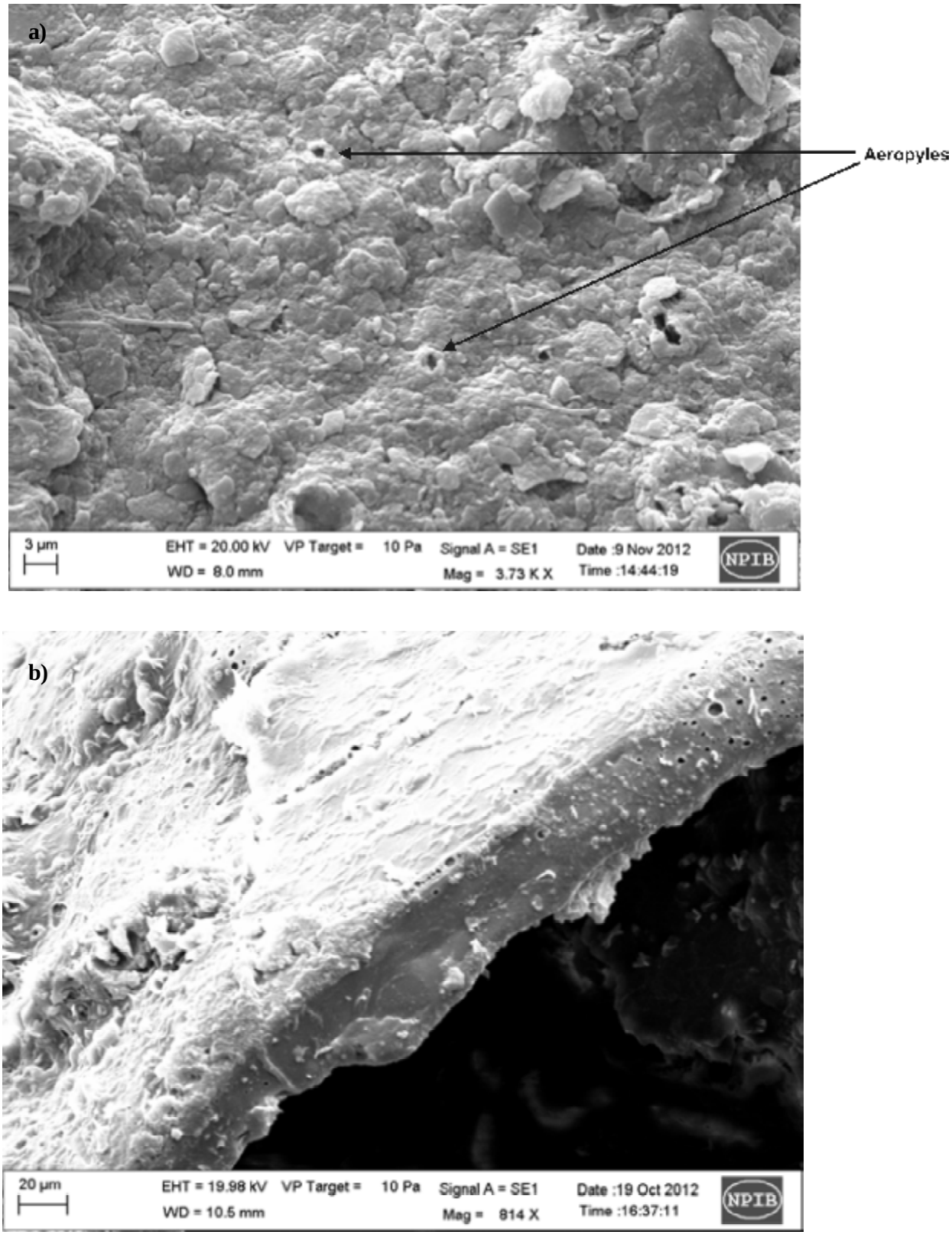

Fig. 1. SEM micrograph of *C. acuta* egg chorionic structure (a) Chorionic surface, (b) cross section of chorion

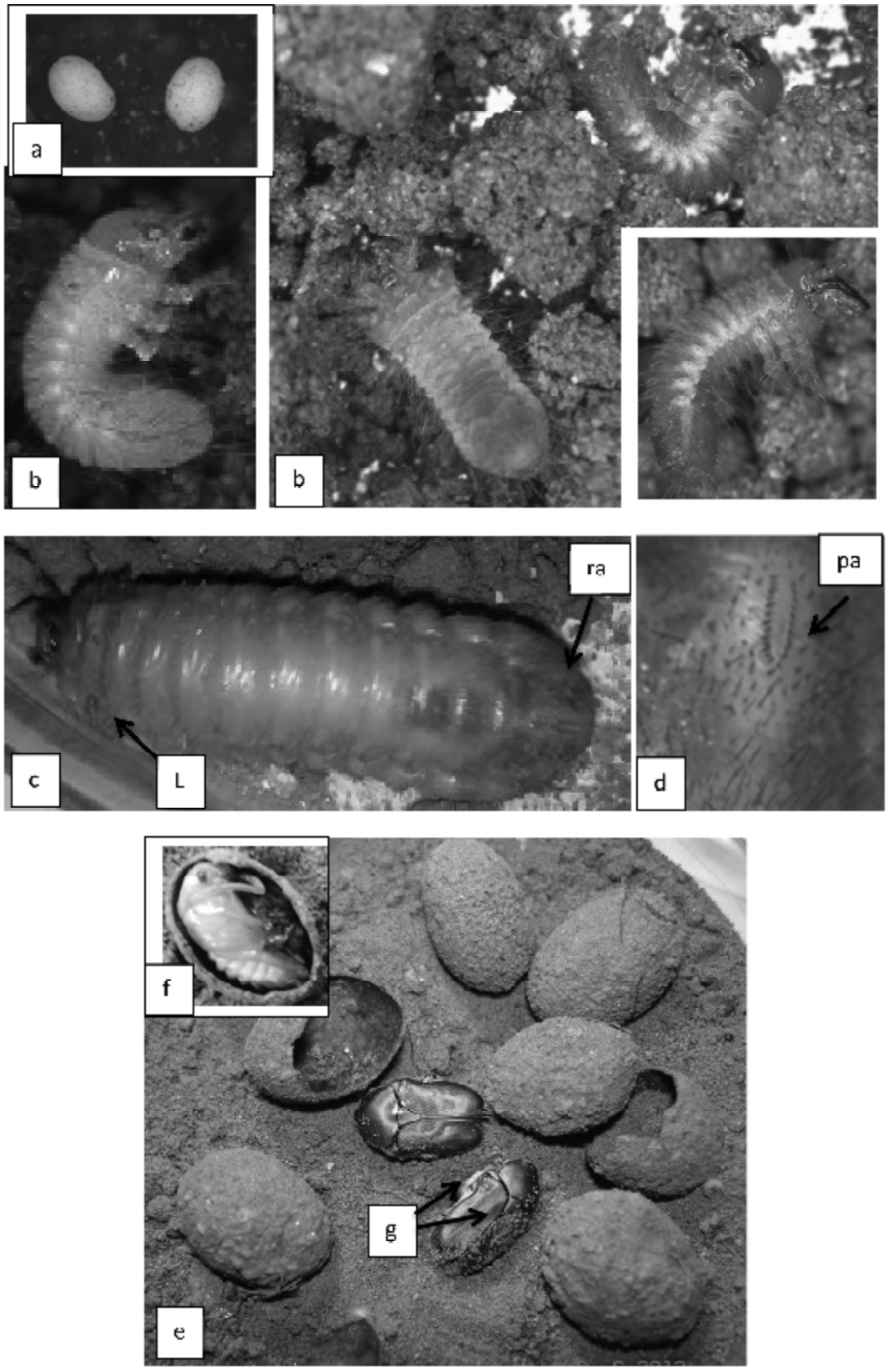

Fig. 2. Different life stages of *C. acuta* a) eggs b) first instar larva c) third instar larva on its back d) raster of larva e) pupal case f) pupa g) adult; L - legs, ra- raster, pa - palidia

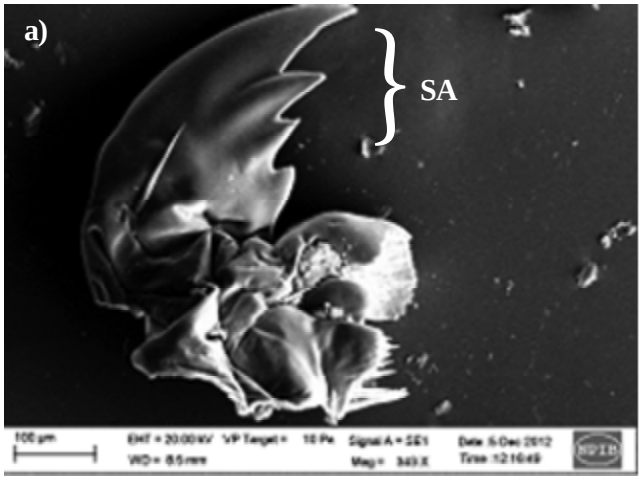

Fig. 3. Larval left mandible of *C. acuta*, a) dorsal view, b) ventral view. SA: Scissorial area, STA: Stridulatory area

MATERIALS AND METHODS

The chafer beetles were collected from the experimental field of Indian Agricultural Research Institute (IARI) and reared at the Division of Entomology, IARI, New Delhi. The adults were released in pairs in to the rearing jars half filled with soil. They were provided with rose flowers daily as food to facilitate feeding and mating. The eggs deposited in the soil were collected and external morphology was observed through light microscopy and length and width were measured with help of ocular micrometer.

Scanning Electron Microscopy (SEM)

The eggs were subjected to SEM to differentiate the chorionic ultrastructures. The eggs were processed for SEM by fixing overnight in 2% glutaraldehyde and carrying out the dehydration process through increased concentration of ethanol and drying at Critical Point Dryer Emitech K850. Scanning Electron Microscopy (SEM) was done on Zeiss EVOMA10 after 24nm thick palladium coating in a SC 7620 sputter coater at 6 x 10^{-2} mbar; images were taken at 20kV EHT amd 10Pa, between 275 and 924x.

Larval Characters

The larvae were observed for leg, raster (the ventral portion of the last abdominal segment just below the anal slit), anal slit, mandibles and spiracles for the diagnostic characters. The larvae later were allowed for pupation and emergence of adults.

RESULTS AND DISCUSSION

Egg morphology

The eggs were very tiny, elliptical in shape and creamish in colour (Fig 2a). The length and width ranged from 1.50mm to 1.75mm and 1.13mm to 1.38mm, respectively measuring on an average 1.6 and 1.25mm, respectively. The incubation period was 15-17 days. The SEM studies of egg chorion revealed that there are no definite patterns but for rough and uneven undulations over the surface. Numerous aeropyles that facilitate gaseous exchange as well as water absorption was observed on egg surface (Fig. 1a). Water absorption essential for growth and embryonic development is closely associated with temperature where water absorption represents about 30% of the total embryogenesis period (Carrillo and Fresard, 2010). This may be related to the wide occurrence and distribution of *C. acuta* in Oriental region and attributed to the egg structure, which is pivotal in success of its survival and race. The cross section of egg revealed that there were no clear distinction of chorionic layers *viz.*, exo and endochorion but was porous (Fig 1b). This is the first study of egg chorion of *C. acuta* through scanning electron microscopy.

Larval morphology

The larva was dull white to greyish white in colour with anterior and posterior ends of the body equally broader. The larvae had three instars and at all instars larva move or crawl on its back (Fig 2c). The raster portion at the ventral side of the last abdominal segment of the larva exhibited a unique 'U' shaped palidia with two rows of pali (Fig. 2d). Palidia consisted of approximately 13 pali on each side. The septula (space

between two rows of pali) is broader at the end closer to the anal slit and narrower towards lower end of palidia. Barbular setae were short, sparse and surrounded the palidia while hamate setae were very less. The anal slit was transverse, which is a typical larval character of subfamilies Rutelinae, Dynastinae and Cetoniinae of Scarabaeidae. Spiracles were 'C' shaped, cribriform and open type. The legs were very much reduced in size in relation to the body and larva always crawled on its back. The legs were proportionately longer in first instar and get reduced gradually as it advanced to later instars, but the movement in first instars also was on their back (Fig 2b). The entire larval body is clothed with setae, denser on dorsal than ventral and has distinct ridges on dorsal side of the body, which aids in movement on its back.

Mandibles were distinct and the left mandible, usually considered for species identity description, showed single scissorial tooth anterior to the scissorial notch, S_1 and three scissorial teeth posterior to the notch, $S_{2+3+4,}$ a species specific character (Fig. 3a). The stridulatory area on ventral side of each mandible was oval with clear, spaced transverse ridges (Fig. 3b). As compared with other genera of Cetoniinae described by Ritcher (1966), the mandible scissorial teeth exhibit distinct pattern, which can be used to delineate the species.

Pupation

Pupation took place in a tough earthen cocoon like structures (Fig 2e). The length and width of the earthen cell measured 1.67cm and 1.14cm, respectively on an average. The pupa was yellowish in color, exarate measuring 1.47cm and 0.83cm length and width, respectively and with larval skin pushed to the side of the pupa in earthen cell (Fig. 2f). The pupation took place in third week of April and slow emergence of the adults was observed in the first week of September. It took nearly 4-5 months for the adult emergence from the cocoon (Fig. 2g). As enough moisture was not provided to the soil containing pupated earthen cells during the observation period, the possibility of non-emergence can be attributed to the dry spell. An excess dryness could have hindered the over summering adult emergence from their cocoons (Fabre, 1987). It either invites that the cetonid requires a good shower for the emergence of adult from the earthen cell or adult undergoing resting phase, which would be an interesting point to explore by the ecologists.

To summarize, *C. acuta*, widely distributed in India showed characteristic egg and larval features, which could be used for diagnostic purposes at field level. The eggs were elliptical, creamish without definite chorionic pattern. The most easily identifiable part, raster of *C. acuta* larvae exhibited 'U' shaped palidia with shorter pali and broader septula. The anal slit of the larva was transverse and pupation observed in earthen cells with larval skin intact in the cell.

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Bio-efficacy of neem products and essential oils against thrips (*Scirtothrips dorsalis* Hood) in Capsicum

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ABSTRACT : Bio-efficacy of two neem products *viz.*, neem soap (1%), pulverised neem seed powder extract (4%) and essential oils (0.2%) of basil (*Ocimum tenuiflorum* syn. *O. sanctum*) mint (*Mentha arvensis*) were evaluated against chilli thrips (*Scirtothrips dorsalis* Hood) in comparison to recommended systemic insecticides *viz.*, acephate (0.075%) and fipronil (0.02%) during 2011-12 *rabi* season in capsicum. Neem soap and pulverised neem seed powder extract prepared in the laboratory and commercially available essential oil samples were used for this study. Ten sprays of the treatments were given at 10 days interval. Thrips damage rating in leaves on a scale of 0-5 was taken once in 10 days to know the treatment effect. Results revealed that neem soap, neem seed powder extract, *basil* and mint were at par with each other and gave significant reduction of *S. dorsalis* damage. While neem seed powder extract, *basil* and mint were at par with acephate and fipronil. Neem soap was at par with fipronil. This study showed that the botanicals like neem soap, neem seed powder extract and essential oils can be used as alternatives to synthetic insecticides for management of chilli thrips, *S. dorsalis* in capsicum.

Keywords: Capsicum, chilli thrips, essential oils, neem soap, pulverised neem seed powder extract

INTRODUCTION

Capsicum, commonly called as bell pepper or sweet pepper, is a very popular vegetable worldwide. Thrips, *Scirtothrips dorsalis* Hood is a major insect pest attacking this crop (Ananthakrishnan, 1973; Jagan Mohan *et al.*, 1980). Nymphs and adults suck the sap of young leaves and cause upward curling of leaves. Severe incidence of thrips may also lead to complete defoliation. Thrips also lacerate and cause scaring on fruits, thus reducing the market value. Generally synthetic insecticides like acephate and fipronil are used to control thrips in capsicum. Our recent studies have shown that botanicals like pulverised neem seed powder extract and neem soap can be effectively used as alternatives to synthetic insecticides in vegetables (Krishna Moorthy and Krishna Kumar, 2002a). Essential oils also have been gaining importance as green pesticides in recent times (Isman, 2000 and Koul *et al*., 2008, Tripathi *et. al.*, 2009). Preliminary studies conducted by us also revelaled that sprays of commercially available essential oil preparations of basil (*Ocimum tenuiflorum* syn. *O. sanctum*) and mint (*Mentha arvensis* Linn.) (both at 0.2%) are effective in reducing onion thrips (*Thrips tabaci* Lindeman) (Krishna Moorthy *et al*., 2013a). With this background, studies were conducted to evaluate these botanicals and essential oils against chilli thrips on capsicum.

MATERIALS AND METHODS

Field experiments were conducted at Indian Institute of Horticultural research, Bengaluru. Capsicum F_1 hybrid *Indra* was planted on 1st October 2011 in plots of 4 x 3 m size with a spacing of 60 x 50 cm at IIHR, Bengaluru. The treatments included sprays of neem soap (1%), pulverised neem seed powder extract (4%), essential oils of mint and *basil* (both at 0.2%), acephate 75SP (0.075%) and fipronil 5SC (0.02%) and an untreated control. While neem soap and pulverised neem seed powder were prepared in the laboratory, essential oil preparations were procured from M/S Aroma Trading Company, Bengaluru. The trials were laid out in a randomized block design with three replications. Ten sprays were given at 10 days interval, using a gator sprayer delivering 1000 l/ha. A pulveriser (manufactured by M/S Surabhee Essar Machines and Co., Bengaluru) was used to powder dry cleaned whole seeds of neem to a very fine consistency before the start of the experiment and it was stored in a cool dry place at room temperature in a gunny bag. Pulverized neem seed powder (40g) was soaked in 200 ml water overnight, filtered through double layered nylon mesh to prevent the powdered neem seed particles clogging the nozzle and volume was made to 1 litre to prepare (4%) solution for spraying. Neem soap developed by us in the laboratory (which is commercially available for agricultural use) was

used for the study. Observations on thrips damage in leaves were taken in the visual damage rating scale of 0 to 5 rating at 10 days interval following the method described by Krishna Kumar (1996) in chilli. In each plot, ten plants were randomly selected and the thrips rating was recorded. Mean thrips rating of different treatments was tabulated and subjected to statistical analysis.

RESULTS AND DISCUSSION

The data on the thrips damage ratings on different dates are provided in Table 1. Data on pre-treatment (I date of observation) and observation after first spray (II date of observation) showed that the thrips damage in all the seven treatments were not significantly different. The observations made after second spray (III date of observation onwards) showed that the treatment of neem soap and fipronil recorded low mean thrips rating of 0.3, but pulverized neem seed powder extract, mint and *basil*, though recorded higher damage ratings, were at par with this treatment on this date. On this date, acephate recorded slightly higher mean thrips rating of 0.7, however it was found to be on par with pulverized neem seed powder extract, Mint and Basil. Control plot recorded significantly high mean thrips rating of 1.3. The observations made after third and fourth spray (IV and V date of observation) showed a similar trend where as all the treatments of botanicals and insecticides were on par with each other and could reduce the thrips incidence significantly. Spray of pulverized neem seed powder extract, Basil and Mint were at par with acephate and fipronil on all observation dates when thrips damage ratings were considered. Neem soap was at par with Mint, Basil and fipronil on all observation dates.

The cumulative thrips damage rating showed that the lowest mean thrips rating was recorded by acephate (4.3) followed by pulverized neem seed powder extract (4.7), fipronil (4.7), Mint (5.3), Basil (5.7) and neem soap (7.4). The treatments of pulverized neem seed powder extract, Basil and Mint were on par with fipronil and acephate. Neem soap was on par with pulverized neem seed powder extract, Mint, Basil and fipronil. All

Table 1. Thrips damage ratings of Capsicum leaves under different treatments

Treatment	Observation dates											
	I (Pre-treat-ment)	II	III	IV	V	VI	VII	VIII	IX	X	XI	Cumu-lative
Neem Soap 1%	0.3 (0.6)	0.8 (1.0)	0.3^{a} (0.6)	0.2^{a} (0.5)	0.5^{a} (0.8)	0.5^{b} (0.8)	1.0^{bc} (1.0)	0.7^{a} (0.9)	1.1^{bc} (1.1)	0.9^{bc} (1.0)	1.4^{bc} (1.2)	7.4^{b} (2.7)
Pulverised Neem seed powder extract 4%	0.7 (0.8)	1.0 (1.0)	0.6^{a} (0.8)	0.4^{a} (0.7)	0.3^{a} (0.6)	0.2^{ab} (0.6)	0.3^{a} (0.6)	0.2^{a} (0.6)	0.5^{ab} (0.8)	0.3^{a} (0.6)	0.8^{a} (0.9)	4.7^{ab} (2.2)
Mint 0.2%	0.7 (0.9)	1.0 (1.0)	0.4^{ab} (0.7)	0.2^{a} (0.5)	0.5^{a} (0.8)	0.3^{ab} (0.7)	0.6^{ab} (0.9)	0.3^{a} (0.7)	0.6^{ab} (0.8)	0.4^{ab} (0.7)	0.9^{ab} (1.0)	5.3^{ab} (2.3)
Basil 0.2%	0.7 (0.8)	1.1 (1.1)	0.5^{ab} (0.8)	0.2^{a} (0.5)	0.5^{a} (0.8)	0.4^{ab} (0.7)	0.6^{ab} (0.8)	0.5^{a} (0.8)	0.6^{ab} (0.8)	0.4^{ab} (0.7)	0.9^{ab} (1.0)	5.7^{ab} (2.4)
Acephate 0.075%	0.4 (0.7)	1.0 (1.0)	0.7^{b} (0.9)	0.2^{a} (0.5)	0.4^{a} (0.7)	0.2^{a} (0.5)	0.3^{a} (0.6)	0.3^{a} (0.7)	0.3^{a} (0.7)	0.2^{a} (0.5)	0.6^{a} (0.9)	4.3^{a} (2.1)
Fipronil 0.02%	0.5 (0.7)	0.9 (1.0)	0.3^{a} (0.6)	0.1^{a} (0.5)	0.3^{a} (0.6)	0.2^{a} (0.5)	0.6^{ab} (0.8)	0.3^{a} (0.6)	0.6^{ab} (0.8)	0.4^{ab} (0.7)	0.9^{ab} (1.0)	4.7^{ab} (2.2)
Control	0.5 (0.7)	0.9 (1.0)	1.3^{c} (1.2)	1.0^{b} (1.1)	1.5^{b} (1.2)	1.2^{c} (1.1)	1.6^{c} (1.3)	1.4^{b} (1.2)	1.6^{c} (1.3)	1.4^{c} (1.2)	1.9^{c} (1.4)	13.9^{c} (3.7)
CD (P=0.05)	NS	NS	0.19	0.21	0.34	0.21	0.31	0.25	0.27	0.31	0.23	0.49
CV (%)	27.7	17.7	13.7	19.5	24.5	16.8	19.8	17.9	16.9	22.3	12.2	11.0

Figures in the parenthesis are square root of (x+0.1) transformed values

these treatments given at 10 days interval recorded a cumulative ratings of less than 7.4 and were found to reduce thrips damage significantly as compared to control, which recorded a cumulative thrips damage rating of 13.9.

Present study clearly showed that neem soap, pulverized neem seed powder extract, essential oil preparations of Basil and Mint can be used as alternative to synthetic insecticides like acephate and fipronil. There are not many reports on the efficacy of botanicals and organics against *S. dorsalis* in Capsicum (Sunitha, 2007). However, pulverised neem seed powder extract and neem soap were found effective in reducing Diamondback moth in cabbage (Krishna Moorthy and Krishna Kumar, 2002a), fruit flies in cucumber (Krishna Moorthy and Krishna Kumar, 2002b). These neem products were also found effective in reducing okra shoot and fruit borer (*Earias vitella*) (Krishna Moorthy and Krishna Kumar, 2012). Use of essential oils in insect pest management is gaining much importance of late and a few formulations have been developed in United States of America, particularly for green house crops (Koul *et al.*, 2008). Recently we have reported that essential oils of Basil and *Geranium* can be used as alternative to insecticides in addition to pulverised neem seed powder extract and neem soap in controlling onion thrips (Krishna Moorthy *et al*., 2013). Present paper is the first report enumerating the field efficacy of essential oils in managing *S. Dorsalis*. Further studies are needed to test the efficacy against a wide range of pests across the crops and to develop suitable formulations.

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Influence of weather factors on the incidence of fruit flies in chilli (*Capsicum annuum* L.) and their prediction model

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ABSTRACT: Seasonal incidence and population fluctuation of fruit flies on chilli (*Capsicum annuum* L.) ecosystem in Mizoram, India revealed that there were four distinct peaks during last week of March (115.33), second week of April (204.67), second week of May (384.00) and first week of June (126.00). The studies clearly showed that minimum temperature (r = -0.546*) was found to be an important predictor of fruit fly catches, while the maximum temperature, relative humidity, rainfall and rainy days had non-significant correlation with trap catches of fruit flies. Further, as a measure of goodness-of-fit, the coefficient of determination (R^2) was used to evaluate the developed empirical models. The abiotic factors jointly had a highly significant impact on population of fruit flies. Validation test showed that the model developed using minimum and maximum temperature, $Y= -52.13 - 46 (X_1) + 39 (X_2)$ (X_1, minimum temperature, X_2, maximum temperature) predicted fruit flies catches reasonably well based on R^2 value (55%). This model can be used for decision making in IPM, but future validation is needed to improve its predictive ability. The present investigation on the fruit flies catches from methyl eugenol traps are new records for India on this chilli ecosystem. The major fruit fly species groups were caught from methyl eugenol trap in chilli ecosystem was *Bactrocera dorsalis* group.

Keywords: *Bactrocera* spp., seasonal incidence, fruit flies, chilli, weather factors

INTRODUCTION

Chilli, *Capsicum annuum* L. is one of the most valuable crops of India. The crop is grown all over India as it is valued for its diverse commercial uses. Among the insect pests infesting chilli, fruit flies, *Bactrocera* spp. (Diptera: Tephritidae) are serious pests incurring considerable post harvest losses in the chilli growing areas of Mizoram (Boopathi *et al*., 2012 and Boopathi *et al*., 2013). The pest not only deteriorates its quality also due to premature dropping of infested fruits. The population buildup of any insect is very intimately associated with the weather parameters prevailing during preceding and corresponding periods. The pest status does not remain static throughout the year, but changes accordingly based on abiotic factors like temperature, relative humidity, rainfall, rainy days, etc. Since the information available on this aspect is meager, studies to understand the role of weather factors in influencing fruit flies incidence is need of the hour in Mizoram. This will not only help in taking right management decisions but also in their execution at right time. Hence, the present study was undertaken to study the role and reliability of weather factors for predicting fruit flies incidence/ catches. Further, the effect of weather data range for accurate assessment of fruit flies incidence/catches was also explored.

MATERIALS AND METHODS

The studies on population dynamics of fruit flies in chilli were carried out at the Research farm, ICAR Research Complex for NEH Region, Mizoram Centre, Kolasib, Mizoram, India. Three methyl eugenol traps were erected with bamboo stick at about 1.5 m above ground level. The monitoring traps were loaded with vials immersed with a cotton wick containing 15 ml of methyl eugenol and malathion in the ratio of 3:1 and the solution was changed once in three months. Fruit fly catches were recorded weekly and collected during March to July 2009. The weekly data on fruit fly catches were subjected to correlation and regression analyses with average weekly weather data to find out the influence of abiotic factors on fruit fly catches. The variable showing significant correlation with fruit flies catches were further analyzed with regression analyses to measure the present variability in fruit flies catches explained by each weather variable (Ryan, 1997). To get further insight into the results obtained, a step-wise regression procedure (Ryan, 1997) was applied to select the most crucial weather factors, which can influence the variability in fruit flies catches. This technique essentially helps in identifying weather factor(s), which are significantly correlated with fruit flies catches (Y) stage by stage. Further, a measure of goodness-of-fit,

the values of co-efficient of determination (R^2) was calculated for developed models (Agostid'no and Stephens, 1986).

RESULTS AND DISCUSSION

The results showed that highest number of fruit flies was caught during April and June months and it was coincided with chilli fruiting period (Fig.1). The major fruit fly species groups were caught from methyl eugenol trap in chilli ecosystem was *Bactrocera dorsalis* group.The catches continued to increase from late March and reached a major peak in mid May (384.00) was followed by a sharp decline in end of June (Fig. 1). There are four distinct peaks were noticed during last week of March (115.33), second week of April (204.67), second week of May (384.00) and first week of June (126.00). Later a minor peak was seen in late June and the catches sharply declined to reach very low numbers in mid/late July. These findings are in agreement with the reports of Sarada *et al*. (2001), Latif and Abdullah (2005), Sithanatham *et al*. (2006) and Selvaraj *et al*. (2006). In the present investigation, more fruit flies were caught during April to May. Similarly, Boopathi *et al*. (2012), Boopathi (2013) and Boopathi *et al*. (2013) reported that fruit flies was more abundant during April and May on tomato, guava and capsicum ecosystems. More fruit flies were attracted towards cotton wick based methyl eugenol traps which is in conformity with the findings of Sithanatham and Boopathi (2010) who reported that fruit flies were attracted towards cotton wick based

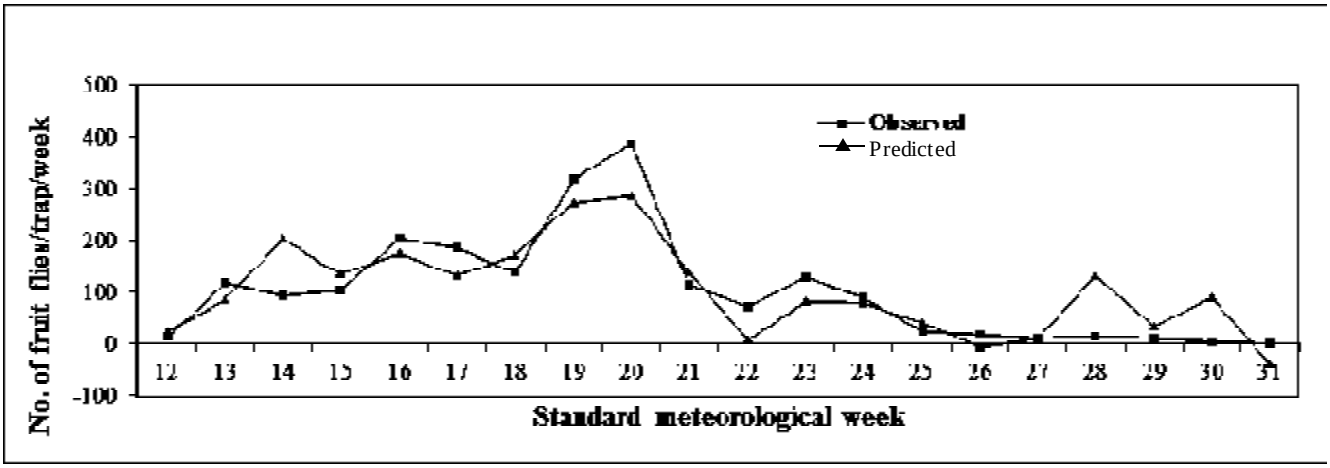

Fig. 1. Graphical representation of the observed vs. predicted fruit flies catches in chilli ecosystem

Table 1. Correlation coefficients of fruit flies in chilli ecosystem with weather parameters

Parameter	Y	X_1	X_2	X_3	X_4	X_5	X_6
Y	1	-0.546*	0.195ns	-0.277ns	-0.055ns	-0.346ns	-0.247ns
X_1		1	0.457*	0.574**	-0.453*	0.182ns	0.023ns
X_2			1	0.402ns	-0.417ns	0.192ns	-0.019ns
X_3				1	0.069ns	0.586**	0.533*
X_4					1	0.242ns	0.262ns
X_5						1	0.650**
X_6							1

ns,*,** non-significant or significant at $P \leq 0.05$ or $P \leq 0.01$

Y = Mean number of fruit flies, X_1 = Minimum Temperature (^{0}C), X_2 = Maximum Temperature (^{0}C), X_3 = Minimum relative humidity (%), X_4 = Maximum relative humidity (%), X_5 = Rainfall (mm), X_6 = Rainy days

Table 2. Results of statistical models along with goodness of fit statistics

Model	Multiple Regression
Full regression model (All weather parameters)	Standard Error : 13.741 (X_1), 13.422 (X_2), 1.624 (X_3), 1.933 (X_4), 0.509 (X_5), 14.440 (X_6) T-value : -4.44** (X_1), 2.33* (X_2), 1.66ns (X_3), -1.23ns (X_4), -1.52ns (X_5), - 0.80ns (X_6) F value : 5.42** R^2 : 0.71** Regression equation : Y= 550.45 - 61 (X1) + 31.00 (X2) + 3.00 (X3) – 2.00 (X4) - 0.80 (X5) - 11.48 (X6)
Model using minimum temperature	Standard Error : 11.285 (X_1) T-value : -2.76* (X_1) F value : 7.63* R^2 : 0.30* Regression equation : Y= 787.84 - 31 (X_1)
Model using minimum temperature and minimum relative humidity	Standard Error : 14.159 (X_1), 1.451 (X_3) T-value : -2.33* (X_1), 0.22ns (X_3) F value : 3.63* R^2 : 0.30* Regression equation : Y= 803.13 - 33 (X_1) + 0.3 (X_3)
Model using minimum temperature and maximum	Standard Error : 11.912 (X_1), 1.933 (X_4) T-value : -3.44** (X_1), -1.83ns (X_4) F value : 5.97* R^2 : 0.41* Regression equation : Y= 1258.39 - 41 (X_1) - 4 (X_4)
Model using minimum temperature and rainfall	Standard Error : 11.268 (X_1), 0.464 (X_5) T-value : -2.53* (X_1), -1.29ns (X_5) F value : 4.79* R^2 : 0.36* Regression equation : Y= 759.44 - 29 (X_1) - 1 (X_5)
Model using minimum temperature and rain days	Standard Error : 11.151 (X_1), 12.617 (X_6) T-value : -2.77* (X_1), -1.20ns (X_6) F value : 4.63* R^2 : 0.35* Regression equation : Y= 820.44 – 31 (X_1) - 15 (X_6)
Optimized model (using minimum temperature and maximum temperature)	Standard Error : 10.487 (X_1), 12.744 (X_2) T-value : -4.37** (X_1), 3.06** (X_2) F value : 10.25** R^2 : 0.55** Regression equation : Y= -52.13 - 46 (X_1) + 39 (X_2)

ns,*,** non-significant or significant at $P \leq 0.05$ or $P \leq 0.01$

Y = Mean number of fruit flies, X_1 = Minimum Temperature (^{0}C), X_2 = Maximum Temperature (^{0}C), X_3 = Minimum relative humidity (%), X_4 = Maximum relative humidity (%), X_5 = Rainfall (mm), X_6 = Rainy days

methyl eugenol traps than other trap systems. The error variation between observed and predicted values was ranged from -116.11 to 98.33. The predicted and observed fruit flies catches is depicted in Fig. 1.

The number of fruit flies attracted was significantly negatively correlated with minimum temperature (r = -0.546*) (Table 1). However, the influence of maximum temperature, relative humidity, rainfall and rainy days was found to be not significant with trap catches. However, Gajalakshmi *et al*. (2011) reported significant correlation between trap catches and maximum temperature. Similarly, Boopathi (2013) reported that maximum temperature had positive correlation with fruit flies catches in tomato ecosystem. Rainfall found negatively correlated with population of fruit flies in present study had been reported by Gupta *et al*. (1990) and Gajalakshmi *et al*. (2011). As a next step, statistical model was developed, by regressing weekly fruit flies catches with all the weather parameters. In the present study, all the abiotic factors jointly had a highly significant impact on fruit flies catches in chilli ecosystem (Table 2). However, Boopathi (2013) reported that all weather parameters jointly had non-significant impact on fruit flies catches in tomato ecosystem. Perusal of Table 2 indicated that about 71% of the variation in fruit flies catches have been collectively explained when all the weather parameters are incorporated in the model. Though the developed model resulted in considerably high R^2 value, one of the regression coefficients corresponding to minimum temperature (-4.44**) was significantly related to fruit flies catches as indicated by the t-test statistic value. Accordingly step-wise regression models were developed and the results are presented in Table 2. Perusal of data indicated that addition of two variables *viz.*, minimum and maximum temperature could explain the variability in the fruit flies catches to the extent of 55% as against 71% (when all weather variables included in the model) as compared to other weather variables. Thus, eliminating other variables did not affect the R^2 value significantly. Therefore, among all weather parameters tried, only minimum and maximum temperature could collectively explain about 55% of the variation in weekly fruit flies catches. Further, the regression coefficient corresponding to this variable in the final model was also statistically significant, as indicated by the t-test statistic value. Thus, $Y= -52.13 - 46 (X_1) + 39 (X_2)$ with minimum variable (X_1, minimum temperature, X_2, maximum temperature) considered as optimized model.

The objective of this study was to develop a reasonable prediction model for fruit flies using reliable and dependable weather variables that have direct influence on fruit flies catches. Only minimum and maximum temperature were found to contribute to the variability in the fruit flies catches to the maximum extent (55%). Further, validation of optimized model (the observed fruit flies catches values were compared to predicted values using optimized model), clearly indicated that the model could predict reasonably well except in weeks when the fruit flies catches was found to be very low or very high. By using the optimized model developed in the present study, it could be possible to workout fruit flies incidence with minimum and maximum temperature. This method is highly useful for estimating the fruit flies incidence and saves precious time by avoiding field observations. However, further studies are needed to standardize the variables for improving the precision of fruit flies incidence estimates.

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Efficacy of certain new acaricides against two spotted spider mite, *Tetranychus urticae* Koch. on ridge gourd

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ABSTRACT : Experiments were conducted in farmers field in Kadapa district, Andhra Pradesh during summer seasons of 2011 and 2012 using ridge gourd variety "Ranjit" to test the bio-efficacy of new acaricides (Fenazaquin, Spiromesifen, Hexythaizox and Fenpyroximate) against two spotted spider mite, *Tetranychus urticae* Koch. The results revealed all the new acaricides tested were effective against two spotted spider mite and recorded 55.55-99.66% mortality up to 14 days after spray as compared to standard acaricide, dicofol during I season (2011) and 66.50-100 % mortality in II season (2012). Fenazaquin and spiromesifen offered superior control over all other acaricides.

Keywords : Fenazaquin, Ridge gourd, Spiromesifen, *Tetranychus urticae*

INTRODUCTION

The two spotted spider mite, *Tetranychus urticae,* a highly polyphagous species it has assumed the status of a major pest on almost all vegetable crops in recent years (Srinivasa *et al.*, 2003). Among the various insect pests *viz.*, beetles, leaf miner and fruit flies infesting cucurbits, the crop is regularly attacked by two spotted spider mite, *Tetranychus urticae* in farmer's field. In spite of relatively small size (100-400µm), the mites can cause considerable loss crop yield and quality losses, because they have short life span and under favorable conditions their populations quickly reach high abundance. Dicofol and sulphur are the common acaricides used for control of mites in major crops but sulphur is not recommended in cucurbits due to phytotoxicity, and dicofol, a conventional acaricide used by farmers requires frequent spraying to control the mites. Therefore there is a need for study on application of new acaricides with novel biochemical modes of action. Keeping in view the new acaricides viz., spiromesifen, fenpyroximate, hexythiazox and fenazaquin were evaluated against two spotted spider mite, *T. urticae* under field conditions.

MATERIALS AND METHODS

Two field trials were conducted to evaluate new acaricides against two spotted spider mite, *T. urticae* for two consecutive seasons during January to March, 2011 and February to March, 2012 in a farmers field on ridge gourd crop cv. Ranjit at Gangarajupuram Village, Kadapa district, Andhra Pradesh. The trials were laid out in a RBD with six treatments *viz.*, spiromesifen @ 96g a.i./ha, dicofol@ 250g a.i./ha, hexythiazox @25g a.i./ha, fenazaquin @100g a.i./ha, propargite@ 612g a.i./ha, fenpyroximate @ 30g a.i./ha and an untreated control replicated thrice. The plot size was $20m^2$ and the crop was grown with recommended package of practices. Two sprays of acaricides were given at 15 days interval starting with the initiation of mite infestation by using knapsack sprayer at 200 L of spray solution per acre. Three matured leaves from each tagged plants (5 plants/ replication) in treated plot and control were collected in a petriplate containing wet cotton to avoid moisture loss. The petriplates were brought to laboratory to count the mites population under stereomicroscope and observations were recorded one day before spraying and at 1, 3, 5, 7, 10 and 14 days of each spraying. The data was used for calculation of mite mortality and the per cent mortality of various mite stages were converted to arcsine values before subjecting them to ANOVA using AGRES STAT package.

RESULTS AND DISCUSSION

Season I: The results indicated that all the new acaricides tested were effective against the spider mite and recorded 55.55-97.66 percent mortality up to 14DAS (days after spray) as compared to standard acaricide, dicofol (34.33-65.54%) (Table 1). Three days after the first spray, all the treatments except dicofol (51.47% mortality over control) have recorded significant mortality of over 75 per cent and highest mortality of *T. urticae* was recorded in fenazaquin treatment (100%) followed by spiromesifen (93.22%), fenpyroximate (92.27%), (hexythiazox 89.74%) and propargite (78.31%). In confirmation of our results, Bhardwaj and Sharma (2010) found that out of seven acaricides evaluated against two spotted spider mite *T. urticae*, fenazaquin (0.001%), hexythiazox (0.0025%) abamectin (0.01%) and propargite (0.05%) provided excellent control in apple. Similarly at

Table 1. Effect of acaricides on *Tetranychus urticae* on ridge gourd (season I)

Treatment	Dose (g a.i./ha)	Percent mortality of mite population after treatment					
		1day	3days	5days	7days	10days	14days
Spiromesifen 22.9SC	96.0	95.51 (77.81)^{d}	93.22 (74.93)^{d}	93.64 (75.47)^{d}	96.09 (80.05)^{d}	97.68 (81.31)^{d}	95.86 (78.75)^{e}
Dicofol 18.5EC	250.0	65.54 (54.05)^{a}	51.47 (45.84)^{a}	54.53 (47.60)^{a}	43.92 (41.50)^{a}	54.42 (47.54)^{a}	34.33 (35.87)^{a}
Propargite 57EC	612.0	81.28 (64.40)^{b}	78.31 (62.27)^{b}	63.59 (52.89)^{b}	61.02 (51.37)^{b}	56.19 (48.56)^{a}	55.55 (48.19)^{b}
Fenpyroximate 5EC	30.0	86.56 (68.59)^{c}	92.27 (73.88)^{d}	90.89 (72.43)^{c}	84.48 (66.80)^{c}	88.28 (70.02)^{c}	82.78 (65.52)^{d}
Hexythiazox 5.45EC	25.0	83.39 (65.98)^{bc}	89.74 (71.40)^{c}	91.27 (72.86)^{c}	88.28 (69.99)^{c}	72.32 (58.26)^{b}	70.55 (57.14)^{c}
Fenazaquin 10EC	100.0	100.00 (88.83)^{e}	100.00 (88.83)^{e}	100.00 (88.83)^{e}	99.63 (90.20)^{e}	100.00 (88.83)^{e}	97.66 (84.39)^{f}
Control	-	0.00 (0.00)^{f}	0.00 (0.00)^{f}	0.00 (0.00)^{f}	0.00 (0.00)^{f}	0.00 (0.00)^{f}	0.00 (0.00)^{g}
CD (p= 0.05)		3.33	2.11	2.20	8.13	1.77	5.01
SE(m)±		1.49	0.95	0.98	3.65	0.79	2.25

Data based on mean of two sprays and three replicates each. Figures in parentheses are arcsine transformed values. Means followed by a common letter do not differ Pd”0.05 by Duncan’s Multiple range test

Table 2. Effect of acaricides on *Tetranychus urticae* on ridge gourd (season II)

Treatment	Dose (g a.i./ha)	Percent mortality of mite population after treatment					
		1day	3days	5days	7days	10days	14days
Spiromesifen 22.9SC	96.0	95.00 (77.62)[d]	97.05 (80.84)[d]	97.68 (81.48)[e]	93.45 (75.30)[d]	92.10 (73.68)[d]	89.34 (70.97)[e]
Dicofol18.5EC	250.0	57.60 (49.37)[a]	59.80 (50.66)[a]	48.60 (44.19)[a]	30.30 (33.38)[a]	28.60 (32.32)[a]	22.94 (28.61)[a]
Propargite 57EC	612.0	84.10 (66.50)[c]	73.50 (59.02)[b]	62.70 (52.36)[b]	55.40 (48.10)[b]	44.30 (41.72)[b]	36.32 (37.05)[b]
Fenpyroximate 5EC	30.0	87.56 (69.35)[c]	89.89 (71.49)[c]	91.66 (73.24)[d]	87.50 (69.30)[c]	75.00 (60.01)[c]	69.87 (56.72)[d]
Hexythiazox 5.45EC	25.0	69.55 (56.52)[b]	86.21 (68.22)[c]	85.76 (67.86)[c]	86.64 (68.65)[c]	72.60 (58.45)[c]	66.50 (54.64)[c]
Fenazaquin 10EC	100.0	97.33 (80.75)[d]	99.25 (85.45)[e]	100.00 (83.83)[f]	100.00 (89.83)[e]	97.68 (81.70)[e]	98.10 (82.18)[f]
Control	-	0.00 (0.00)[e]	0.00 (0.00)[f]	0.00 (0.00)[g]	0.00 (0.00)[f]	0.00 (0.00)[f]	0.00 (0.00)[g]
CD(p = 0.05)		4.34	4.11	2.40	2.43	2.34	1.96
SE(m)±		1.94	1.84	1.08	1.09	1.05	0.88

Data based on mean of two sprays and three replicates each. Figures in parentheses are arcsine transformed values. Means followed by a common letter do not differ Pd”0.05 by Duncan’s Multiple range test

5 days after spraying fenazaquin followed by spiromesifen (93.64%) showed higher efficacy by recording cent percent mortality over control whereas propargite recorded only 63.59%. Though the percent mortality varied in fenpyroximate and hexythiazox on 5th and 7th days after spray, they were statistically at par with each other (Table 1). On 7th and 10th after spraying, fenazaquin recorded mortality of 99.63-100 percent which was statistically significant over other treatments but on 14th day, though the percent mortality decreased (97.66%), but it was superior over other acaricides. Similarly Senapati *et al.*, (2010) reported that fenazaquin gave best control of yellow mite up to 14days in chilli. The standard acaricide, dicofol though initially recorded higher mortality on 1st (65.54%) and 3rd day (51.47%) but gradually the efficacy declined and reached 34.33 percent mortality on 14th day observation compared to fenpyroximate (82.78%), hexythiazox (70.55%) and propargite (55.55%). The novel acaricide, spiromesifen a tetronic acid derivative with new biochemical mode of action caused more than 90% mortality throughout the observation period.

Season II: In the second season also all the new acaricides were highly effective against all motile stages of *T. urticae* and recorded 66.50 to 100 per cent mortality over control up to 14 DAS (days after spray) (Table. 2) as compared to 22.94 - 57.60% and 36.32 to 84.10% in dicofol and propargite, respectively. A day after spray, highest mortality was recorded in fenzaquin followed by spiromesifen but both were statistically at par with each other whereas 3 days after spray the former acaricide showed significant highest mortality of 99.25% over latter acaricide with 97.05 percent. A field trail conducted by Misra (2011) during 2008-09 and 2009-10, against *T. urticae* in tomato recorded that fenzaquin at 125 and 150g ai/ha registered significantly highest population reduction (90.27-92.13%) followed by dicofol. Hexythiazox recorded lower mortality of 69.55% on first day after spray as compared to fenpyroximate (87.56%), whereas on 3rd, 7th and 10th day after spray, though the percent mortality varied between these two acaricides, they were statistically at par with each other and both these gave significant mortality over propargite and dicofol (Table 2). In support of our findings, Fenpyroximate 5%EC@ 500-625 ml/ha found to be more effective in controlling tomato and bottle gourd mite incidence compared to dicofol 18.5%EC@ 1375ml/ha as reported by Murthy and Shashi Bhushan (2010). In II season too, spiromesifen gave mortality of more than 90 % mortality upto 14 days after spray compared to all other acaricides except fenazaquin.

Five days after spray 100 per cent mortality of mites was recorded with fenazaquin treatment and continued up to 7 days after spray there after it declined and reached 98.00 percent mortality on 14th day after spray compared to spiromesifen with only 89.34% mortality. Similarly the mite mortality was <40% in dicofol and propargite treatments whereas it was 66.50 and 69.87% in fenpyroximate and hexythiazox, respectively on 14th day after spray. Based on the two seasons' data, the present study revealed that among various acaricides tested, fenazaquin and spiromesifen offered superior control over other acaricides. The rotation of all these new acaricides each having new mode of action can be used in the field for prolonged efficacy and also to avoid resistance development in the long term against *T. urticae* infesting ridge gourd.

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Management of nematode induced disease complex in seedlings of cauliflower (*Brsassica oleraceae* var. *botrytis*) using bio-pesticides

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ABSTRACT : Experiments were conducted to produce bio-agent colonized seedlings using effective formulations of bio-agents *viz., Trichoderma viride* Pers., 1794 (CFU $2x10^6$ /g), *Pseudomonas fluorescence* Migula., 1895 (CFU 2×10^8/g) and *Purpureocillium lilacinum* Luangsa., 2011 (*Paecilomycis lilacinus*) (CFU 2×10^6/g). Observations were made on evaluation of the compatibility of these bio-agents and their bio-efficacy against root-knot nematode (*Meloidogyne incognita* Chitw.) and Fusarium wilt (*Fusarium oxysporum* f. sp. conglutinans) on seedlings of cauliflower (*Brsassica oleraceae* var. *botrytis*). Significant increase in plant growth and reduction in disease incidence (Fusarium wilt) were observed. Formulations of bio-pesticides were used in combinations for production of organic seedlings of cauliflower. Treatments with combination of formulation of 3 bio-pesticides proved to be effective promoting plant growth with reduction in disease incidence of Fusarium wilt in seedlings satge. Seedlings were transplanted to pots under controlled conditions showed vigorous growth and were able to manage the disease complex caused by *Meloidogyne incognita* and *Fusarium oxysporum*. Results proved that these bio-agents were effective in sustainable management of disease complex in cauliflower.

Keywords: Bio-management, Cauliflower, *Meloidogyne incognita*, *Fusarium oxysporum* f. sp. conglutinans, nematode.

INTRODUCTION

Infection of root knot nematodes in various crops is more severe than any other pathogens since it mainly attacks root system of plants (Sikora and Fernandez 2005). The nematode infestation on plants leads to the secondary infection of other soil borne pathogens Many times nematodes attack from the establish of other pathogens in the root system leading to the manifestation of disease complex caused by nematode, fungal / bacterial pathogens (Taylor, 1990).

Certain bacterial and fungal bio-control agents are being used to control a wide range of plant parasitic nematodes (Spiegel and Chet, 1998; Kerry, 2000; Hallamann *et al.*, 2001; Meyer *et al.*, 2001). Plant Growth Promoting Rhizobacteria (PGPR) is a heterogeneous group of bacteria that can be found in the rhizosphere, on the root surface and in association with roots. PGPR- *P. fluorescence* was found to Induced Systemic Resistance in plants and this phenomenon was responsible for management of root-knot nematodes and nematode induced disease complex in various horticultural crops (Rao *et al.*, 2002a.). *Pseudomonas fluorescence* was found effective against root-knot nematodes (Santhi and Sivakumar, 1995; Perveen *et al.*, 1998; Siddiqui *et al.*, 1999; Rao *et al.*, 2002a, b) and soil borne pathogens (Rao, M. S., 2002b, 2007). *Trichoderma viride* is a bio-control agent effective against several fungal pathogens (Kapoor Shashi *et al.*, 2010). *Trichoderma viride* also proved to be effective against Meloidogyne spp. It showed a significant reduction in egg mass number (Abd Al-Fattah A. *et al.*, 2007).

Various researchers reported the fungus *P. lilacinum* as a potential biological control agent of Meloidogyne spp (Samson, 1975; Jatala, *et al.*, 1982, 1986; Adiko, 1984; Cardona and Leguizamon, 1997 and Khan and Williams, 1998). In these investigations, efforts were made to evaluate the effect of combination of three bioagents *viz., T. viride, P. fluorescence* and *P. lilacinum*, in the management of nematode induced disease complex of cauliflower which is caused by *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. conglutinans. As the single bio-agent has not proved effective in the management of nematode induced disease complex, it was thought to evaluate the combination of nematophagus fungus *P. lilacinum* with other bio-agents. *viz. T. viride* & *P. fluorescence*. As such *T. viride* alone or in combination

with *P. lilacinum* could not prove very effective in the management of nematode induced disease complex in cauliflower. So in our earlier studies additionally *P. fluorescence* which was found effective in the management of nematode induced disease complex was included in the consortia for the use in the experiments.

MATERIALS AND METHODS

Culture conditions and maintenance of bio-control agents

Trichoderma viride (ITCC No. 6889), *P. fluorescence* (ITCC No. B0034) and *P. lilacinum* (ITCC No. 6887) were used in the experiments. These cultures were maintained on following media *viz.,* Nutrient agar for Bacterial culture and Potato dextrose agar (PDA) for fungi by cryopreservation method as described by Sudheer, 2010 and isolates were sub-cultured and used for current study.

Sub-culture of test inoculants

Freshly sub-cultured *P. fluorescens* were inoculated in 250 ml in Erlenmeyer flask with 100 ml Kings'B broth (King, 1954) and incubated at room temperature for overnight at 37±2°C on rotating shaker incubator at 150 RPM and used for the experiments. Fresh cultures of *T. viride* and *P. lilacinum* were prepared by placing 5 mm culture disc made by using a cork borer. The discs were inoculated in plates solidified with PDA and incubated for 5 days at 27 ± 2 °C to obtain fresh cultures.

Compatibility of *T. viride, P. lilacinum* and *P. fluorescens in vitro*

A direct confrontation assay was conducted to evaluate the compatability of *T. viride P. fluorescens,* and *P. lilacinum, in vitro*. Five mm diameter circular culture discs of *T. viride* and *P. lilacinum* were punched in the periphery of the 5 day old fungal growth in the petri dishes. Petri dishes (90 mm) plated with PDA solidified plates were used in the experiments. All petridishes were inoculated with *P. fluorescens* initially and after 6 hrs *T. viride* and *P. lilacinum* were inoculated 20 mm from the periphery of the petridish. Observations were recorded on the diameter of each fungal culture growth for 7 days. The diameter of both the fungal colonies in each Petri dish was recorded by taking the average of two measurements at right angles per plate. Percent compatibility was calculated from the measurements recorded on 7th day.

Pseudomonas fluorescens strain was streaked (zigzag) 25 mm from periphery and allowed to grow for 48 h in the centre of a Petri dish containing PDA. Five mm diameter culture discs from an actively growing colony of *T.viride* and *P. lilacinum* were taken from PDA plates and then placed in the dish 25 mm from both the periphery on the either side of *P. fluorescens* and the plates were sealed with parafilm. Five replicates (5 plates) were maintained for each treatment. The plates were incubated in BOD for 7 days at 27 ± 2 °C. Observations were recorded after every 24 h on the growth of *T.viride* and *P. lilacinum* in the presence of *P. fluorescens* strain.

Calculation of percent compatibility of *T. viride, P. lilacinum* and *P. fluorescens in vitro*:

Percent compatibility was calculated by using the formula: Percent compatibility = 100 - Percent 'I'

Where 'I' is % Increase in diameter of colony.

Whereas the percent increase in the colony diameter was calculated using the formula:

$$\text{Percent increase} = \frac{\text{Colony diameter in the treated - Colony diameter in control}}{\text{Colony diameter in control}} \times 100$$

Evaluation of bio-efficacy in combination of *T. viride, P. lilacinum* and *P. fluorescens in-vivo*

Seedlings of cauliflower were produced under glass house conditions for evaluation of the compatibility of *T. viride, P. lilacinum* and *P. fluorescens* in terms of root colonization by these bio-agents. The compatibility protocol as standardized by Sangeetha *et al.,* 1993 was followed. Talc based formulation of *T. viride, P. lilacinum* and *P. fluorescens* were produced by using standard protocols (Vidhyasekaran and Muthamilan, 1995 and Vidhyasekaran *et al.,* 1997).

Twenty grams of talc formulation of each *T. viride* (2×10^6 CFU/g), *P. lilacinum* (2×10^6 CFU/g) and *P. fluorescens* (2×10^8 CFU/g) were used to treat the 1 kg cauliflower seeds. one kg substrate (sterile coco peat) mixed with 5 g of respective bio-pesticides were used to fill each seedling pro-tray for sowing seeds of cauliflower (*Brassica oleraceae* var. *botrytis*).

T1- Seeds & substrate treated with *T. viride* (TV+SB+SD)

T2- Seeds & substrate treated with *P. fluorescens* (PF+SB+SD)

T3- Seeds & substrate treated with *P. lilacinum* (PL+SB+SD)

T4- Seeds & substrate treated with *T. viride* and *P. lilacinum* (TV+PL+SB+SD)

T5- Seeds & substrate treated with *T. viride* and *P. fluorescens* (TV+PF+SB+SD)

T6- Seeds & substrate treated with *P. fluorescens* and *P. lilacinum* (PF +PL +SB+SD)

T7-Seeds & substrate treated with *T. viride, P. fluorescens* and *P. lilacinum* (TV+PF +PL +SB+SD)

T8- Chemical treatment

T9 - Control

All the experimental trays were kept in glass house at a temperature of 30-35°C. Each treatment was replicated five times in a completely randomized block design. The same experiment was repeated twice.

Estimation of plant growth parameters

Thirty day old cauliflower seedlings (5numbers) were randomly uprooted from each seedling tray (from all the replicates) and observations on plant growth parameters were made as described Bridge and page 1980. The plants root length and weight (from base of the stem to the bottom of the roots) and fresh shoot length and weight (from base of stem to the tip of leafs) were recorded.

Estimation of root colonization of bio-agents

Observations were also made on rhizospheric competency of the bio-agents by recording the Colony Forming Units (CFUs) of each organism from the root sample. Thirty day old cauliflower seedlings (5 numbers) were colleted from randomely block design and washed under running distilled water. One gram of root samples were collected and grounded in the mortar & pestle. The CFU was checked by the serial dilution followed by pour plate method. Dilutions up to 10^{-6} were prepared and 1 ml from each of 10^{-4}, 10^{-5} and 10^{-6} dilutions were poured into the petri dishes and spread completely in the plate (Tarun Kumar *et al.,* 2005). Freshly prepared PDA for fungal cultures or Kings B (King *et al.,* 1954) agar media for bacterial cultures was poured into each plate and by pour plate method and allowed for solidification (Mitchell *et al*. 1987). For each dilution, 3 replicates (3 respective media plates) were maintained with controls and CFU was recorded after 24 and 96 hrs for *T. viride, P. lilacinum* and *P. fluorescens* respectively (Table 2).

Inoculation of *Meloidogyne incognita*

The eggmasses were collected from cauliflower culture pots. These egg masses were kept for hatching as following Baermann's funnel (Petri dish) modified method (Schindler,1961). The nematode suspension collected in the above manner was used in the experiments and inoculated to the 30 days old cauliflower seedlings. After 30 days of inoculation these seedlings were transplanted from protrays to pots which contained 1kg sterile soil.

Inoculation of pathogenic fungus inoculum

Fusarium oxysporum f. sp. *conglutinans* was isolated from fusarium infected cauliflower plants. Inoculum of this fungus was prepared by culturing the isolate in Potato Dextrose Broth (PDB) amended with streptomycin sulphate for 7 days at 25 ± 27 °C. Mycelium was collected on strile blotting paper, and excess water and nutrients were removed by pressing between 2 folds of sterile blotting paper. Five ml of 3.2 x10^6 CFU / g fungal suspension was inoculated in the rhizosphere of 45 days old cauliflower seedlings after nematode inoculation. After 45 days the inoculated seedlings were transplanted to pots which is contains 1kg sterile soil.

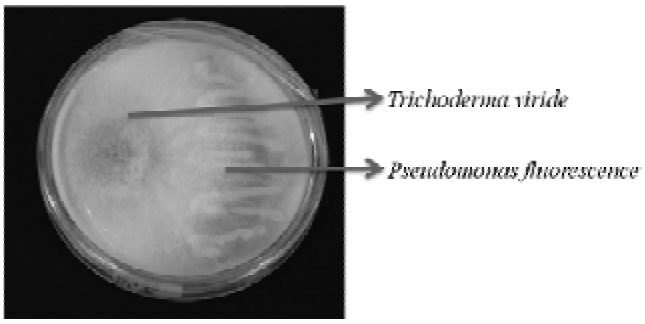
RESULTS AND DISCUSSION

The results of direct confrontation assay to evaluate the compatabiltity of *T. viride, P. fluorescens* and *P. lilacinum* indicated a higher percentage of compatability (78.24%) (Table 1 and Fig 1,2). The method which is necessary to check the compatibility of bioagents *in vitro* for combined use were standardized for application of two or more bio-agents in combination for the effective management of disease complex of crops (Rao *et al.,* 1997; 1998a; Nagesh and Parvatha Reddy, 2000; Anusuya and Vadivelu, 2002; Manoj Kumar *et al*., 2010). The results were analyzed for compatibility test *in vivo*, treatment with combination of three bio-agents T7-

Table 1. Compatibility of strains of the *Trichodserma viride* (Tv) and *Pseudomonas fluorescens* (Pf) and *Paecilomycis lilacinum in vitro*, in dual culture method

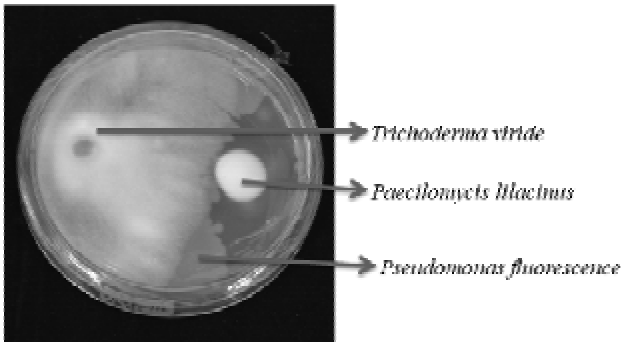
Treatment (Strain combination)	Average compatibility (%)
T1.TV+ PF	73.25[a]
T2.TV+ PL	76.42[c]
T3.PL+ PF	75.51[b]
T4. TV+ PF+PL	78.24[d]
CD 1%	4.54

Mean followed by the same letter in each column are not significantly different (P < 0.01) according to Duncan's multiple range test.



TV-*Trichoderma viride*; PF-*Pseudomonas fluorescence*

Fig. 1 : Compatibility of TV+PL



TV-*Trichoderma viride*; PF-*Pseudomonas fluorescence*; PL-*Paecilomycis lilacinum*

Fig. 2 : Compatibility of TV+PF+PL

TV+PF+PL+SB+SD had a significant effect on plant growth and reduction in fusarium disease incidence *in vivo* (Table 3,4). Treatments with combination of 3 bio-agents recorded as higher plant growth parameters than other treatments (Table 3).

Lesser disease incidence was observed in treatment T7-TV+PF+PL+SB+SD followed by T5-TV+PL+SB+SD. In case of root galling index in cauliflower was significantly lesser in treatment T7-TV+PF+PL+SB+SD 1.96 (Table 4). Significantly lesser disease incidence (10.90%) of *F. oxysporum* in cauliflower with the combination of 3 bio-agents than control which was recorded highest as 49.28 percent (Table 4). Observations were also made on percentage reduction of disease incidence in cauliflower. Treatments with bio-agents (single & in combination) showed significant reduction in disease incidence when compared with control. The highest reduction (57.96%) was observed in case of T7-TV+PF+PL+SB+SD inoculated with *M. incognita* (Table 4). Seed and substrate treatment using the combination of *T. viride*, *P. lilacinus* and *P. flourescence* enriched by mixing of neem cake was more effective than treatments with individual bio-agents in reducing *M. incognita* population and disease incidence caused by *F. oxysporum*. *Paecilomyces lilacinus* colonizes the root surface and parasitizes eggs, egg-masses, juveniles and females of *Meloidogyne* spp. by direct hyphal penetration (Jatala *et al.*,1979; Mohd *et al.*, 2009; Prakob *et al.*, 2009; Mucksood and Tabriez, 2010; Sowmya *et al.*, 2012). Siddiqui and Shaukat (2004) reported the synergistic effect of strains used in the combination

Table 2. Effect of bio-agents on root colonization

Treatment	CFU of *T. viride* ($\times 10^5$/1 g root)	CFU of *P. lilacinum* ($\times 10^5$/1 g root)	CFU of *P. fluorescens* ($\times 10^6$/1 g root)
T1-TV+SB+SD	1.73^{c}	1.73^{b}	0^{a}
T2-PF+SB+SD	0.00^{a}	0.00^{a}	1.82^{c}
T3-PL+SB+SD	0.00^{a}	0.00^{a}	0.00^{a}
T4-TV+PF+SB+SD	1.65^{b}	0.00^{a}	1.72^{b}
T5-TV+PL+SB+SD	1.82^{d}	1.40^{b}	0.00^{a}
T6-PF+PL+SB+SD	0^{a}	0.35^{a}	1.84^{c}
T7-TV+PF+PL+SB+SD	2.21^{e}	1.37^{b}	2.06^{d}
T8-Control	0.00^{d}	1.61^{b}	0.00^{a}
CD-5%	0.058	0.061	0.063

Mean followed by the same letter in each column are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

Table 3. Effect of different treatments of biopesticides on cauliflower plant growth

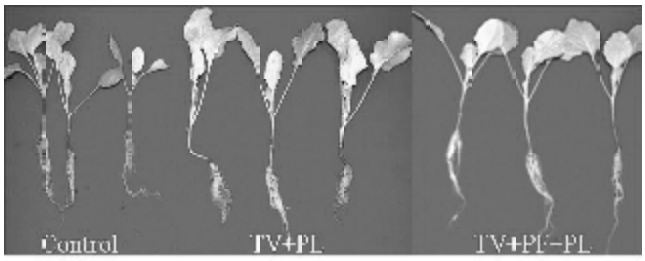
Treatment	Shoot length(cm)	Root length(cm)	Shoot weight (g)	Root weight (g)
T1-TV+SB+SD	15.634[d]	9.416[cd]	1.41[c]	0.728[bc]
T2-PF+SB+SD	15.22[c]	9.366[c]	1.32[ab]	0.704[ab]
T3-PL+SB+SD	16.484[e]	9.622[de]	1.47[d]	0.814[d]
T4-TV+PF+SB+SD	18.418[f]	9.53[cd]	1.52[d]	0.876[ef]
T5-TV+PL+SB+SD	22.51[g]	10.924f	1.628[e]	0.906[f]
T6-PF+PL+SB+SD	18.686[f]	9.732[e]	1.578[e]	0.84[de]
T7-TV+PF+PL+SB+SD	25.92[h]	12.706[g]	1.958[f]	0.956[g]
T8-Carbofuran	13.92[b]	8.764[b]	1.374[bc]	0.752[c]
T9-Control	10.458[a]	8.222[a]	1.302[a]	0.664[a]
CD-5%	0.31	0.28	0.07	0.05

TV= *T. viride*; PF= *P. fluorescens*; PL= *P. lilacinum*; SB = Substrate treatment; SD = Seed treatment. All the figures are the mean average values of five replicates of pooled data. Mean followed by the same letter in each column are not significantly different (P < 0.05) according to Duncan's multiple range test.

Table 4. Effect of various treatments of bio-pesticides on disease complex caused by *M. incognita* and *F. oxysporum* in cauliflower

Treatment	Nematode	Fusarium	*M. incognita* and *Fusarium oxysporum.f. sp.*, conglutinans			
	Fusarium alone Root knot-nematode (*M. incognita*) index on 1-10 scale	alone Percent disease incidence (*F.oxy-sporum*.)	Root knot-nematode (*M.incog-nita*) index on 1-10 scale	Reduction in infestation by *M. incognita* (%)	% disease incidence (*F. oxysporum)* (%)	Reduction in disease incidence caused by *F. oxysporum* (%)
TV+SB+SD	2.5[bc]	13.08[e]	2.62[bc]	44.38 (41.78)	14.25[e]	73.04 (58.69)
PF+SB+SD	2.63[c]	13.21[e]	2.65[cd]	43.74 (41.38)	14.28[e]	72.98 (58.63)
PL+SB+SD	2.62[c]	13.53[f]	2.68[cd]	43.10 (41.03)	14.61[f]	72.36 (58.24)
TV+PF+SB+SD	2.51[b]	12.36[c]	2.56[b]	45.65 (42.48)	13.47[c]	74.51 (59.67)
TV+PL+SB+SD	2.65[c]	11.89[b]	2.69[d]	42.89 (40.86)	13.09[b]	75.23 (60.13)
PF+PL+SB+SD	2.53[b]	12.75[d]	2.57[b]	45.44 (42.36)	13.81[d]	73.87 (59.21)
TV+PF+PL+SB+SD	1.96[a]	10.9[a]	1.98[a]	57.96 (49.54)	12.74[a]	75.89 (60.53)
Carbofuran	3.64[e]	14.37[g]	3.72[f]	21.01 (27.28)	16.26[g]	50.32 (45.17)
Control	4.68[f]	49.28[h]	3.72[g]	0.00	52.86[i]	0.00
CD-5%	0.09	0.43	0.088	1.83	0.96	1.83

Mean followed by the same letter in each column are not significantly different (P d" 0.05) according to Duncan's multiple range test. The values in paranthesis are log 10 transformed.



TV-*Trichoderma viride*; PF-*Pseudomonas fluorescence*; PL-*Paecilomycis lilacinum*

N+F-Nematode and Fusarium; untreated-without bioagents treatment; treated- with bioagents treated; without N+F-wothout inoculation of Nematode and Fusarium.

Fig. 3 : Effect of bioagents on Plant growth parameters

treatment where the presence of *Trichoderma sp.* enchanced the biological control of nematodes by *P. fluorescence* when produced together then leading to greater reduction in nematode population.

Highest reduction (75.89%) was observed in treatment T7-TV+PF+PL+SB+SD in case of *F. oxysporum* (Table 4). The treatment of seeds and substrate with combination of formulation of *T. viride*, *P. fluorescence* and *P. lilacinum* was significantly effective than other treatments in controlling nematode induced wilt disease complex in seedlings of cauliflower. Seedlings being colonized by bio-agents play a key role in organic seedlings production as transplanted seedlings carry these bio-agents to the main field. Their efficacy was enhanced in the presence of neemcake. This botanical (neem cake) served to have synergistic effect on the growth of these bio-agents. So they are protected the seedlings from the soil borne pathogens.

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Effect of *Bacillus subtilis* on suppression of disease complex in bell pepper (*Capsicum annum* L.)

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ABSTRACT: Field experiments were conducted to evaluate the effect of *Bacillus subtilis* (Cohn) formulation, on the disease complex of bell pepper (*Capsicum annum* L.) caused by *Meloidogyne incognita* (Kofoid *et* White *et* Chitwood) with the bacterium *Ralstonia solanacearum*, under field conditions. A method for the management of this disease complex, was standardized by the use of *B. subtilis* 1% W.P. 20g for the seed (1 kg) treatment, 10 g for treating 1 kg of substrate (coco peat) or 50 g for treating 1 m^2 nursery bed and 2 kg for the enrichment of vermicompost (200 kg) which was applied at the rate of 50 g/m^2 as a substrate treatment before transplanting bell pepper seedlings. This method reduced significantly the population of *M. incognita* and the disease incidence of *R. solanacearum*. This bio-pesticide significantly increased plant growth parameters (shoot and root length) and also crop yield to the tune of 66%.

Keywords: *Bacillus subtilis*, bell pepper, *Meloidogyne incognita*, *Ralstonia solanacearum*, vermicompost

INTRODUCTION

Plant parasitic nematodes, such as *Meloidogyne* spp. cause extensive damage to crops every year (Casas and Herrera, 2007). The root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White *et* Chitwood) was found to significantly reduce the yields of bell pepper (*Capsicum annum* L.) (Di Vito *et al.*, 1985). The incidence of wilt disease caused by *Ralstonia solanacearum* Yabuuchi was found to be significantly higher in the presence of root-knot nematodes (Rao *et al.*, 2009). Excessive use of chemicals to control soil-borne pathogens is expensive and affecting soil bio-diversity adversely and making plants even more susceptible to pests and diseases (Bhanti and Taneja, 2007, Mancini *et al.*, 2008). Considering the negative impact of the synthetic chemicals commonly used for the management of nematodes and bacteria on the environment, it was thought to evaluate the effect of formulation of *Bacillus subtilis* 1% W.P. developed at Indian Institute of Horticultural Research, Bangalore, in the management of wilt disease complex in bell pepper (*Capsicum annuum* L.) under field conditions.

Bacillus subtilis (Cohn, 1872), is an adept rhizobacterium and has gained global attention as a bio-pesticide (Edgecomb and Manker, 2006) for control of several plant diseases. It is known to inhibit a number of soil borne pathogens (Asaka and Shoda, 1996; Edgecomb and Manker, 2006). The potential of this bio-control bacterium has been reported to be effective against plant pathogenic nematodes (Siddiqui and Mahmood, 1999; Siddiqui and Ehteshamul, 2001) and other soil borne pathogens (Asaka and Shoda, 1996; Edgecomb and Manker, 2006). However, there are no reports on the management of this disease complex on bell pepper using this bio-pesticide. Hence, we attempted to develop a suitable bio-management strategy for the disease complex in bell pepper by using talc formulation of *B. subtilis*.

MATERIALS AND METHODS

The local isolate of *B. subtilis* (IIHR - Bs-2 NAIMCC- B-01211), maintained in the collection of NAIMCC (National Agriculturally Important Microbial Culture Collection, Mau, Uttar Pradesh, India) was mass produced through liquid fermentation processes and prepared as a talc formulation with $2x10^8$ CFU/g. Initially the seeds of bell pepper (cv. Orobel) were treated with bio-agent at the rate of 20g/kg. They were sown in seedling trays containing substrate (cocopeat) treated with formulation at the rate of 10g/kg or in raised bed treated with 50g/m^2. 30 days old seedlings were uprooted to record entire seedling length and weight and assess root colonization by the *B. subtilis*. Two kg of *B. subtilis* was added to 200 kg of vermicompost and left under shade for 15 days by maintaining optimum moisture (25-28%). The bio-pesticide enriched vermicompost was added to the field plots at different doses. The experiment was conducted at the Indian Institute of Horticultural Research farm in a field infested with *M. incognita* and *R. solanacearum*. During the experimental periods in two

seasons there were rainy days with total rain received up to 63 mm (first season) and 76 mm (second season) and soil temperatures were in the range 25-37°C. Treated seedlings were transplanted into 1 m × 2 m plots. There were eight treatments (Tables 1-3).

Seedlings derived from seed, substrate or nursery bed treatment were transplanted in plots amended with enriched vermicompost at different dosages of 25g, 50g or 100g per m^2. Seedlings derived from non treated seeds or substrate and transplanted in plots not amended with vermi-compost served as controls. The treatments were T1 – Seed treatment with *B. subtilis* @ 20g/kg of seed (BS-S); T2 – T1+ Substrate treatment with *B. subtilis* at the rate of 10g/kg (BS-ST); T3 - T1 + nursery bed treatment with *B. subtilis* at the rate of 50g/m^2 (BS-SN); T4 – T2 + application of *B. subtilis* enriched vermicompost to plots at the rate of 25g/m^2 (BS-ST+VC-25); T5 – T2 + application of *B. subtilis* enriched vermicompost to plots at the rate of 50g/m^2 (BS-ST+VC-50); T6 – T2 + application of *B. subtilis* enriched vermicompost to plots at the rate of 100g/m^2(BS-ST+VC-100); T7 – T3 + application of *B. subtilis* enriched vermicompost to plots at the rate of 25g/m^2 (BS-SN+VC-25); T8 – T3 + application of *B. subtilis* enriched vermicompost to plots at the rate of 50g/m^2 (BS-SN+VC-50); T9 – T3 + application of *B. subtilis* enriched vermicompost to plots at the rate of 100g/m^2 (BS-SN+VC-100); T10- Application of carbofuran (3G) 5g/kg substrate or at the rate of 5g/m^2 nursery bed +Streptomycin; T11- Control (CHECK) (CH).

The crop was maintained by following regular agronomic practices. The experiment was carried out in two different seasons and the following observations were made. Seedlings (30-day-old) of bell pepper were transplanted in the plots with a spacing of 50 × 50 cm in 4 rows (2 plants in each row) and there were 10 replicates per treatment arranged in a randomized block design (RBD). The crop was maintained by applying recommended dosages of fertilizers and plant protection chemicals. Observations of the root-knot index on a 1-10 scale (Bridge and Page, 1980), nematode infestation, disease incidence, yield of bell pepper at harvest (90 days after transplanting), root colonization by *B. subtilis* were recorded.

Root colonization by *B. subtilis* was assessed by following the standard serial dilution technique. One gram root sub-samples were taken and washed gently to remove the soil. The dilutions were prepared up to 10^{-5} and 0.1 ml aliquots of the 10^{-4} and 10^{-5} dilutions were spread on Petri dishes containing nutrient agar medium and incubated at 27 ± 1 °C. The colonies were counted and calibrated to 10^{-6} CFU/ml. Soil samples were collected at random by taking 100g soil from five different spots in each plot and thoroughly mixed. Then the nematodes in a 100cm^3 subsample were extracted using Cobb's sieving and decanting technique combined with the modified Baermann funnel technique (Flegg, 1967).

Five plants per plot were uprooted at random and the nematode infestation of the roots was estimated from the rating chart of Bridge and Page (1980), using 25g of roots (five g from each of the five plants per plot collected at random). The roots were stained in boiling 0.1% acid fuchsin solution (acid fuchsin dissolved in a 1:1:1 mixture of glycerol, lactic acid and distilled water) for five minutes, comminuted in a homogenizer and the adult nematodes count was taken under a stereo-microscope. The reductions in the nematodes density due to the application of *B. subtilis* was calculated. Further, data on the mortality of the plants in the field, due to wilt disease complex, were recorded by counting the plants wilted in the field (during flowering, the number of plants completely wilted out of total number of plants was expressed as a percentage). The plant death due to wilt disease was confirmed by ooze test (Patrice *et al.*, 2009) and re-isolating the pathogen on 2,3,5 TTC medium (2,3,5 Triphenyl Tetrazolium Chloride medium) (Kelman 1954).

Based upon experimental design, a one-way analysis of variance (ANOVA) was performed using SPSS ver. 10.0. As a follow-up of ANOVA, the treatment means were separated using Fisher's least significant difference (LSD) and Duncan's multiple range tests. Data from repeated trials were pooled and presented.

RESULTS AND DISCUSSION

The seedlings were vigorous and there was a significant increase in the growth of those raised after seed and substrate treatments with the formulation of *B. subtilis* than in the treatments with seed or substrate treatment alone (Table 1). It was also observed that the plant weight was higher in case of seedlings grown in nursery beds compared to those in the seedling trays it was *vise versa* in case of plant height. There was no significant difference in colonisation between these seedlings roots by the bio-agents (Table 1) and, after transplanting, colonization persisted until harvest of the crop (Table 2).

Bell pepper seedlings obtained from *B. subtilis* treated seed and substrate both from nursery beds and

seedling trays were transplanted into the plots amended with vermicompost enriched with formulation. They showed significant reductions in galling index, disease incidence, and numbers of nematodes in roots and soil compared with individual treatments (Table 2). This treatment was also effective significantly in increasing yield of the crop and reducing mortality of the plants in the plots (Table 3). There are few reports on the bio-control of *B. subtilis* on root knot nematodes (Siddiqui and Mahmood, 1999; Siddiqui and Ehteshamul-Haque, 2001; Ali *et al*., 2002; Xia *et al*., 2011) and bacterial pathogens (Bais *et al*., 2004; Chen *et al*., 2013). Reduction in the root-knot nematode population by *B. subtilis* was responsible for the significant reduction in the mortality of the plants due to wilt disease complex (Table 3). No significant differences in nematode infestation, disease incidence and yield were observed between the plants transplanted from seedling trays and nursery beds (Table 1).

It is important to develop a suitable delivery system for the application of *B. subtilis* formulation in the field as huge quantities of formulation is required to treat the soil. Vermicompost is the most suitable organic material which could be enriched with this bio-agent for the application in the main field conditions as it can help in the overall plant growth (Lazcano *et al*., 2009) simultaneously enhancing the microbial growth in the rhizosphere (Norman and Edwards, 2005) which is a pre-requisite for the successful management of nematode and pathogenic bacteria on any crop. Effect of vermicompost containing humic substances at low concentrations has been explained by various theories, the most convincing of which hypothesizes a "direct" action on the plants, which is hormonal in nature, together with an "indirect action" on the metabolism of soil microorganisms (Norman and Edwards, 2005).

Our study revealed that vermicompost could be easily enriched with *B. subtilis*. Delivery of the bio-agents through vermicompost helps in the application of *B. subtilis* to the rhizosphere of bell pepper crop. This is the first report on multiplication of *B. subtilis* in vermicompost. Use of enriched vermicompost showed an increase in the root colonization of *B. subtilis* with the increased dose of application of bio-agent enriched vermicompost from 25g to 100g/m^2 (Table 1 and 2). Further, it was also found to reduce the root-knot nematode infestation (Table 3). The efficacy of the dosage 50g/m^2 and 100 g/m^2 of *B. subtilis* enriched vermicompost were on par with each other. They were effective in reducing root-knot index, nematode population in the soil as well as roots and also the colonization of bio-agent on roots. However, at these dosages the bio-agent enriched vermicompost was significantly effective in the management of the disease complex on bell pepper when compared to the lower dosage of application of 25g/m^2. Seed or substrate treatment alone did not prove significantly effective in the management of disease complex on bell pepper (Table 1 and 2). The seedlings grown on seedling trays and nursery beds were on par in terms of management of disease complex and yield. The experiment was repeated twice to confirm the results on the effect of application of *B. subtilis* enriched vermicompost on the management of *M. incognita* and *R. solanacearum* on bell pepper under the field conditions. The results of these field experiments clearly demonstrate the potential use of *B. subtilis* in management of the disease complex. This is the first report on the management of disease complex

Table 1. Effect of *B. subtilis* on the growth of the seedlings of bell pepper

Treatment	Seedling length (cm)	Seedlingweight (g)	Colonization of *B. subtilis* (CFU/g root)
BS – S	14.8^b	3.5^a	15642^b
BS – ST	15.4^b	3.9^a	17423^c
BS – SN	18.2^c	4.8^b	18349^d
CARB	10.8^a	2.6^b	0.0^a
CH	10.5^a	2.5^c	0.0^a
C.D. at 5%	0.61	0.52	0.57

S = Seed treatment; ST = Seed + Substrate treatment, SN = Seed + Nursery bed treatment
Means followed by the same letter in each column are not significantly different (P < 0.05) according to Duncan's multiple range test.

Table 2. Effect of vermicompost on the rhizospheric colonization of *B. subtilis* in bell pepper under field conditions.

Treatment	CFU of *B. subtilis*	
	CFU/g root	CFU/g soil
BS – S	14523[b]	10985[b]
BS – ST	15232[c]	11345[c]
BS – SN	16473[d]	11452[d]
BS-ST+VC-25	18995[f]	13458[f]
BS-ST+VC-50	19535[g]	14567[g]
BS-ST+VC-100	19842[j]	14953[i]
BS-SN+VC-25	18825[e]	13400[e]
BS-SN+VC-50	19642[h]	14642[h]
BS-SN+VC-100	19740[i]	14934[i]
CARB	0.0[a]	0.0[a]
CH	0.0[a]	0.0[a]
C.D. at 5%	6.6	3.4

S = Seed treatment; ST = Seed + Substrate treatment, SN = Seed + Nursery bed treatment, VC = Enriched vermicompost application

Means followed by the same letter in each column are not significantly different (P < 0.05) according to Duncan's multiple range test.

Table 3. Effect of *B. subtilis* on the population of *M. incognita* in roots and soil, root-knot index, mortality due wilt disease complex and the yield of bell pepper under field conditions.

Treatment	Nematode population		Root-knot index (1-10)	Mortality due wilt disease complex (%)	Bell pepper yield (kg/2 m^2)
	In 100 ccsoil	In 5 g root			
BS – S	90[d]	24[b]	3.7[bc]	23.6[c]	6.1[c]
BS – ST	80[c]	21[b]	3.5[b]	22.8[bc]	6.7[cd]
BS – SN	82[c]	23[b]	3.3[b]	22.6[b]	6.9[de]
BS-ST+VC-25	64[b]	19[b]	2.9[ab]	19.2[a]	7.4[def]
BS-ST+VC-50	59[ab]	13[a]	2.1[a]	18.6[a]	8.1[gh]
BS-ST+VC-100	56[a]	11[a]	1.9[a]	18.5[a]	8.3[gh]
BS-SN+VC-25	65[b]	19[b]	2.9[ab]	19.4[a]	7.6[gh]
BS-SN+VC-50	55[a]	12[a]	2.0[a]	18.5[a]	8.3[gh]
BS-SN+VC-100	57[a]	11[a]	2.0[a]	18.6[a]	8.2[gh]
CARB	92[d]	22[b]	4.5[c]	38.9[d]	5.3[bb]
CH	138[e]	35[c]	7.9[d]	43.7[e]	5.0[za]
C.D. at 5%	8.32	4.99	0.59	1.1	0.98

S = Seed treatment; ST = Seed + Substrate treatment, SN = Seed + Nursery bed treatment, VC = Enriched vermicompost application

Means followed by the same letter in each column are not significantly different (P < 0.05) according to Duncan's multiple range test.

caused by *M. incognita* and *R. solanacearum* on bell pepper using *B. subtilis*. It was also found to increase the yield to a tune of 66 per cent. Hence, the seed and substrate treatment with *B. subtilis* at the rate of 20g/kg of seed and 50g/m^2 respectively followed by field treatment with *B. subtilis* enriched vermicompost at the rate 50g/m^2 is very useful for the management of the nematode induced disease complex in bell pepper.

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Visual analysis of field data on papaya ring spot virus (PRSV) infection by high density multi-parametric charts using CIRCOS

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ABSTRACT: Multi parameters were collected from a papaya field infected with ring spot virus (PRSV). To visualise the individual contributions of each parameter, a technique of drawing circular charts with CIRCOS which is a program developed in PERL language was developed. The circular charts were designed to chart the data both in normalized as well as in non-normalized ways, thus the overall effects of each character was clearly differentiated. The circular format allowed the viewing of 12 parameters of each plant as defined by its row and column position in a single hi-density graph which provided a way to visualise all the measured parameters across the entire field as a single chart.

Keywords: CIRCOS, Papaya ring spot virus, Visual analysis

INTRODUCTION

Papaya Ring Spot (Jenson, 1949) causes a devastating epidemic in India (Tripathi *et al.*, 2008) and the plants in many cases do not produce any economic yield. We have attempted to visualize the collected data from a PRSV infect filed as a single multi-parametric graphic. Tables are natural containers for data. Whenever information is presented, chances are more that it would be communicated by means of a table. In many cases when this information became complex (and the table, therefore, became larger and more complicated) a tabular presentation was difficult to parse visually and so patterns in the tabulated data remained hidden. Morphometric data has been required to give a complete picture of the viral disease and its effects. The data so collected usually had been presented in separate tables. While tables are very good in organizing the data they are not so much effective in presentation of the underlying patterns nor in the interactions among data. Acombined data table was not comprehensible as numerical data do not permit the recognition of patterns. Individual charts were good to make the patterns visible but creation of a large number of charts has not helped in the identification of underlying patterns. Moreover changes in the magnitude of the data values many a times made it impossible to graph on a single scale. Circular graphs with each ray representing an individual plant provided a high density pattern which was comprehensible due to its visual characteristics, moreover pre-processing of data helped to normalize the data leading to charting of data of differing magnitudes in a single chart.

A software package, CIRCOS (http://www.circos.ca), was found to be ideal for visualizing data and for processing data into information as CIRCOS visualized data in a circular layout which made it ideal for exploring relationships between objects or their positions. CIRCOS has been ideal for creating publication quality info graphics and illustrations with a high data to ink ratio, richly layered data and pleasant symmetries. Fine control over each element in the figure to tailor its focus points and detail became possible. CIRCOS was noted to be flexible as images created with CIRCOS, could illustrate links, ribbons, tiles and a variety of 2D data tracks Although CIRCOS had been originally designed for visualizing genomic data, it could create figures from data in any field data that described relationships or multi-layered annotations of one or more scales. CIRCOS could be automated due to its control by plain text configuration files, This automation made it easily incorporate able into data acquisition, analysis and for reporting pipelines of a multi-step process in which data would need to be analysed by multiple and typically independent tools, each passing their output as the input to the next step (Martin *et al.*, 2009)

CIRCOS had been developed as a free software and it has been licensed under GPL. As CIRCOS has been designed utilizing PERL(http://www.perl.org/), a modern computational language it could be deployed on any operating system for which Perl has been made available (e.g. Windows, Mac OS X, Linux and other UNIX flavours) and consequently CIRCOS has been designed to produce bitmaps (PNG) and vector (SVG) images

using plain text configuration and input files which could be edited in a text processor. The circular form naturally supported a range of resolutions, since the circumference of a track would be proportional to its radial position. The ability for colouring the links using text input based on each of the elements, helped to follow relationships to/from an element visually. (http: // circos.ca / presentations / articles / vis_tables1 / # methods)

MATERIALS AND METHODS

Papaya seedlings (variety Red Lady) were raised in nursery and were transplanted to the field, they were grown following all recommended cultural practices. In this experiment the following data pertaining to a PRSV infected field was collected, 1. Plant Height (cm), 2. Stem Circumference (cm), 3. Leaf Number (No), 4. Leaf Area (cm^2), 5. Petiole Length (cm), 6. 1st harvest (No), 7. 2nd harvest (No), 8.Total fruits (No), 9. Total yield (Kg), 10.PRSV Incidence (%), 11.*Phytopthora* incidence (%), 12. Mealy bug Incidence (No).). The data was collected on the 360th day after planting (Table 1). Data was collected form a grid composing o 6 rows and 8 columns. Ways and means to present the data using a circular chart in CIRCOS were assessed.

(i) Using Installed CIRCOS for High Density Charting

The process of installation of CIRCOS have been given in detail in the Annexure. Data collected for each parameter which was present in a Row (1-6) and Column (1-8) format was combined into a single Excel spreadsheet and was saved as a text file. The values for Plant Height (cm), Stem Circumference (cm), Leaf Number (no), Leaf Area (cm^2), Petiole Length (cm), 1st harvest (no), 2nd harvest (no), total fruits (no), Total yield (Kg), PRSV Incidence (%) *Phytopthora* incidence (%) Mealy bug Incidence (%) were multiplied by the following factors to normalize the values 2.8,12,8,0.4,2,12,12,6,0.25,2,45. The data in the text sheet was used to draw the charts using the multi histogram of CIRCOS either with the Papaya Ring Spot Virus Incidence ribbons at the centre or without it. Legends were created using ColorPic software.

ii) Using the online CIRCOS table viewer http:// mkweb.bcgsc.ca/tableviewer/

Data which had been already collected for each parameter and which was present in a Row (1-6) and Column (1-8) format was converted to a matrix Row (X) Col format using an Excel spreadsheet (Table 2,3). The online table viewer used to draw individual ribbon charts (12 numbers)

RESULTS AND DISCUSSION

i) Results using Installed CIRCOS for High Density Charting

Using CIRCOS a combined chart of 12 characters which had been Normalised, with PRSV incidence at centre, with Column 8 in 12 'O' clock position, columns 7,6,5,4,3,2,1, Rows 1,2,3,4,5,6 in in clockwise direction was drawn. Each ray in the chart represented a single plant defined by its Row and Column coordinates. Similarly another chart without the Papaya Ring Spot Virus Incidence ribbons at the centre were also drawn (Fig. 1, 2). Another set of CIRCOS charts were drawn for the Non Normalized data (Fig.3,4). Normalization of the data yielded well-proportioned clear and legible chart elements which helped to understand the individual components made by each parameter. As the number of rows and columns were unequal it helped in quick

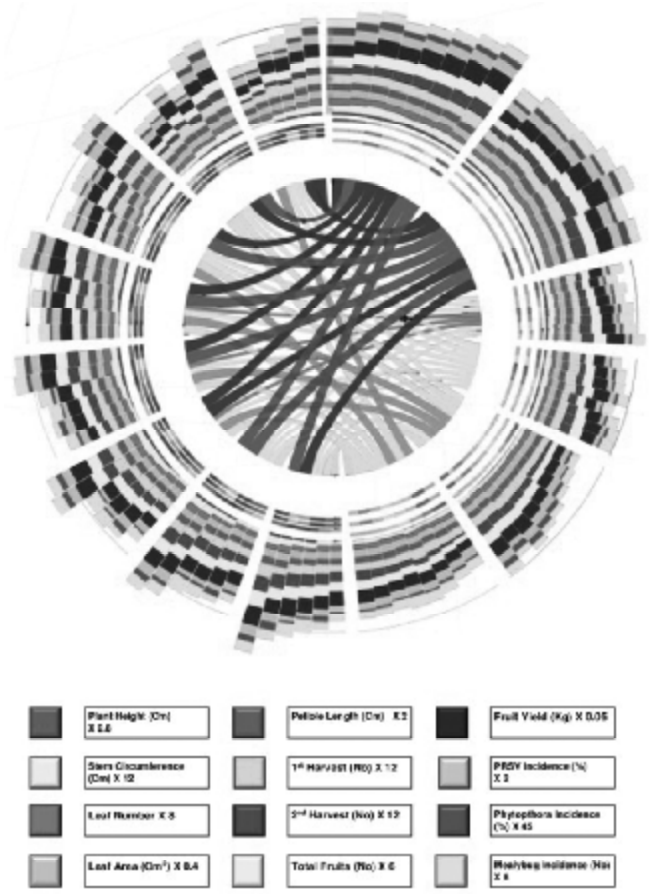

Fig. 1. Combined CIRCOS Graph of 12 Characters (Normalised, with PRSV incidence at center), Column 8 is in 12 'O' clock position, columns 7,6,5,4,3,2,1, Rows 1,2,3,4,5,6 are in clockwise direction. Each ray represents a single plant defined by the Row and Columns.

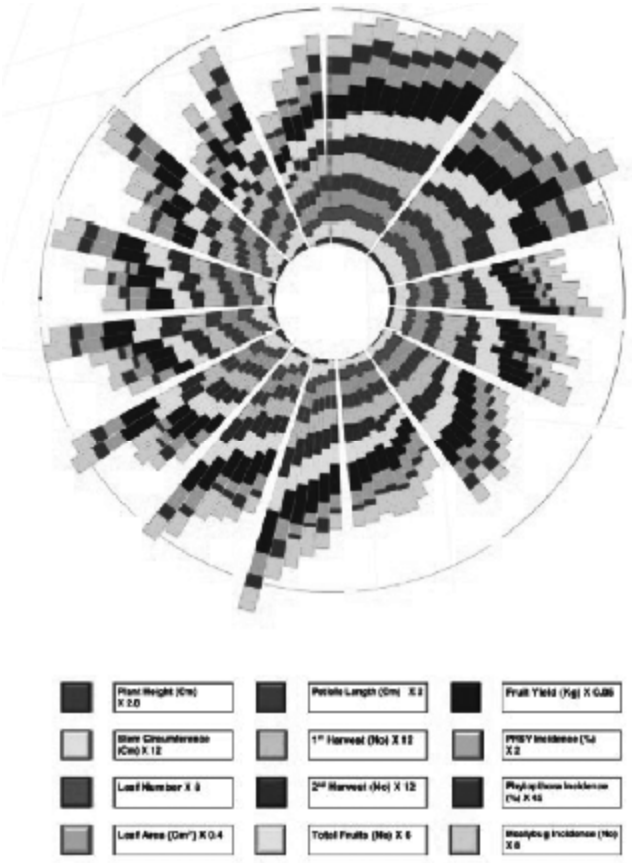

Fig. 2. Combined CIRCOS Graph of 12 Characters (Normalised), Column 8 is in 12 'O' clock position, columns 7,6,5,4,3,2,1, Rows 1,2,3,4,5,6 are in clockwise direction. Each ray represents a single plant defined by the Row and Columns

identification of the row and column Identities. While the ribbon graph of PRSV incidence in the centre was helpful to correlate PRSV incidence with the parameters, absence of this ribbon chart made the multi part histogram clearer due to an increase in the size of individual rays. Normalization provided an easy way to visualize in detail the contribution of a particular parameter and its variations. Normalization also provided a way to spotlight particular data.

ii) Results using the online CIRCOS table viewer http://mkweb.bcgsc.ca/tableviewer/

Using the online CIRCOS table viewer http:// mkweb.bcgsc.ca/tableviewer/ a total of twelve individual circular charts were made. As the online viewer does not support histogram functions they were not drawn. All the 12 parameters were divided into 40 colour bands at 2.5 percentiles, helped to visualize the entire field. Only one chart is described in detail (due to space constraints) . Figure 5 of Plant Height Data with Column 8 in 12 'O' clock position, columns 7,6,5,4,3,2,1, Rows 1,2,3,4,5,6 in clockwise direction with each ribbon representation a single plant defined by the Row and Columns is shown in Chart 5. The inherent colouring was as percentile groups grouped by 2.5% in similarity, enabling 40 different colours of ribbons with a false colour scheme of red corresponding to the lowest value and purple to the highest. (The same colour scheme was followed in Figures 5 -17). Plant height was seen to be minimum in the second row where 5 plants (3,4,5,6) were shorter than all others, using the circus Fig. 5,6, it could be easily visualized that the Plant height was minimum in the 2nd individual plant in the columns 7,6,5,4,3 of the field, indicating a probable common cause in this Row and Column affecting plant growth. Maximum height was seen in Row 4 Col 7 (4th plant).

Nowadays there is a real requirement for morphometric measurement and to analyse the parameters in field to identify source of resistance and

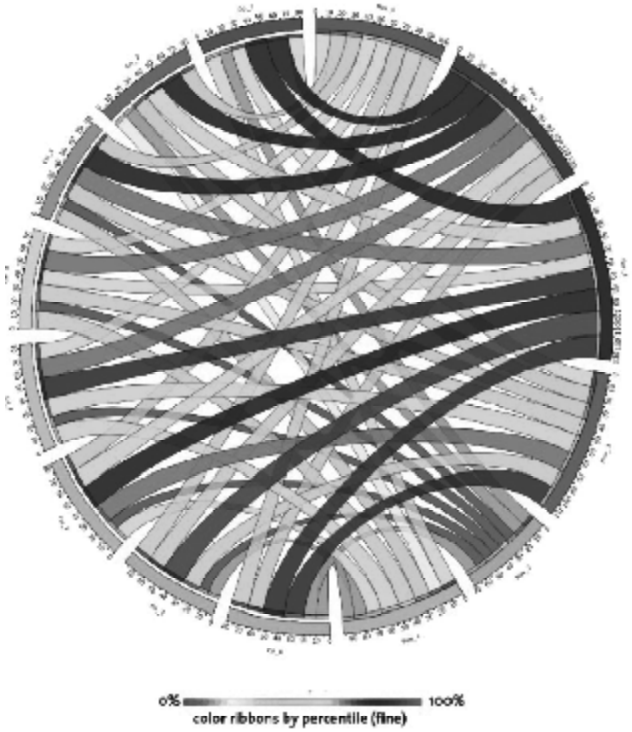

Fig. 5. CIRCOS Graph of Plant Height Data. Column 8 is in 12 'O' clock position, columns 7,6,5,4,3,2,1, Rows 1,2,3,4,5,6 are in clockwise direction. Each ribbon represents a single plant defined by the Row and Columns. Percentile groups are 2.5% in size and so 40 different colors of ribbons would be seen. A false color scheme is used, with red corresponding to the lowest value and purple to the highest.

also to evaluate the effects of interventions. This approach provides a way to visualize both the individual parameters and their combined effects. In effect it bring the entire field and its data to the printed page. Thus this method of High Density Multi-parametric Charts Using CIRCOS will be immensely useful in analysing field experimental data. CIRCOS uses a circular ideogram layout to facilitate the display of relationships between pairs of positions by the use of ribbons, which encode the position, size, and orientation of related genomic elements. CIRCOS is capable of displaying data as scatter, line, and histogram plots, heat maps, tiles, connectors, and text. Bitmap or vector images can be created from GFF-style data inputs and hierarchical configuration files, which can be easily generated by automated tools, making CIRCOS suitable for rapid deployment in data analysis and reporting pipelines. Using the CIRCOS charts the variation among the parameter for each individual plant can be easily measured as the all the data appears on the same segment. This also facilitates to detect whether the same plant is scoring low on many parameters. We are sure that charts made by CIRCOS will help in the analysis of field data.

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(Note : The details on installation of CIRCOS and Command Algorithms are available in online copy of article at www.aaphme.in)

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Management of black-rot in cauliflower caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson

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ABSTRACT : Field trials were carried out during *rabi* season 2010-11 and 2011-12 for the management of black rot disease in cauliflower with thirteen treatments at the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore. The treatments included, seed treatment with hot water, bactericide and bioagent alone before sowing and in combination with sprays of bactericide, chemicals and bioagent in the main field. Of the different treatments tested, the combined treatment of hot water treatment of seeds at 50°C for 15 minutes followed by two applications of Streptocycline (100 ppm) + Copper oxychloride (0.3%) at fifteen days interval starting from fifteen days after transplanting recorded lowest disease incidence of 19.37 per cent and significantly highest yield of 33.3 t/ha, as compared to maximum disease incidence of 61.92 per cent and lowest yield of 15.33 t/ha in untreated control plots. Seed treatment with streptocycline (100 ppm) before sowing followed by sprays of Streptocycline (100 ppm) + Copper oxychloride (0.3%) was found to be next best by recording 20.87 per cent disease incidence and 31.00 t/ha cauliflower yield.

Keywords: Black-rot, cauliflower, management

INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis* L) is one of the important winter vegetable crops cultivated throughout the world. In India, its commercial cultivation is limited by various factors among which disease plays an important role. The crop is subjected to attack by a variety of pathogens both in the nursery as well as in field. Black rot, caused by the bacterium *Xanthomonas campestris* pv *campestris* (Pammel) Dowson (Alvarez 2000), is the most common and serious disease of cauliflower and other crucifers like cabbage, radish, mustard, broccoli and turnip. The bacterium is seed borne and can infect the plant through roots, wounds, hydathodes and leaf stomata (Schaad and Alvarez, 1993). The disease is characterized by V-shaped, chlorotic to necrotic lesions at the leaf margins and blackened vascular tissues (Vicente *et al*., 2001). The disease affects primarily the above ground parts of plants during any stage of growth causing yield and quality losses, especially in tropical and subtropical regions during rainy season. It has been reported that under severe outbreak, the disease can cause substantial yield loss of up to 70.0 per cent (Jorgensen and Walter, 1955). Managing the disease is very difficult as the bacterium spreads within and between fields by water splashes, wind, insects, machinery and irrigation. There are no resistant cauliflower cultivars available for black rot disease in India. Therefore, an experiment was conducted at the Indian Institute of Horticultural Research farm, Hessaraghatta, Bangalore, to develop a management strategy for the bacterial black rot disease in cauliflower.

MATERIALS AND METHODS

Field trials were carried out during *rabi* season of 2010-11 and 2011-12 with cauliflower variety Tetris (black rot susceptible) for the management of black rot disease. The experiment had thirteen treatments with four replications each in a randomized block design with each plot measuring 3.0 m x 4.0 m. The treatments included, i) hot water treatment at 50°C for 15 minutes, ii) seed treatment with streptocycline @ 100ppm, iii) seed treatment with *Pseudomonas fluorescens* @ 5g/kg of seed, iv) T_1 + spraying with streptocycline @ 100ppm twice at 15 days interval, v) T_1 + two sprays with streptocycline @ 100ppm + Copper oxychloride @ 0.3% at 15 days interval, vi) T_1 + root dipping (10g/L) + foliar spray (0.5%) with *P. fluorescens*, vii) T_2 + spraying with streptocycline @ 100ppm twice at 15 days interval, viii) T_2 + one spray with streptocycline @ 100ppm + Copper oxychloride @ 0.3%, ix) T_2 + root dipping (10g/L) + foliar spray (0.5%) with *P. fluorescens*, x) T_3 + spraying with streptocycline @100ppm twice at 15 days interval, xi) T_3 + one spray with streptocycline @ 100ppm + Copper oxychloride @ 0.3%, xii) T_3 + root dipping (10g/L) + foliar spray (0.5%) with *P. fluorescens*, xiii) untreated control (Table 1).

Table 1. Effect of various treatments on black rot incidence (%) in cauliflower

Treatment		Mean PDI			* disease reduction over control (%)
		2010-11	2011-12	Pooled Mean	
T1.	Seed treatment with hot water at 50^0 C for 15minutes	34.60[d] (36.00)	27.60[d] (31.67)	31.10[c] (33.84)	49.67
T2.	Seed treatment with Streptocycline (100ppm)	38.46[e] (38.31)	30.46[e] (33.46)	34.50[d] (35.89)	44.28
T3.	Seed treatment with *Pseudomonas fluorescens* @ 5g/kg seed	55.34[g] (48.06)	47.20[g] (43.37)	51.27[f] (45.72)	17.19
T4.	T_1 + spraying with Streptocycline (100ppm) twice at 15 days interval	30.79[c] (33.66)	23.20[c] (28.74)	26.99[b] (31.2)	56.41
T5.	T_1 + two sprays with Streptocycline (100ppm) + Copper oxychloride (0.3%) at 15 days interval	20.13[a] (26.62)	18.60[a] (25.51)	19.37[a] (26.07)	68.71
T6.	T_1 + root dipping (10g/l) + foliar spray (0.5%) with *P. fluorescens*	36.13[de] (36.93)	30.43[e] (33.44)	33.28[cd] (35.19)	46.25
T7.	T_2 + spraying with Streptocycline (100ppm) twice at 15 days interval	28.70[c] (32.37)	20.79[bc] (27.10)	24.75[b] (29.74)	60.02
T8.	T_2 + two sprays with Streptocycline (100ppm) + Copper oxychloride (0.3%)	25.60[b] (30.35)	16.13[a] (23.62)	20.87[a] (26.99)	66.29
T9.	T_2 + root dipping (10g/l) + foliar spray (0.5%) with *P. fluorescens*	52.80[f] (46.59)	44.80[g] (41.99)	48.80[ef] (44.29)	21.18
T10.	T_3 + spraying with Streptocycline (100ppm) twice at 15 days interval	38.60[e] (38.38)	31.42[e] (34.06)	35.01[d] (36.22)	43.45
T11.	T_3 + two spray with Streptocycline (100ppm) + Copper oxychloride (0.3%)	36.73[de] (37.28)	29.50[de] (32.88)	33.12[cd] (35.08)	46.41
T12.	T_3 + root dipping (10g/l) + foliar spray (0.5%) with *P. fluorescens*	50.42[f] (45.22)	43.45[f] (41.22)	46.94[e] (43.22)	24.19
T13.	Untreated Control	65.63[h] (54.10)	58.20[h] (49.70)	61.92[g] (51.90)	-
CD (P=0.05)		2.53	2.59	2.56	-
CV % 4.57		5.24	4.91	-	
SEm (±)		0.88	0.90	0.89	

*Mean of four replications

Figures in parentheses are arc sine transformed values

Table 2. Effect of various treatments on yield of cauliflower (tonnes/ha)

Treatment		Mean yield (tonnes/ha)*		
		2010-11	2011-12	Pooled Mean
T1.	Seed treatment with hot water at 50^0 C for 15minutes	27.91[f]	25.41[g]	26.66[g]
T2.	Seed treatment with Streptocycline (100ppm)	25.00[g]	23.75[i]	24.38[i]
T3.	Seed treatment with *Pseudomonas fluorescens* @ 5g/kg seed	17.50[j]	16.66[k]	17.08[l]
T4.	T_1 + spraying with Streptocycline (100ppm) twice at 15 days interval	29.33[d]	27.83[d]	28.58[d]
T5.	T_1 + two sprays with Streptocycline (100ppm) + Copper oxychloride (0.3%) at 15 days interval	34.16[a]	32.50[a]	33.33[a]
T6.	T_1 + root dipping (10g/l) + foliar spray (0.5%) with *P. fluorescens*	27.91[f]	26.66[f]	27.28[f]
T7.	T_2 + spraying with Streptocycline (100ppm) twice at 15 days interval	30.83[c]	28.33[e]	29.58[c]
T8.	T_2 + two sprays with Streptocycline (100ppm) + Copper oxychloride (0.3%)	31.66[b]	30.33[b]	30.99[b]
T9.	T_2 + root dipping (10g/l) + foliar spray (0.5%) with *P. fluorescens*	18.00[i]	17.41[j]	17.70[k]
T10.	T_3 + spraying with Streptocycline (100ppm) twice at 15 days interval	25.00[g]	24.16[h]	24.58[h]
T11.	T_3 + two spray with Streptocycline (100ppm) + Copper oxychloride (0.3%)	28.33[e]	27.16[e]	27.74[e]
T12.	T_3 + root dipping (10g/l) + foliar spray (0.5%) with *P. fluorescens*	18.33[h]	17.50[j]	17.91[j]
T13.	Untreated Control	16.33[k]	14.33[l]	15.33[m]
CD (P=0.05)		0.12	0.16	0.14
CV % 2.49		3.38	2.94	-
SEm (±)		0.43	0.06	0.25

*Mean of four replications

Hot water treatment of seeds at 50°C was given only for 15 min. as longer duration of 25-30 min. may affect seed vigour in cauliflower (Clayton, 1924) as quoted by Williams, 1980). The treatments were imposed at the time of seed sowing in the nursery bed, during transplantation and also in the main filed during cropping period. Agricultural operations generally recommended for cauliflower cultivation were followed from nursery till harvest. Observations were made on the incidence of black rot on leaves at weekly intervals by using disease index standards on a 0 – 5 scale (0 = no disease; 1 = up to 20 per cent of leaf area with disease symptoms; 2 = 21- 40 per cent disease; 3 = 41- 60 per cent; 4 = 61 - 80 per cent and 5 = > 80 per cent leaf area with disease symptoms) and PDI was worked out as per standard protocol (Kashyap and Dhiman, 2010). All the experimental data were subjected to statistical analysis. Data on percentage values were transformed in to arcsine and analysis of ANOVA was carried out with transformed values. The means were compared to statistical significance using DMRT (Snedecor and Cochran, 1957).

RESULTS AND DISCUSSION

The results of the trials showed that all the treatments were effective in reducing the black rot incidence and increasing the cauliflower yield as compared to untreated control (Table 1). The black rot incidence was significantly reduced to a mean PDI of 19.37, a decrease of 68.71 per cent over control, and recorded a highest yield of 33.3 t/ha, an increase of 54.00 per cent over control, in treatment-T5, where seed treatment with hot water at 50°C for 15 min. before sowing followed by two sprays of Streptocycline (100ppm) + Copper oxychloride (0.3%) at fortnightly interval (Tables 1and 2) was imposed in the main field. This was followed by the treatment-T8, which included seed treatment with Streptocycline (100ppm) before sowing followed by two sprays of Streptocycline (100ppm) + Copper oxychloride (0.3%) at fortnightly interval which recorded a mean PDI of 20.87 and cauliflower yield of 31.00 t/ha. The untreated control plot, however, recorded significantly highest PDI of 61.92 and lowest yield of 15.33 t/ha (Tables 1 and 2).

Seed treatment with hot water at 50°C for 15 min. alone recorded 31.10 per cent disease incidence, which shows that hot water treatment alone is not sufficient to manage black rot in cauliflower, as it can only take care of the bacterium present inside the seed. Hence the spread of the disease in field can be managed by spraying Streptocycline (100 ppm) and copper oxychloride (0.3%). Though the treatment- T7, i.e. seed treatment with streptocycline before sowing and field application of streptocycline + copper oxychloride has given significant result in reducing the disease incidence and increasing the yield, it is best only next to treatment-T5, i.e. hot water treatment of seeds followed by application of streptocycline + copper oxychloride. It has been observed that five diseased seedlings per 10,000 seeds sown can result in a relatively high incidence of black rot in crucifers and hence it is strongly advised to treat the seeds in hot water before sowing in order to kill the bacterium in the seeds, if there are any (Schaad, *et al.*, 1980).

In the present study, treatment with *Pseudomonas fluorescens* did not give encouraging result in controlling black rot in cauliflower. Beura *et al.* (2006) also made similar observation with *P. fluorescens*, which gave 15.61 per cent control over the disease and cauliflower yield of 17.5 t/ha. However, Mishra and Arora (2010) found that seed and seedling treatment with *P. fluorescens* gave significant control over black rot in cauliflower. This may be due to strain variations among *P. fluorescens* isolates, as out of 54 bacterial strains isolated from the rhizospheric soils, only one isolate T07 was found to be effective in controlling black rot in cauliflower. In conclusion the study showed that black rot of cauliflower was successfully managed by hot water treatment of seeds at 50°C for 15 min. followed by two application of streptocycline (100 ppm) + Copper oxychloride (0.3%) at fortnightly interval starting from 15 days after transplanting.

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FOCUS ARTICLE

Impact of climate change on insect pests, pathogens and nematodes

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ABSTRACT: Anthropogenically induced climatic change arising from increasing levels of atmospheric greenhouse gases would likely to have a significant effect on agricultural pests. Changes in climate may trigger changes in geographical distribution, increased overwintering, changes in population growth rates, increases in the number of generations, extension of the development season, changes in crop-pest synchrony, changes in interspecific interactions, pest biotypes, activity and abundance of natural enemies, species extinction, increased risk of invasion by migrant pests and efficacy of crop protection technologies. Global warming will also reduce the effectiveness of host plant resistance, transgenic plants, natural enemies, biopesticides, and synthetic chemicals for pest management. Therefore, there is a need to generate information on the likely effects of climate change on pests to develop robust technologies that will be effective in future under global warming and climate change.

Keywords: Climate change, insect pests, mites, plant pathogens, nematodes

Crop plants live in a very complex ecosystem. They are attacked by viruses, bacteria, fungi, insects, mites and nematodes. All of these species interact with each other. Globally, pests are estimated to destroy about one third of our crops and are an increasingly serious constraint to crop production, in spite of the advances in pest control technology over the last half century. Many people believe that global warming as predicted would increase pressure from pests and diseases. Higher temperatures and longer growing seasons could result in increased pest population in temperate regions of Asia. In general, however, most pest species are favoured with warm and humid conditions. Pest infestations often coincide with changes in climatic conditions, such as early or late rains, drought, or increases in humidity, which can reduce yields. Climate change might have an influence on increased pesticide use due to presence of diseases and pests.

Global warming and climate changes will result in:

- Extension of geographical range of pests and pathogens.
- In cooler latitudes, global warming brings new species.
- Increased risk of invasion by migrant pests.
- Reduced effectiveness of crop protection technologies.
- A 2.4 to 2.7-fold increase in pesticide use by 2050 (Tilman *et al.*, 2001).
- Increased probability of pests developing faster resistance to pesticides.
- Warmer winter temperatures would reduce winter kill, favouring the increase of pest populations.
- Rising temperatures extend the growing season.
- Overall temperature increases may influence crop-pest interactions by speeding up pest growth rates which increases reproductive generations per crop cycle.

INSECTS AND MITES

Insect habitats and survival strategies are strongly dependent on patterns of climate. Therefore, it is highly expected that, the major drivers of climate change *i.e.* elevated CO_2, increased temperature and depleted soil moisture can impact population dynamics of insect-pests and the extent of crop losses, significantly. It has been reported that, global climate warming may lead to altitude wise expansion of the geographic range of insect pests (Elphinstone and Toth, 2008), increased abundance of tropical insect species (Diffenbaugh *et al*., 2008), decrease in the relative proportion of temperature sensitive insect population (Sharma *et al*., 2010), more incidence of insect transmitted plant diseases through range expansion and rapid multiplication of insect vectors (Petzoldt and Seaman, 2010). Thus, with changing climate it is expected that the growers of crops have to face new and intense pest problems in the years to come.

The aggravating pest problems under changing climate regimes are expected to intensify the yield losses; threatening the food security of the countries with high dependency on agriculture. The climate change is likely to affect the extent of entomophilies pollination by

disrupting the synchrony between plant-pollinator life cycles, with an estimated risk of reduction in world food production by one-third (Klein *et al.*, 2007).

Increased temperature

Insects are particularly sensitive to temperature because they are stenotherms (cold-blooded). In general, insects respond to higher temperature with increased rates of development and with less time between generations. Warmer winters reduce winterkill and consequently induce increased insect populations in the subsequent growing season. It has been estimated that with a 2°C temperature increase insects might experience one to five additional life cycles per season (Yamamura and Kiritani, 1998). Increased temperatures will accelerate the development of cabbage maggot, onion maggot, European corn borer, Colorado potato beetle–possibly resulting in more generations (and crop damage) per year. Rising temperatures will lengthen the breeding season and increase the reproductive rate. That, in turn, will raise the total number of insects attacking a crop and subsequently increase crop losses. In addition, some insects, such as the Southwestern corn borer, will be able to extend their range northward as a result of the warming trend (Chippendale, 1979). In colder regions (higher latitudes) with distinctive seasons, insects have broader thermal tolerance and are living in climates that are currently cooler than their optima (Deutsch *et al.*, 2008). Global warming might therefore benefit many insect species in the temperate regions.

Studies on aphids and moths have shown that increasing temperatures can allow insects to reach their minimum flight temperature sooner, aiding in increased dispersal capabilities (Zhou *et al.*, 1995). Accelerated metabolic rates at higher temperatures shorten the duration of insect diapauses due to faster depletion of stored nutrient resources (Hahn and Denlinger, 2007). Warming in winter may cause delay in onset and early summer may lead to faster termination of diapauses in insects, which can then resume their active growth and development. This gives an important implication that increase in temperature in the range of 1°C to 5°C would increase insect survival due to low winter mortality, increased population build-up, early infestations and resultant crop damage by insect-pests under global warming scenario (Harrington *et al.*, 2010). Higher average temperature might result in some crops being able to be grown in regions further north – it is likely that at least some of the insect pests of those crops will follow the expanded crop areas. Rising temperatures could result in more insect species attacking more hosts in temperate climates (Bale *et al.*, 2002).

Natural enemy and host insect populations may respond differently to changes in temperature. Parasitism could be reduced if host populations emerge and pass through vulnerable life stages before parasitoids emerge. Hosts may pass though vulnerable life stages more quickly at higher temperatures, reducing the window of opportunity for parasitism. Skirvin *et al.* (1997) modeled the interaction of ladybird (*Coccinella septempunctata*) with aphid populations (*Sitobion avenae*) and predict that in hot summers coccinellids reduce aphids more strongly than in moderate summers. The effects of higher temperature on the impact of the microsporidia *Nosema lymantriae* on the gypsy moth (*Lymatria dispar*) clearly showed a much higher and earlier mortality of gypsy moth larvae at higher temperatures (Pollan, 2009). In temperate zones milder winters might enhance survival of parasitoids. Legrand *et al.* (2004) have shown that parasitoids of cereal aphids are active in winter and this winter activity can considerably reduce spring aphid populations.

Increasing temperatures may result in a greater ability to overwinter in insect species limited by low temperatures at higher latitudes, extending their geographical range (Elphinstone and Toth, 2008). In Japan, warmer climate led to the northward migration of the green stinkbug (*Nezara viridula*) a major agricultural pest damaging soybean, rice, cotton and many other crops (Musolin, 2007). The environmental factors like high temperature have been found affecting transgenic expression in Bt cotton resulting in reduced production of Bt toxins. This lead to enhanced susceptibility of the crops to insect-pests like bollworms *viz.*, *Heliothis virescens* (Kaiser, 1996), *Helicoverpa armigera* and *H. punctigera* (Hilder and Boulter, 1999).

Certain pesticides like pyrethroids, organophosphates and especially the biopesticides being highly thermo-unstable degrade faster at higher temperatures. Altered temperature regimes may render many of these products to be less or not effective in pest control, necessitating frequent insecticide applications for effective control (Musser and Shelton, 2005). It is apparent that for sweet corn pests, warmer temperatures translate to increased insecticide applications to produce a marketable crop. With more insecticide applications required, the probability of applying a given mode of action insecticide more times in a season will increase, thus increasing the probability of insects developing resistance to insecticides. Chen and

McCarl (2001) reported that hotter weather increases pesticide costs for corn, cotton, potato, and soybean but decreases the cost for wheat.

Elevated CO_2 levels

Whittaker (1999) concluded from his review of studies on insects and elevated CO_2 that so far, population density of chewing insects are unaffected or decrease, but do not increase while sap sucker (phloem feeder) population density might increase. Chen *et al.* (2005) reared larvae of *H. armigera* on milky grains of spring wheat grown in ambient CO_2 concentration, at 550 ppm and at 750 ppm. The results show that the larvae developed quite similarly under all CO_2 concentrations, even though the larvae under elevated CO_2 consumed much more than those under ambient CO_2. Quite interesting is the fact that the adult moth raised under elevated CO_2 lived longer, but laid significantly less eggs. A multiple generation experiment compared consumption, growth and performance of *H. armigera* feeding on transgenic Bt cotton versus conventional cotton grown under elevated CO_2 (750 ppm) versus ambient CO_2 (375 ppm). The results suggest that on the one hand damage caused by the cotton bollworm might be higher under elevated CO_2 conditions, regardless of the cotton variety. On the other hand population abundance might be lower under elevated CO_2 compared to that under ambient CO_2 (Chen *et al.*, 2007). The researcher explains both observations with nutritional changes under elevated CO_2 (e.g. compensatory feeding).

Bollworm (*H. armigera*) larvae feeding on elevated CO_2-grown pea plants at 700 ppm were significantly smaller than those grown on ambient-grown plants. The predatory omnivorous bugs performed best when fed on bollworm larvae from the elevated-CO_2 treatment apparently because these prey were smaller and thus easier to overcome. Taken together, results indicate that elevated CO_2 may benefit generalist predators through increased prey vulnerability, which would put pest species under higher risk of predation (Coll and Hughes, 2008). During the early season, soybeans grown in elevated CO_2 atmosphere had 57% more damage from insects (primarily Japanese beetle, potato leaf hopper, western corn rootworm and Mexican bean beetle) than those grown in today's atmosphere, and required an insecticide treatment in order to continue the experiment. It is thought that measured increases in the levels of simple sugars in the soybean leaves may have stimulated the additional insect feeding (Hamilton *et al.*, 2005). Increased atmospheric carbon dioxide is expected to alter the nutritional makeup of crops, thereby affecting the severity of attack from insects.

Researchers have observed that insects sometimes feed more on leaves that have lowered nitrogen content in order to obtain sufficient nitrogen for their metabolism (Hunter, 2001). Increased carbon to nitrogen ratios in plant tissue resulting from increased CO_2 levels may slow insect development and increase the length of life stages vulnerable to attack by parasitoids (Coviella and Trumble, 1999). In one study two-spotted spider mites (*Tetranychus urticae*) were raised on common beans grown at 600 and 700 ppm CO_2. A significant decrease in the number of the offspring in the first and second generations (34% and 49%) respectively was observed compared to ambient CO_2 (Joutei *et al.*, 2000). A similar experiment was conducted, where *T. urticae* were raised on clover (*Trifolium repens*) grown at different CO_2 levels (395-748 ppm). The results showed a quite opposite effect: under elevated CO_2 spider mite reproduction increased significantly compared to lower CO_2 (Heagle *et al.*, 2002).

Precipitation changes

Precipitation – whether optimal, excessive, or insufficient – is a key variable that also affects crop-pest interactions. Drought stress sometimes brings increased insect pest outbreaks. It is well known that drought can change the physiology of host species, leading to changes in the insects that feed on them (Mattson and Haack, 1987). Abnormally cool, wet conditions can also bring on severe insect infestations, although excessive soil moisture may drown out soil-residing insects. Some insects are sensitive to precipitation and are killed or removed from crops by heavy rains - in some northeastern US states, this consideration is important when choosing management options for onion thrips (Reiners and Petzoldt, 2005). For some insects that overwinter in soil, such as the cranberry fruit worm and other cranberry insect pests, flooding the soil has been used as a control measure (Vincent *et al.*, 2003). One would expect the predicted more frequent and intense precipitation events forecasted with climate change to negatively impact these insects. Fungal pathogens of insects are favoured by high humidity and their incidence would be increased by climate changes that lengthen periods of high humidity and reduced by those that result in drier conditions. Guiterrez *et al.* (2008) found out that during the normally wet northern California winter, the fungal pathogen (*Pandora neoaphidis*) causes catastrophic mortality to pea aphid (*Acyrthosiphon*

pisum). Chen and McCarl (2001) investigated the relationship of precipitation and pesticide costs for several crops in the USA and concluded that increases in rainfall leads to increases in average pesticide costs for corn, cotton, potato, soybean, and wheat.

PLANT PATHOGENS

The classic disease triangle recognizes the role of climate in plant diseases as no virulent pathogen can induce disease on a highly susceptible host if climatic conditions are not favourable. Climate influences all stages of host and pathogen life cycles as well as development of disease. Disease severity over a period can fluctuate according to climatic variation (Fig. 2).

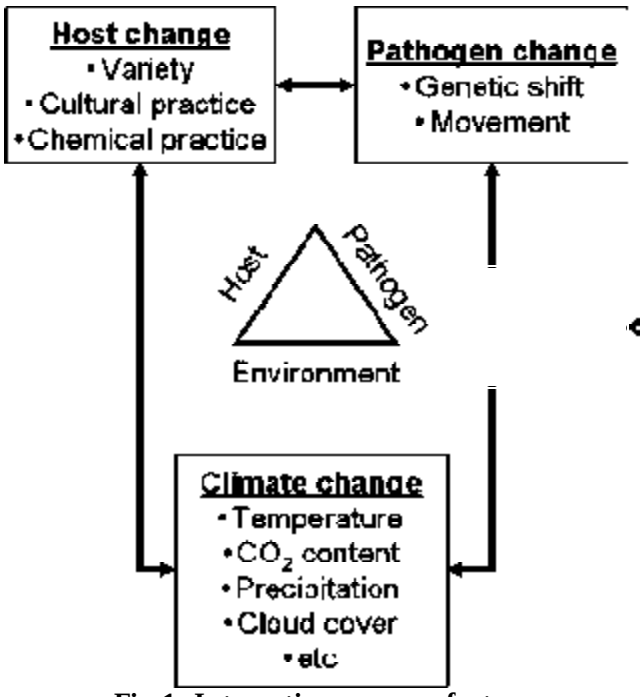

Fig 1. Interactions among factors

Climate factors that influence the growth, spread, and survival of crop diseases include temperature, precipitation, humidity, dew, radiation, wind speed, circulation patterns, and the occurrence of extreme events. Higher temperature and humidity and greater precipitation result in the spread of plant diseases, as wet vegetation promotes the germination of spores and the proliferation of fungi and bacteria, and influences the life cycle of soil nematodes. In regions that suffer aridity, however, disease infestation lessens, although some diseases (such as the powdery mildews) thrive in hot, dry conditions, as long as there is dew formation at night. Most crop diseases have greater potential to reach severe levels under warmer conditions. Fungal and bacterial pathogens are also likely to increase in severity in areas where precipitation increases. Under warmer and more humid conditions cereals would be more prone to diseases such as *Septoria*. In addition, increases in population levels of disease vectors could lead to increased epidemics of the diseases they carry. Under the warmer-but-drier conditions projected for North America, crop losses due to plant diseases are expected to decline as much as 30 percent. However, under the wetter conditions projected for Africa, losses to diseases will increase by more than 100 percent for some crops.

Elevated CO_2 levels

Elevated CO_2 would increase canopy size and density of plants, resulting in a greater biomass production and microclimates may become more conducive for foliar pathogens (rusts, mildews, leaf spots and blights) development. Because of high C: N ratio of litter as a consequence of plant growth under elevated CO_2, decomposition will be slower. Increased plant biomass, slower decomposition of litter, and higher winter temperature could increase pathogen survival on over-wintering crop residues and increase the amount of initial inoculum available for subsequent infection.

Research on rice leaf blast and sheath blight in the temperate climates of Japan showed that elevated CO_2 increased the potential risks for infection from leaf blast and epidemics of sheath blight (Kobayashi *et al.*, 2006). In soybeans, elevated CO_2 alone or in combination with ozone (O_3) significantly reduced downy mildew (*Peronospora manshurica*) disease severity by 39–66% across a 3 year study. In contrast, elevated CO_2 alone or in combination with O_3 significantly increased brown spot (*Septoria glycines*) severity (Eastburn *et al.*, 2009). In wheat, grown at elevated atmospheric CO_2 (700 ppm), the mean per cent leaf area infected with mildew was significantly reduced under elevated atmospheric CO_2, compared to ambient CO_2. The enlarged canopy of *Stylosanthes scabra* plants grown under elevated CO_2 trapped more conidia of *Colletotrichum gloeosporioides* that, together with increased humidity in the denser canopy, led to more severe anthracnose than on plants grown under lower CO_2 (Fig. 2) (Chakraborty *et al.*, 2000).

Following penetration, established colonies of *Erysiphe graminis* grew faster and sporulation per unit area of infected tissue was increased several-fold under elevated CO_2. It has been also observed that under elevated CO_2 out of the 10 biotrophic pathogens studied,

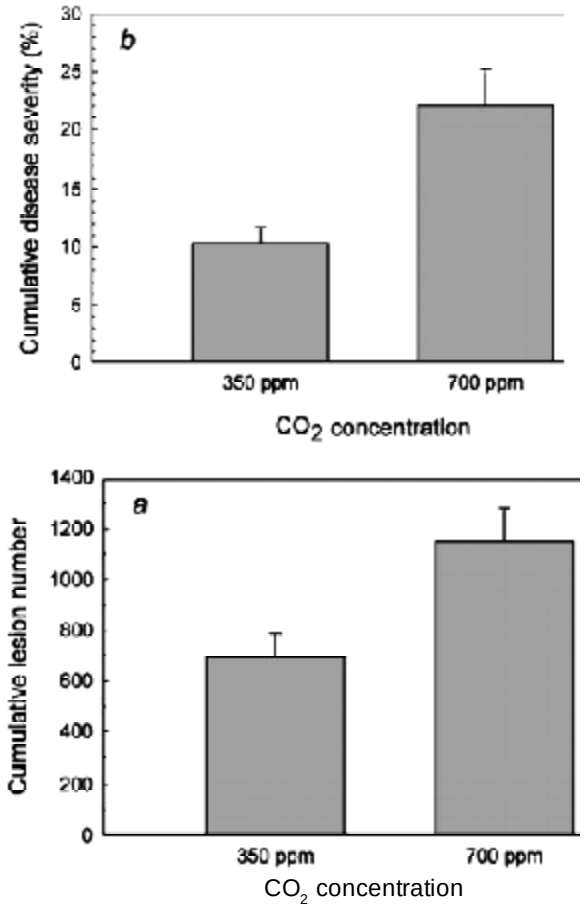

Fig 2. Cumulative number of lesions (a) and disease severity expressed as percent leaf area affected (b) caused by *Colletotrichum gloeosporioides* on susceptible *Stylosanthes scabra* plants (Chakraborty *et al.*, 2000)

disease severity was enhanced in six and reduced in four and out of 15 necrotrophic pathogens, disease severity increased in nine, reduced in four and remained unchanged in the other two.

Elevated temperatures

In most locations, temperature changes had significant effects on disease development. However, these effects varied between different agro-ecological zones. In cool sub-tropical zones such as Japan and northern China, elevation of ambient temperature resulted in greater risk of rice blast epidemics. Situations in the humid tropics and warm humid subtropics were opposite to those in cool areas. A lower temperature resulted in greater risk of blast epidemics.

Temperature change might lead to appearance of different races of the pathogens hitherto not active but might cause sudden epidemic. Change in temperature will directly influence infection, reproduction, dispersal, and survival between seasons and other critical stages in the life cycle of a pathogen. At higher temperature, lignification of cell walls increased in forage species and enhanced resistance to fungal pathogens. Research has shown that host plants such as wheat and oats become more susceptible to rust diseases with increased temperature; but some forage species become more resistant to fungi with increased temperature (Coakley *et al.*, 1999). Predictive models for potato and tomato late blight (caused by *Phytophthora infestans*) show that the fungus infects and reproduces most successfully during periods of high moisture that occur when temperatures are between 7.2°C and 26.8°C (Wallin *et al.*, 1950). Earlier onset of warm temperatures could result in an earlier threat from late blight with the potential for more severe epidemics and increases in the number of fungicide applications needed for control.

In mild winters, high intensity of aphid movement during spring and a high frequency of PVY-infected potatoes have been reported. Aphid vectors are expected to have increased survival with milder winter temperatures, and higher spring and summer temperatures will increase their development and reproductive rates and lead to more severe disease. Systemic fungicides could be affected negatively by physiological changes that slow uptake rates, such as smaller stomatal opening or thicker epicuticular waxes in crop plants grown under higher temperatures.

Precipitation patterns

Some pathogens such as apple scab, late blight, and several vegetable root pathogens are more likely to infect plants with increased moisture – forecast models for these diseases are based on leaf wetness, relative humidity and precipitation measurements. Other pathogens like the powdery mildew species tend to thrive in conditions with lower (but not low) moisture. Bacteria are spread to their host plants mainly by water, usually in the form of rain splash and insects. In humid, wet conditions, infected plant tissues can exude masses of bacteria that are spread from host to host by rain splash and insects. Therefore, the warmer drier summers expected with climate change should limit bacterial diseases. Precipitation following fungicide application may improve its distribution (Schepers, 1996) but an increase in rainfall intensity can deplete fungicide residue on the foliage. The more frequent rainfall events predicted by climate change models could result in farmers finding it difficult to keep residues of contact fungicides on plants, triggering more frequent applications.

Expansion of geographical range

The northward expansion of the soybean sudden death syndrome (a soil-borne fungal disease caused by *Fusarium solani* f. sp. *glycines*) is an example (Roy *et al*., 1997). The disease was first reported in Arkansas in 1971; in the early 1980s it was found in southern Missouri, Illinois, and Indiana; by the early 1990s it was also found in southern Iowa, northern Illinois and northern Indiana; and in 1998 it was found in Ontario, Wisconsin and Ohio.

The gray leaf blight of corn caused by the fungus *Cercospora zeae-maydis* ranks number one in causing yield losses of corn in recent years. It is also a disease whose range expansion was first noticed in the 1970s; in the last two decades, the disease has gradually developed into a major production problem in the Corn belt. Although the increase in the abundance and epidemics of this disease may be due, in part, to the increase in the use of conservation tillage, the observed trends in minimum temperatures and precipitation in the region may also have contributed.

NEMATODES

Plant pathogenic nematodes are one of the important biotic constraints in crop production. Climate change due to increased emission of greenhouse gases is posing a serious challenge to sustainability of crop production by interfering with biotic and abiotic components and their interactions with each other. Global warming resulting in elevated carbon dioxide (CO_2) and temperature in the atmosphere may influence plant pathogenic nematodes directly by interfering with their developmental rate and survival strategies and indirectly by altering host plant physiology. Severe droughts resulting in a reduction of soil water will most likely negatively affect soil nematodes.

Increased temperatures

Temperature is the most important factor influencing the biology of nematodes. Nematode developmental rate is directly influenced by the temperature with slower development at cooler and faster growth rate at warmer soil temperatures. Therefore, increase in atmospheric temperature due to global warming is expected to result in more number of generations per season and expansion of their geographical distribution range. Other potential effects of elevated temperature on parasitic nematodes include altered sex ratio, host defense responses and interference in their survival strategies like dauer juveniles or egg diapauses in extreme environments.

Drier temperatures are expected to increase symptoms of water stress in plants infected with nematodes such as the soybean cyst nematode. Overwintering of nematodes is not expected to be significantly affected by changes in climate, although for some, such as the soybean cyst nematode, egg viability may be reduced in mild winters.

Warming will generally cause a pole-ward shift of the risk of damage from potato nematodes which would increase in all regions and may become a serious problem with additional generations per year.

Data from lab and field experiments demonstrated that higher *Radopholus similis* nematode population densities and greater root damage to banana at the projected temperature increases.

Elevated CO_2 levels

Herbivorous nematodes showed neutral or positive response to CO_2 enrichment effects with some species showing the potential to build up rapidly and interfere with plant's response to global warming. The number of herbivore, bacterivore and fungivore nematodes was significantly higher under winter wheat and sugar beets grown under elevated CO_2 (550 ppm), while the number of carnivore was not changed. The total numbers of herbivore, bacterivore and fungivore nematodes were higher under elevated CO_2 wheat than under elevated CO_2 sugar beet, most likely due to the very different root system of both plant species (Sticht *et al*., 2009).

Expansion of geographical range

Studies have also demonstrated that the geographical distribution range of plant pathogenic nematodes may expand with global warming spreading nematode problems to newer areas. Before 1970, the soybean cyst nematode was mainly distributed in the Mississippi River Delta area, northern Arkansas, southern Missouri, southern Illinois, and western Kentucky. It is now distributed throughout the main soybean production area and has become the number one soybean pest in the U.S. In Iowa alone it caused an estimated yield loss of 201 million bushels (worth about US$ 1.2 billion) during the 1998 growing season. *Radopholus similis*, currently absent from cooler, higher altitude areas is likely to expand its range.

ADAPTATION AND MITIGATION

Adaptation refers to an adjustment in natural or human systems in response to actual or expected climatic stimuli or their effects that moderate, harm or exploit beneficial opportunities.

Climate change mitigation encompasses the actions being taken, and those that have been proposed, to limit the magnitude and/or rate of long-term global warming induced climate change.

- Biosecurity, quarantine, monitoring, and control measures can be strengthened to control the spread of pests and diseases under a warming climate.
- More resilient/adaptable crop genotypes needed, especially with durable resistance to pests.
- Adoption of environmental conserving pest controlling activities such as organic farming, bio-control and integrated pest management.
- Application of natural mulches helps in suppression of harmful pests and diseases.
- A diverse fauna of natural enemy species can successfully suppress pests.
- Mulching and reduced tillage for example increases spider abundance
- Specific relationships between pests and host plants are interrupted by crop rotations.
- A 'healthy' soil, with optimal physical, chemical and biological properties increases plant resistance to insects and diseases.
- Avoidance of excess use of nitrogen which can increase the severity of certain diseases and make a crop more susceptible to pests.
- Organic agriculture system uses crop rotation, green manure, compost, biological pest control and mechanical cultivation to control for pests. Organic systems avoid the use of synthetic pesticides and rely on practices such as crop rotations which break up pest cycles and encourage beneficial insects.
- Polyculture techniques such as crop rotation, multi-cropping and intercropping are less susceptible to pests than monoculture crops.
- In addition to the prudent application of pesticides, increased use of nonchemical pest controls (crop rotations, biological controls, altering planting dates and fertilizer and irrigation applications, and soil management and tillage) would help minimize crop losses and thereby help maintain crop yields.
- Genetically engineered plants have been designed to resist pests, diseases and nematodes without the need for pesticides.
- The growers of the crops have to change pest management strategies by rescheduling the crop calendars in accordance with the projected changes in pest incidence and extent of crop losses in view of the changing climate.
- Geographic Information System (GIS) is an enabling technology for crop protection scientists, which help in relating pest outbreaks to biographic and physiographic features of the landscape, hence can best be utilized in area wide pest management programmes.
- Pesticides with novel mode of actions such as neonicotinoid insecticides for controlling sucking pests which induces salicylic acid associated plant defense responses. Such more compounds needs to be identified for use in future crop pest management.
- Integrated pest management (IPM) is an effective and environmentally sensitive approach to pest management that uses current, comprehensive information on the life cycles of pests and their interaction with the environment to manage pest damage by the most economical means, and with the least possible hazard to people, property, and the environment.

CONCLUSION

Global warming and climate change will have serious consequences on diversity and abundance of pests, and the extent of losses due to pests, which will impact both crop production and food security. Prediction of changes in geographical distribution and population dynamics of pests will be useful to adapt the pest management strategies to mitigate the adverse effects of climate change on crop production. Pest outbreaks might occur more frequently, particularly during extended periods of drought, followed by heavy rainfall. Some of the components of pest management such as host plant resistance, biopesticides, natural enemies, and synthetic chemicals will be rendered less effective as a result of increase in temperatures and UV radiation, and decrease in relative humidity. Climate change will also alter the interactions between the pests and their host plants. As a result, some of the cultivars that are resistant to pests, may exhibit susceptible reaction under global warming. Adverse effects of climate change on the activity and effectiveness of natural enemies will be a major concern in future pest management programmes. Rate of pest multiplication might increase with an increase in CO_2 and

temperature. Therefore, there is a need to have a concerted look at the likely effects of climate change on crop protection, and devise appropriate measures to mitigate the effects of climate change on food security.

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RESEARCH NOTE

Relative incidence of leaf webber, *Orthaga exvinacea* Hamp. on varieties and hybrids of mango (*Mangifera indica* L.)

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Mango is an important fruit crop that is extensively grown in India and in many other tropical countries. The crop is attacked by about 492 species of insects, 17 species of mites and 26 species of nematodes in the world level of which 188 species have been reported from India (Tandon and Verghese, 1985; Srivastava, 1998). Among the insect pests, mango leaf webber, *Orthaga euadrusalis* and *O. exvinacea* Hampson, (Lepidoptera: Pyralidae) is a major pest responsible for low productivity. The heavily infested trees present a burnt look and severe infestation results in complete failure of flowering (Verghese, 1998). It is widely distributed in different agro-climatic zones of India and has gained the status of serious pest in UP, Uttarachchal and AP (Singh *et al.*, 2006). The mango leaf webber is a major pest in intensively cropped areas of Andhra Pradesh and heavy infestation by this pest adversely affects the flower as well as the growth of new flush (Kavita *et al.*, 2005). Ninety percent of the completely defoliated/skeletonized shoots dried and did not fruit in coming season (Gajendra Singh, 1988). Considering the importance of the insect pest, a study was initiated to understand the response of mango varieties and hybrids against leaf webber *O. exvinacea* incidence.

Twenty two varietal collections of mango *viz.*, Alphonso, Safeda, Kesari, Manoranjan, Yellamanda, Dasheri, Royal special, Mehamooda Vikarabad, Pulihora, Proddutur Avakai, Alipasand, Peter, Banglora(Totapuri), Neelum, Baneshan Rumani Prabhasankar, Ratna, Dasheri x Vikarabad, Mallika, Amrapali and Manjeera were screened for leaf webber incidence. The webber infestation begins from June and continues up to December and peak infestation is observed in September. The numbers of live webs per tree were recorded in different varieties and hybrids for a period of four years in September month to know the damage intensity and the data was pooled to get total number of live webs.

Results revealed that none of the 16 varieties and 6 hybrids screened were resistant to the leaf webber. The number of live webs ranged from 0-18, 0-24, 0-20, and 1-33 per tree in 2009, 2010, 2011 and 2012, respectively indicating that there was some variation in year to year infestation with 2012 readings showing maximum incidence of leaf webber compared to other years under observation. No varieties or hybrids of mango screened for leaf webber incidence were highly resistant to the insect pest (Kavitha *et al.*, 2005, Kannan and Venugopala Rao, 2006). Among all the sixteen varieties tested, Pulihora was highly susceptible with maximum infestation of 94 webs per tree followed by Peter (89), Banglora (71), Alipasand (64), Yellamanda (53), Neelum (49), Produtur Avakai(34) and least susceptible variety was Safeda with only 3 webs/ tree. The varieties *viz.*, Dasheri (UP), Alphonso (Maharashtra) and Kesar (Gujarat) popularly cultivated in these states were also screened and found that the former varieties had less number of webs *i.e.* 6 webs per tree compared to Kesar which has 13 webs per tree. In contrary to our findings, studies conducted by Srivastava and Rizvi (2002) shown that the higher level of infestation was recorded for Dasheri and lowest pest incidence was recorded for late cultivar Fazli. The higher incidence in Dasheri may be due to the predominant cultivar grown in that region.

Among the popular varieties *viz.*, Banglora, Baneshan, Neelum and Alphonso cultivated in Rayalaseema region of AP, the highly susceptible variety was Banglora followed by Neelum and Baneshan and least susceptible was Alphonso with only 6 webs/tree in all the years under study. The data of Singh and Verma (2013) showed that the least susceptible variety was Alphonso with 1.01 webbed mass/tree. Similarly Reddy *et al.*, (2001) evaluated ten mango genotypes for resistance to *O. exivanacea* and noticed that Banglora and Panchadara Kalasa x Willard were highly susceptible. Similarly in the mango hybrids screened for resistance,

Table 1. Incidence of leaf webber, *Orthaga exvinacea* on different cultivars of mango

S.No.	Variety	2009	2010	2011	2012	Total	Mean
1	Alphonso	2	-	-	4	6	1.5
2	Safeda	2	-	-	1	3	0.75
3	Kesar	1	2	4	6	13	3.25
4	Manoranjan	5	3	2	6	16	4.00
5	Yellamanda	10	12	13	18	53	13.25
6	Dasheri	3	-	-	3	6	1.50
7	Royal Special	6	3	4	7	20	5.00
8	Mehamooda Vikarabad	6	4	6	9	25	6.25
9	Pulihora	18	24	20	32	94	23.50
10	Proddutur avakai	8	6	5	15	34	8.50
11	Allipasand	16	15	12	21	64	16.00
12	Peter	17	21	18	33	89	22.25
13	Banglora	19	16	14	22	71	17.75
14	Neelum	12	8	10	19	49	12.25
15	Baneshan	3	6	5	8	22	5.50
16	Rumani	5	5	6	8	24	6.00
17	Prabhasankar (Bombay green x Kalepadu)	2	-	-	3	5	1.25
18	Ratna (Neelum x Alphonso)	4	2	4	9	19	4.75
19	Mallika (Neelum x Dasheri)	3	-	-	3	6	1.50
20	Amrapali (Dasheri x Neelum)	4	-	-	4	8	2.00
21	Manjeera (Rumani x Neelum)	3	1	2	2	8	2.00
22	Dasheri x Vikarabad	0	-	-	3	3	0.75

Dasheri x Vikarabad had minimum no.of webs (3) followed by Prabhasankar (5). The popular hybrids cultivated in different states *viz.*, Ratna, Amrapali, Manjeera and Mallika were also susceptible to leaf webber with former hybrid recorded maximum number of webs (19) and lowest in latter hybrid (6), where as other two hybrids observed to be equally susceptible with same number of webs (8) indicating that Mallika has less number of webs compared to Amrapali. It was also further interesting to observe in hybrids that though the female parent was common viz., Neelum, the susceptibility varied for example in Ratna and Mallika. The results of Singh and Verma (2013) for screening of mango hybrids against leaf webber recorded that Amrapali (2.62 webbed mass/tree) and Mallika (1.67webbed mass/tree) received moderate infestation. Similarly Singh *et al.*, (2006) reported mango hybrids, Mallika received low incidence of leaf webber and Amrapali was completely free from infestation, whereas Kavitha *et al.*, (2005) screened twelve mango cultivars against mango leaf webber *O. euadrusalis* and found variety Peddarasam and Mallika hybrid were highly susceptible which recorded maximum number of webs per tree. In conclusion, all the varieties and hybrids screened under the study were susceptible to leaf webber incidence and in varieties Pulihora whereas in hybrids Ratna were highly susceptible.

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RESEARCH NOTE

Evaluation of insecticides against citrus leaf miner, *Phyllocnistis citrella* Stainton in acid lime

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The citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracilleridae) is one of the serious pests of nursery as well as young plantations of citrus. The activity of the pest is normally observed throughout the year due to its overlapping generation, however new flushes are more exposed. The larvae feed on the epidermis of the tender leaves making serpentine mines due to which leaves become distorted and crumpled. This adversely affects the photosynthesis activity which results in reduced vigour and growth of the plant. The infestation and severity of citrus canker is more in plants with leaf mined leaves. It was estimated that nearly 45 percent of new leaf area was lost due to citrus leaf miner mining (Garcia-Mari *et al.*,2002). More than (80%) of nurseries in Central India are affected by leaf miner. During serious infestation leaf damage exceeds (87%) and moderate infestation lowered yield by (30-40%) (Shivankar *et al.*, 2002). To combat this pest damage and for qualitative and quantitative increase of yield, sustainable approaches for pest management are very much essential. Various control methods have been proposed for the management of citrus leaf miner, these include cultural practices, chemical control, biological control, etc. (Muthaiah *et al.*,1998 and Shivankar *et al.*,2002) and several workers studies on the knock down effect of different types of insecticides for controlof this pest (Batra,1991 and Katole *et al.*,1993). In the present investigation on efficacy of newer insecticides was tried for management of this pest and to overcome the problem of resistance and resurgence.

Field experiments were conducted to evaluate the efficacy of different novel groups of insecticides for the management of citrus leaf miner on acid lime (*Citrus aurantifolia* Swingle) at Research Farm of Citrus project during 2010-11 and 2011-12. The experiment was conducted in randomized block design with thrice replications on 8-year-old acid lime (*Kagzi* lime). The treatments evaluated in the experiment were T_1:Abamectin 1.9 EC at the rate of (0.0007 %), T_2:Spinosad 45 SC at the rate of (0.002%), T_3:Novaluron 10 EC at the rate of (0.005%), T_4:Diafenthiuron 50 WP at the rate of (0.05%), T_5:Triazophos 40 EC at the rate of (0.06 %), T_6:Acephate 75 SP at the rate of (0.1125 %) along with untreated control. The insecticides applied as foliar spray twice at 15 days interval initiating during the emergence of new flush in the month of August during both years. Two plants were designated as the unit for each replication per treatment. Observations were taken on total leaves and damaged leaves (total damaged with leaf curled and twisted) in thirty terminal shoots per treatment on one day before spray and 3, 7 and 14 days after spray (DAS) application. The data were transferred to arc-sin transformed values. The data of two year trials were subjected to ANOVA with least significant difference (P=0.05) as test criterion. Yield data and economics of each treatment were also worked out.

The mean data of two years (2010-11 and 2011-12) in respect of leaf infestation caused by citrus leafminer in acid lime are present in Table 1, revealed that all the insecticidal treatments significantly recorded less infestation (33.62-45.49, 27.29-37.35 and (20.10-31.61%) over untreated control (71.30, 69.77 and 68.98%) on 3rd, 7th and 14th DAS application. Abamectin 1.9 EC at the rate of 0.0007 percent was recorded minimum infestation of leaves (33.62, 27.29 and 20.10%) followed by novaluron 10 EC at the rate of 0.005percent (36.24, 29.64 and 22.22%) and spinosad 45 SC at the rate of 0.002 percent (37.48, 29.40 and 24.58%) recorded on 3^{rd,} 7th and 14th DAS, respectively and it was at par with abamectin. The rest of the treatments *viz.*, diafenthiuron 50 WP at the rate of (0.05%), acephate 75 SP at the rate of (0.1125%) and triazophos 40 EC at the rate of (0.06%) also reduced the leaf miner infestation and it was 42.98, 32.32 and 26.95; 45.49, 35.64 and 30.46 and 45.40, 37.35 and 31.61 percent on 3^{rd,} 7th and 14th DAS, respectively. The present

Table 1. Efficacy of different insecticides against citrus leaf miner in acid lime

Treatment	Percent infestation of leaves				Yield (t/ha)	B : C ratio
	1 DBS	3 DAS	7 DAS	14 DAS		
Abamectin1.9 EC @ 0.0007 %	72.48 (62.80)**	33.62 (34.44)	27.29 (30.68)	20.10 (24.78)	8.83	1.38
Spinosad 45 SC @ 0.002%	70.09 (58.31)	37.48 (37.77)	29.40 (31.59)	24.58 (27.32)	8.68	1.29
Novaluron 10 EC @ 0.005%	70.24 (61.49)	36.24 (37.91)	29.64 (32.44)	22.22 (27.29)	8.33	1.32
Diafenthiuron 50 WP @ 0.05%	71.59 (63.90)	42.98 (39.48)	32.32 (33.15)	26.95 (29.40)	8.00	1.26
Triazophos 40 EC @ 0.06 %	70.10 (60.31)	45.40 (44.68)	37.35 (35.96)	31.61 (33.36)	7.68	1.26
Acephate 75 SP @ 0.1125 %	73.09 (62.03)	45.49 (44.67)	35.64 (36.82)	30.46 (33.48)	7.73	1.26
Untreated control	71.90 (64.81)	71.30 (63.91)	69.77 (63.00)	68.98 (62.79)	6.85	1.15
SE ±	1.70	2.32	2.26	2.79	0.25	-
CD at 5 %	NS	7.16	6.98	8.61	0.76	-

Figures in parenthesis are arcsine transformed values.

findings are in accordance with the results reported by earlier workers (Howard, 1993; Rae *et al.*, 1996; Shivankar *et al.*, 2002, Rao *et al.*, 2002 and Hammad and Antar, 2003).

As regards yield and economics, maximum yield (8.83 t/ha) of acid lime was recorded in abamectin 1.9 EC at the rate of (0.0007%) followed by spinosad 45 SC at the rate of (0.002%) (8.68 t/ha) followed by novaluron 10 EC at the rate of (0.005%) (8.33 t/ha) and it was at par with abamectin. Maximum B:C ratio was also recorded in Abamectin 1.9EC at the rate of (0.0007%) (1.38) followed by novaluron 10 EC at the rate of (0.005%) (1.32) and spinosad 45 SC at the rate of (0.002%) (1.29). The rest of the treatments *viz.*, diafenthiuron 50 WP at the rate of (0.05%), acephate 75 SP at the rate of (0.1125%) and triazophos 40 EC at the rate of (0.06%) also recorded higher yield of 8.00, 7.73 and 7.68 t/ha with B:C ratio of 1.26), respectively as against untreated control (6.85 t/ha with B:C ratio 1.15). The infestation level and reduction in yield of fruits are similar with the studies of Coleman (1994) according to whom the infestation of 1-2 larvae/leaf was sufficient to reduce the number of leaves and lower the yield by (30-40%). Knapp *et al.* (1993) also reported the reduction in yield up to 50% due to infestation caused by citrus leaf miner. These results are lend support to the present findings.

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RESEARCH NOTE

Severe incidence of hairy caterpillar, *Trabala vishnou* (Lefedvre) (Lepidoptera: Lasiocampidae) on pomegranate, *Punica granatum* L.

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Regular monitoring of pomegranate, *Punica granatum* L. (cv. Bhagwa) during 2013-14, for pest occurrence revealed severe defoliation by a hairy caterpillar *Trabala vishnou* (Lefedvre) (Lepidoptera: Lasiocampidae) during monsoon period in an unsprayed experimental orchard of Indian Institute of Horticultural Research, Bangalore, Karnataka, India. Caterpillars of mixed instars were found feeding heavily on the tender foliage causing severe defoliation. Preliminary observations revealed that the infestation was observed to be severe, with 55.80% plants in the experimental orchard being infested (48 out of 86 plants) with an average defoliation of 37.5% per tree. Up to 15 larvae were observed feeding voraciously on plants. The caterpillars had long tufts of urticating hairs all over body that can cause extreme itching, skin irritation when touched (Fig. 1b). Literature reported this caterpillar, *T. vishnou* as a sporadic pest of castor. It has also been found feeding on almond, jamun, guava, pomegranate (Butani, 1976) and populus (Tewari and Namgail, 1999). Further, it has been recorded as a new pest on Himalayan cedar, *Cedrus deodara* from Himachal Pradesh during 2002 (Kalia and Pandey, 2002). Besides India, this lasiocampid moth has been reported in Oriental realm mainly from Sri Lanka, Myanmar, Malaysia, Thailand, Indonesia, China and Taiwan. Further, it has been reported to feed on a wide range of host plants such as *Acacia confuse* (Leguminosae), *Santalum album* (Santalaceae), *Castanea* and *Quercus* spp. (Fagaceae) and *Eucalyptus globules* (Myrtaceae).

Eggs were creamy-white and covered with brown hairs laid either on leaves or fruit calyx (Fig 1a,b) and the incubation period varied from 8 to 10 days (Rathore and Verma, 1976). The full grown larvae measured on an average 5.06 cm in length with fine network of

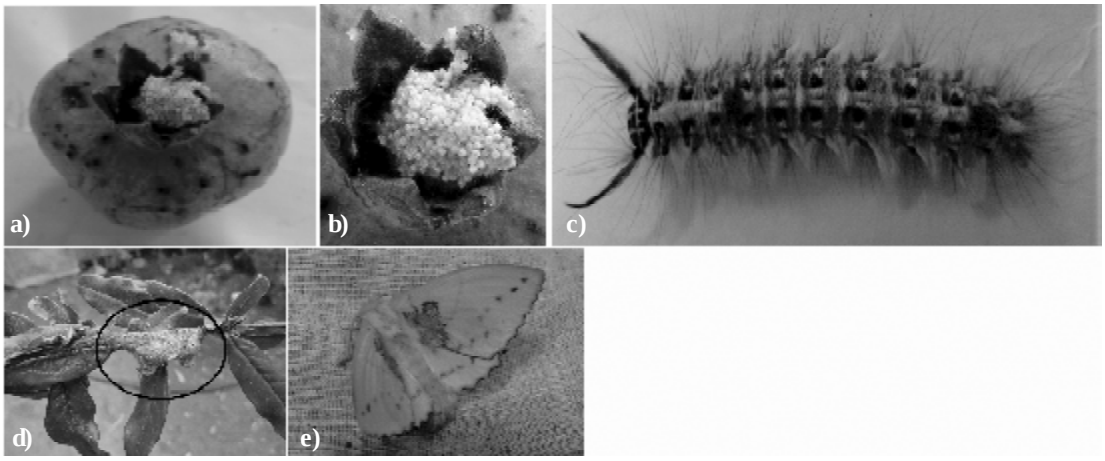

Fig. 1. Different stages of lasiocampid moth, *T. vishnou;* (a, b) Eggs laid on fruit calyx; (c) Hairy caterpillar (d) Pupa (encircled) on pomegranate shoot (e) adult female moth

vertical/horizontal lines and up to six larval instars were reported with an average larval period of 28.3 days for both the sexes (Sevastopulo, 1939; Beeson, 1941). Pencil like dark brown hairs arising from the first somite was noticed in all instars (Fig 1c). Pupation took place on plant itself. Pupa was reddish brown and pupation took place inside a saddle like cocoon, usually cocoons were found on the petiole of leaves (Fig 1d). The colour of the cocoon was same as that of the larva. Each cocoon had two humps on dorsal side and two openings, one on each side. Male cocoons were smaller in size than the female. The total pupal period was 13 – 18 days (Rathore and Verma, 1976; Vishwanath and Visweswara Gowda, 1974).

The adult is a medium sized moth exhibiting sexual dimorphism. The male moth is greenish, whereas, the female is stouter and greenish yellow/yellow with large anal tufts (Fig 1e). Bipectinate antennae are present in both the sexes but bristles are longer in males. The adult lived for 5-6 days and up to 3 generations per year were reported (Raghunatha, 1999). Earlier report of the occurrence of the hairy caterpillar *T. vishnou* in considerable numbers on pomegranate in and around Bangalore was in 1998 by Mani *et al.*, (2000). They also reported natural parasitization (up to 100%) by the tachinid *Blepharipa zebina* Walker and also natural pathogenisis with NPV (5%) in the field. The current observation on severe defoliation of pomegranate foliage by this lasiocampid shows that it has potential to become serious pest of pomegranate under organic cultivation where no insecticidal sprays are exercised.

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RESEARCH NOTE

Species composition of fruit flies, *Bactrocera* spp. (Diptera: Tephritidae) infesting guava in Maharashrtra

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Guava, *Psidium guajava* L. is one of the most important fruit crops. The infestation of fruit fly is a major limiting factor in the production of guava. It is in the range of 20 - 46% (Hasseb, 2007). Fruit flies belong to the family Tephritidae and order, Diptera. Tephritidae is the largest family, comprising more than 4000 species in 500 genera (White and Elson-Harris, 1992). There are about 325 species of fruit flies occurring in the Indian subcontinent, of which 205 are from India alone (Kapoor, 2005). Fruit fly infestation being the major obstacle in the production and marketing of the guava, the successive cultivation and export are highly dependent on sound control of fruit fly. Trapping fruit flies is one of the alternatives to manage pest in an ecofriendly way. Use of chemical attractants like methyl eugenol and cue lure along with appropriate trapping technique has been reported effective in monitoring, suppressing and even complete eradication of various fruit flies (Steiner *et al.*, 1970, Grewal and Kapoor, 1987). The correct identification of species has immense value to promote sound biological evidence for the specific status of sibling species which ultimately helps in developing and adopting effective control strategies. Most of the fruit flies are having limited distribution perhaps due to physical, climatic and vegetative factors and host specificity.

Fruit fly traps with cotton wicks treated with methyl eugenol and cue lure were installed at guava orchard of Department of Horticulture, MPKV, Rahuri. In guava orchard, traps with methyl eugenol and cue lure were hanged at 3 meter distance from ground level and by maintaining the 40 meter distance between the traps to avoid trap interference effect. The fruit fly traps *i.e.*, methyl eugenol and cue lure were installed at guava orchards and fruit flies recorded during weekly interval and species composition was done at the same time. The fruit flies were obtained from fallen infested fruits in the laboratory from orchards. The fallen fruits were examined for the emergence of flies. Five kg of fallen fruits were collected from guava orchard and were brought to the laboratory and kept in rearing cages, provided with a bed layer having mixture of sand and cocopit (1:1) ratio was prepared at the bottom of cage to facilitate the pupation. Then infested fruits were kept on the prepared layer in fruit fly rearing cages for emergence of fruit flies. After 12 - 13 days the fruit fly emergence was started and on first day 55 - 60 % flies were emerged while the rest of the flies were emerged on second day. The fruit flies were handled carefully and care was taken to prevent escaping from the rearing cages. The emerged fruit flies were provided food by hanging cotton swab soaked in 10 % honey solution for a week. The flies were allowed for sclerotization and development of body colour. The collected flies were killed by using chloroform. The flies emerged from the damaged fruits were examined under microscope and were got identified with the help of taxonomical keys.

The collected specimen of fruit flies was identified with the help of taxonomical keys. The highest population of *B. dorsalis* was trapped to methyl eugenol traps installed in guava orchard and lowest population of fruit fly *B. verbascifoliae* was trapped due to the least prevalence of this species in the guava orchards of Ahmednagar district while in cue lure traps, only one species *B. cucurbitae* was noticed throughout the year. The fruit fly species, *B. zonata* and *B. correcta* were also observed in the fruit fly methyl eugenol trap.

Verghese and Jayanthi (2001) reported the catches of *B. dorsalis* and *B. correcta* in the trap baited with methyl eugenol. Madhura and Viraktamath (2003) recorded five species of fruit flies *viz.*, *B. dorsalis, B. correcta, B. verbascifoliae, B. affinis* and *B. zonata* which were attracted to methyl eugenol traps located at Bangalore. Morde (2003) observed *B. caryeae, B. dorsalis* and *B. zonata* in the trap installed in guava orchard in Konkan area. Kawashita et al. (2004) reported catches of *B. correcta, B. dorsalis* and *B. zonata* in methyl eugenol while *B. cucurbitae* in cue lure. Satarkar

et al. (2009) reported four fruit flies species *i.e*. *B. caryeae, B. zonata, B. affinis* and *B. correcta* attracted to the methyl eugenol trap in coastal region of Goa.

Data recorded on number of flies emerged from infested fruits from cages in the laboratory are presented in Table 1. It is revealed that guava fruits were infested with four different fruit fly species. Among the four species, *B. dorsalis* was observed to be dominant species with highest mean number of flies 50.25 (49.95%) emerged out per cage. *B. zonata* was found next dominant species recorded 38.5 flies (31.36%) emerged out per cage. *B. correcta* recorded 24.5 flies (19.95%) emerged out per cage. Very low infestation of *B. verbascifoliae* found during the present investigation which was recorded 9.5 flies (7.73%) per cage. The different fruit fly species obtained from the cages were collected and get identified with the help of taxonomical keys.

From Table 1 it is revealed that different species of fruit flies such as, *B. zonata, B. dorsalis, B. correcta* and *B. verbascifoliae* infest guava in Ahmednagar District. These findings are in agreement with the reports of earlier research workers. Kapoor (1993) found the fruits of guava infested by *B. dorsalis, B. zonata, B. correcta* and *B. caryeae*. Jalaluddin *et al*. (1999) recorded fruit fly species *B. correcta, B. dorsalis* and *B. zonata* from guava fruits and out of them *B. correcta* was predominant one. Gupta and Bhatia (2000) reported *B. dorsalis* and *B. zonata* in guava orchards. Jalaluddin *et al*. (2001) collected the guava fruit fly *B. correcta* from guava orchard. Khan *et al*. (2005) observed *B. zonata* on guava to the extent of 49.62%.

There is no information on the fruit fly species of Ahmednagar region of Maharashtra. However, Gawade (1994) in his study conducted to find out the effectiveness and efficacy of some pheromone formulations, observed two species *viz., B. dorsalis* and *B. cucurbitae* infesting different crops in Konkan region. Similarly, Morde (2003) reported six fruit fly species *viz., B. dorsalis, B. correcta, B. zonata, B. caryeae, B. cucurbitae* and *B. tau* from Konkan region. Satarkar *et al*. (2006) studied the fruit fly species complex in agro-ecosystem of Goa, with the help of methyl eugenol trap and reported five fruit fly species *i.e. B. caryeae, B. zonata, B. affinis, B. dorsalis* and *B. correcta*. However, Galande and Ukey (2011) had recorded seven fruit fly species *viz., B. dorsalis, B. correcta, B. versicolor* and *B. zonata* attracted to methyl eugenol trap while in cue lure three fruit flies species *i.e., B. cucurbitae, B. gavisa* and *B. tau* in the vicinity of guava orchard in Pune region. It can be concluded that guava orchards of Ahmednagar District were infested with the fruit fly species, *B. dorsalis, B. zonata, B. correcta* and *B. verbascifoliae*. Among these *B. dorsalis* was the most predominant species, followed by *B. zonata*

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Table 1. Species proportion of fruit flies emerged from guava fruits

Species	Rearing cage 1	Rearing cage 2	Rearing cage 3	Rearing cage 4	Total	Per cent
Bactrocera zonata	36	42	32	44	154	31.36
B. dorsalis	56	45	52	48	201	49.95
B. correcta	20	27	30	21	98	19.95
B. verbascifoliae	8	10	13	7	38	7.73

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RESEARCH NOTE

Report on the occurrence and biology of *Thalassodes pilaria* Guenée (Lepidoptera: Geometridae) on litchi (*Litchi chinensis* Sonn.) in Bihar, India

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Litchi or lychee (*Litchi chinensis* Sonnen) is one of the most important subtropical fruit trees of the family Sapindaceae. The translucent, flavoured aril or edible flesh of the litchi is popular as a table fruit in India, while in China and Japan it is preferred in dried or canned state. India is the second largest producer of litchi in the world after China with an area and production of 82,000 ha and 555,000 tonnes, respectively during 2012-13 (DAC, 2013). Pests are one of the major constraints affecting production and quality of litchi. In recent years, a change in pest dynamics has been observed in litchi (Kumar *et al.*, 2011). The occurrence of a green looper, identified as *Thalassodes pilaria* (Lepidoptera: Geometridae) was recorded on litchi trees in Bihar state during scouting surveys of farmer fields. The moths are commonly called Big emerald. The population of the pest was monitored in a fixed plot at National Research Centre for Litchi (NRCL) Experimental Farm during 2012. The mean number of insects (count) per 100 leaves was recorded.

Rearing of the pest was done in laboratory on litchi leaves at a temperature of 28 ± 1°C, relative humidity 70 ± 5% and photoperiod 12:12 h L:D conditions. The young larvae (1st and 2nd instars) were collected from field and reared in the laboratory in Petri dishes (18 cm diam.). The pupae were transferred to insect rearing jar (8.5 cm × 8.5 cm × 15 cm) for emergence of adults. A filter paper disc with a cotton swab dipped in honey was kept in each rearing jar. Morphometric measurements of different stages were taken and biology was studied. Insect morphology was studied with the help of a stereo-binocular microscope. The data on looper counts were subjected to analysis of variance (ANOVA) after square root transformation. The least significant differences (LSD) between means at 5% significance level (P = 0.05) and the standard error (SE) of means were computed. The diagnostic symptoms of damage and characteristics of the pest as well as summaries of studies conducted to assess the occurrence of pests in litchi are described.

The larvae of *T. pilaria* fed on tender foliage and resembled a green stick similar to the midrib of leaves or thin shoots that served as a camouflage for the pest (Fig. 1). Studies on the biology of the pest revealed that larvae were green resembling to colour of young leaves which gradually changed to darker green as the leaves became older. The dimensions of fully grown larvae (last instar) were approximately 2 mm wide and 3.6-4.3 cm (mean 3.86 ± 0.109) long. The width of head capsules in last instar larvae were 1.4-1.6 mm (mean 1.52 ± 0.055). The pupae were 1.4-1.7 cm (mean 1.54± 0.047) long and the adults with fully spread wings were 2.7-3.0 cm (mean 2.83 ± 0.047) wide. The developmental period from larva to adult was completed in 18-23 days (mean 20.11 ± 0.962), out of which the larval period was 9-13 days (mean 10.89 ± 0.981), the pupal period was 6-7 days (mean 6.67 ± 0.272) and adult period was 2-3 days (mean 2.56 ± 0.192). The adults, however, failed to mate in the laboratory (temp. 28 ± 1 °C, RH 70 ± 5%, and 12:12 h L:D. The newly formed pupa was greyish brown in colour. The adult moth had sea-green colour wings which were semitransparent having fine white lines (Fig. 2). Fore wing was broad, triangular and leading edge was yellowish brown, crossed by two distinct white lines and a number of faint transverse marks which was also white. The distinct fine white lines continued up to terminal edge of hind wings, converging to form a 'W' ¾ way along length. The hind wings had an angular margin. Underside of the moth was greenish white. Legs and antennae were yellowish brown. The first half of antennae was feathery while second half was threadlike.

The period of occurrence of this pest was from July to December. The peak infestation was observed from 15th September to 15th October that coincided with the occurrence of another geometrid pest of litchi, *Perixera illepidaria* Guenée. During September and October 2012,

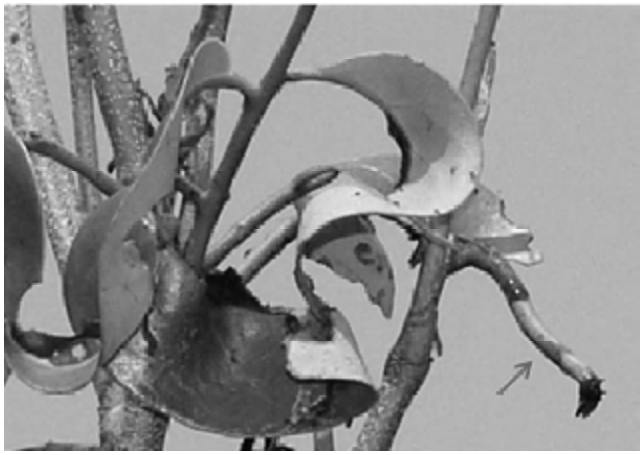

Fig. 1. Larva of *T. pilaria* feeding on young leaves of litchi.

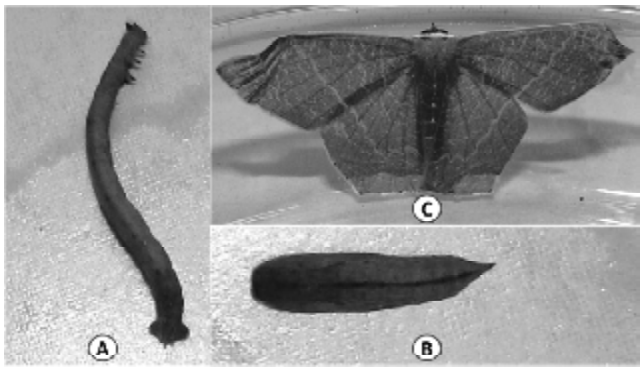

Fig. 2. The developmental stages of *T. pilaria*: A. Larva, B. Pupa and C. Adult

the mean temperatures were 25.3-32.2 °C and 21.1-31.6 °C, and the mean relative humidities were 70.6-89.7% and 56.4-84.8%, respectively. The photoperiod conditions at experimental farm of NRCL on 15th September, and 15th October was 12.18:11.42 h L:D and 11.33:12.27 h L:D, respectively. The data on larval count at 15 days interval during 2012 are given in Fig. 3. A statistically significant difference in mean larval counts per 100 leaflets (SEm 0.34 ± 0.12, $P = 0.05$) at different interval during the period of occurrence was observed. It was evident from the data that the mean number of looper larvae per 100 leaflets significantly varied from 1.66 to 13.56. The highest mean count (13.56/100 leaflets) was on 15th October in the litchi orchard block at NRCL Farm. The population increased after 15th July and and decreased significantly after 30th November.

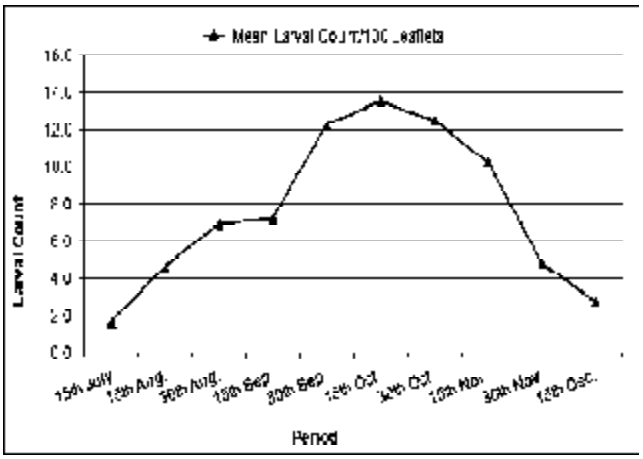

Fig. 3. Temporal distribution of *T. pilaria* in litchi at Muzaffarpur

The species, *T. pilaria* was first described by Guenée (1857). The adult moths of this species are members of the group called 'Emeralds'. This species occurs across the south Pacific basin, including Fiji, Society Islands, Tahiti, Queensland in Australia (Anonymous, 2011), Indonesia (Sulawesi), Vanuatu, and Rarotonga on Cook Islands (Anonymous, 2007). This is a polyphagous pest feeding on several host tree species and is known to damage litchi in New Caledonia (Anonymous, 2007). Hung *et al*. (2006) had recorded a related species, *Thalassodes immissaria* Walker feeding on litchi (*L. chinensis*) and longan (*Dimocarpus longans*) in Taiwan. So far, this species has not been reported from India as a pest of litchi. Though, the incidence of this pest currently is not at an alarming level, with the change of the cropping pattern vis-à-vis climate change, there is a chance that the pest status of this species in litchi ecosystem may also change and hence warrants a regular monitoring.

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RESEARCH NOTE

Field efficacy of insecticides and integrated pest management modules against litchi leaf roller, *Dudua aprobola* Meyrick

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Litchi (*Litchi chinensis* Sonn) is attacked by more than 54 insect and mite pests (Singh *et al.*, 2001) and the litchi leaf roller, *Dudua aprobola* Meyrick is one of the economically important pests. It causes severe damage to litchi foliage in Bihar, Uttar Pradesh and West Bengal (Singh, 1971; Chakraborty and Samanta, 2005). The peak infestation period of the pest was recorded during July to February when new flush is available and its restricted breeding takes place during off-season (March - April) on alternate hosts such as Kath - Jamun (*Zizigium jambolona*) and Chhota amaltas of litchi orchards (Lall and Mallik, 1976). In order to evaluate the effects of pesticides alone and in combination with other pest management tools against the litchi leaf roller, *D. aprobola*, a field trial was carried out in randomized block design (RBD) at the research farm of Rajendra Agricultural University, Bihar, Pusa during the years 2010 -11 and 2011-12.

The infested leaves and shoots were pruned and destroyed by burning in the month of June and November in both the years. The cakes were applied during the first fortnight of July in both the years. All the three pesticides were sprayed alone or in combination with other tools, twice at the time of emergence of new flushes (September-October). After the application of treatments, mean per cent leaf infestation was worked out by sampling and counting the infested twigs randomly. The fruit yield (kg / tree) was worked out at the time of fruit harvest and finally their observations were statistically analyzed. The cost effectiveness of the treatments was also worked out on the basis of their benefit - cost ratio.

Studies revealed that spraying of dichlorvos (0.07%) twice at the time of flush (September and October) along with pruning and destruction of affected twigs and soil application of neem and castor cake (4 + 1 kg / tree), after first shower in the first fortnight of July afforded maximum protection to the litchi tree from the incidence of litchi leaf roller and recorded lowest leaf infestation (6.10 and 4.70 per cent) followed by spraying of carbaryl (0.2 %) along with pruning and application of cakes during 2010 – 11 and 2011 – 12 (Table - 1). Spraying of dichlorvos (0.076 %) alone also recorded lower leaf infestation (7.80 and 6.80 per cent) compared to fenpropathrin (0.03 %) either in combination with pruning and cakes (10.30 and 10.50 per cent) or in alone (12.70 and 13.60 per cent) and spraying of carbaryl (0.2 %) alone (11.70 and 9.00 per cent). The treatment of pruning and destruction of affected twigs twice (June and November) recorded, 28.70 per cent and 25.90 leaf infestation but when this treatment was combined with soil application of cakes recorded slightly lower mean per cent leaf Infestation (20.00 and 21.40 per cent). However, all the treatments were found significantly superior over the untreated control (44.30 and 49.50 per cent) while the interaction of treatments with year had no significant effect when tested under pooled analysis whereas the treatments differed significantly. Similarly, fruit yield (kg / tree) was recorded maximum with the application of spraying of dichlorvos (0.076 %) combination (125.60 and 119.60 kg. per tree) followed by spraying of carbaryl (0.2 %) combination recording fruit yield 117.80 and 113.80 kg per tree, respectively. However, the other treatments did not vary significantly with fruit yield due to pest incidence. It is obvious that the severity of pest incidence was restricted to newly emerged leaves (Lall and Rahman, 1975) and spraying dichlorvos (0.076 %) combination afforded maximum protection from the pest and recorded maximum fruit yield and proved to be cost effective (C : B = 1: 9) also. Due to its higher toxicity (LD_{50} = 80 mg / kg) dichlorvos cannot be suggested for the litchi fruit crop. The other pesticide, carbaryl is comparatively less toxic (LD_{50} = 850 mg / kg) and also afforded effective protection (8.70 and 5.90 per cent leaf infestation) to the pest and was cost effective (C / B = 1: 7.3) when sprayed in combination of pruning and cake application. Thus the application of carbaryl with its combination can be preferred for effective and sustainable pest management strategy against litchi leaf roller, *D. aprobola*

Table 1. Effect of pest management modules against litchi leaf roller, *Dudua aprobola* and fruit yield during 2010 - 11 and 2011 - 12

	Treatment	Mean leaf infestation (%) (2010 - 11)	Yield (kg / tree)	Mean leaf infestation (%) (2011 - 12)	Yield (kg / tree)	Mean leaf infestation (%) (Pooled)	Yield (kg / tree) (Pooled)	Benefit : Cost ratio
T_1	Pruning and destruction of all the affected twigs/ leaves by burning during June and November	28.70 (31.60)	88.80	25.90 (30.50)	82.60	27.30 (31.10)	85.70	6.5
T_2	T_1 + soil application of neem cake (4 kg) + castor cake (1 kg) just after 1st shower of rainfall	20.00 (26.10)	93.70	21.40 (27.40)	87.10	20.70 (26.30)	90.40	4.7
T_3	T_2 + spraying of fenpropathrin 10 EC (0.01 %)	10.30 (18.00)	105.10	10.50 (18.50)	101.30	10.40 (18.40)	103.20	5
T_4	T_2 + spraying of carbaryl 50 WP (0.2 %)	8.70 (16.50)	117.80	5.90 (13.50)	113.80	7.30 (15.00)	115.80	7.3
T_5	T_2 + spraying of dichlorvos 76 EC (0.076 %)	6.10 (13.40)	125.60	4.70 (11.60)	119.60	5.40 (12.50)	122.60	9
T_6	Spraying of fenpropathrin 10 EC (0.01 %)	12.70 (20.60)	80.80	13.60 (21.20)	74.20	13.20 (20.90)	77.50	08
T_7	Spraying of carbaryl 50 WP (0.2 %)	11.70 (19.60)	83.80	9.00 (17.20)	74.80	10.30 (18.30)	80.30	1.9
T_8	Spraying of dichlorvos 76 EC (0.076 %)	7.80 (15.70)	115.80	6.80 (13.70)	90.50	7.30 (14.70)	92.60	8.4
T_9	Untreated control	44.30 (41.70)	94.70	49.50 (44.70)	71.20	46.90 (43.20)	73.90	-
CD (P = 0.05)		(7.98)	21.39	(6.34)	26.25	(7.40)	28.68	-
CV	(20.42)	12.83	(16.63)	16.74	(16.95)	15.67	-	
Year x treatment		-	-	-	-	NS	NS	-

Values in parenthesis ( ) are angular values

Lall and Rahman (1975) observed that pruning and destruction and soil application of cakes and ploughing eliminate the shelter places and alternate hosts of the pest and thereby alter the favourable ecology for the pest. Lall and Mallik (1976) and Hameed *et al.* (1999) reported that the spraying of dimethoate (0.045 %) recorded maximum reduction of the pest population (up to 69.42 %). Sahoo and Maiti (1992) and Waite and Elder (1996) also observed that spraying of carbaryl, fenitrothion, endosulfan and phosphamidon were effective against this pest. But in the present situation, the above measures could be less effective as many of these insecticides were either restricted (endosulphan, phosphamidon) or banned because of their harmful effects on non targets. While in the present study both cultural and safer pesticides were combined together and tested against the pest activity and significant results were obtained. Thus, the findings of the present study have definitely contributed towards the effective pest management strategy developed earlier by several workers.

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RESEARCH NOTE

Efficacy of insecticides and NSKE against American serpentine leaf miner, *Liriomyza trifolii* Burgess on cucumber (*Cucumis sativus* L.)

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Cucumber (*Cucumis sativus* L.) is one of the most widely grown vegetable crops during *kharif* and summer season in all parts of the country. In Maharashtra, cucumber is grown in all the districts. American serpentine leaf miner (*Liriomyza trifolii*) has attained a pest of economic importance in the recent past. Control of leaf miner with insecticides is usually difficult because of its biology *i.e.* short life cycle, smaller in size and high mobility of adults, relatively long pupal stage in soil, high reproductive capability *i.e.* fecundity and concealed larval stages. In addition, mines created by larvae remain on the leaf as long as the leaf survives. Different conventional insecticides were tried for the control of serpentine leaf miner infesting cucumber (Wakchaure,1998 and Nadagouda *et al.*, 2010). The conventional insecticides recommended against this pest gave low to moderate control and hence there is a need to test newer insecticides and with this the present study was undertaken.

The field experiment was conducted during 2011 at Entomological Section, PGI Block, MPKV, Rahuri. All the recommended agronomical practices were followed in raising the crop and uniform plant population was maintained .The experiment was laid out in a randomized block design with three replications and nine treatments including an untreated control. The observations on live mines were recorded on randomly selected three plants in each plot. Pre-treatment counts of *L. trifolii* were recorded one day before the insecticides applications and subsequent observations for post treatment counts were recorded on 7thday after insecticidal application of each spray. Three sprays were applied at 14 days at an interval .The difference of live mines between the pre count and post count observations was worked out. The data on per cent mortality of maggot of *L. trifolii,* per cent decline of damaged leaves of cucumber and yield were statistically analyzed.

The data on the per cent mortality of maggot (percent decline of live mines) influenced by various insecticidal treatments are presented in Table 1. The data revealed that all the insecticidal treatments found significantly superior over untreated control and it was at par with each other. Amongst the insecticides, abamectin 1.9 EC at (0.00057%) significantly reduced maggot population of leaf miner by 65.68, 71.90 and 75.26 percent mortality on 7thdays after application of each spray at1st, 2nd and 3rd spray ,respectively followed by cartap hydrochloride 50 SP at 0.05 percent (62.40, 68.66 and 73.68%) and it was at par with abamectin. The rest of the treatments *viz.*, NSKE percent (60.06, 64.43 and 67.55%), fipronil 5 SC at 0.0075 percent (59.76, 65.86 and 70.40%), spinosad 45 SC at 0.018 percent (57.28,64.06 and 67.66%), triazophos 40 EC at 0.08 percent (57.03,61.93 and 65.59%), imidacloprid 17.8 SL at 0.0071 percent (56.48, 59.35 and 59.30%) and carbosulfan 25 EC at 0.025 percent (53.21%)also effective in minimizing the pest population on 7thdays after each spray application *i.e.*1st, 2nd and 3rd spray, respectively.

Mean of per cent mortality of maggots due to three sprays ranged from 56.36 to 70.95% in different treatments. The highest mortality (70.95%) of maggot was recorded with abamectin 1.9 EC at (0.00057%) which was at par with cartap hydrochloride 50 SP at 0.05 percent (68.25%). The effectiveness of remaining treatments in decreasing order was fipronil (65.34%)>NSKE (64.01%)> spinosad (63.00%) > triazophos (61.52%) > imidacloprid (58.38%) > carbosulfan (56.36%).

The data of mean percent decline in damaged leaves of cucumber influenced by various insecticidal treatments are presented in Table 2. The results revealed that all the treatments gave significantly higher per cent decline in

Table 1. Efficacy of different insecticides against *L. trifolii* on cucumber

Treatment	Percent mortality of maggot at 7 days after sprays			
	First spray	Second spray	Third spray	Mean
Abamectin 1.9 EC (0.00057%)	65.68(54.15)	71.90(58.00)	75.26(60.18)	70.95(57.44)
Cartap hydrochloride 50 SP (0.05%)	62.40(52.18)	68.66(55.97)	73.68(59.15)	68.25(55.77)
Spinosad 45 SC (0.018%)	57.28(49.19)	64.06(53.17)	67.66(55.35)	63.00(52.57)
Fipronil 5 SC (0.0075%)	59.76(50.63)	65.86(54.27)	70.40(57.06)	65.34(53.99)
Imidacloprid 17.80 SL(0.0071%)	56.48(48.73)	59.35(50.39)	59.30(50.36)	58.38(49.83)
Carbosulfan 25 EC(0.025%)	53.21(46.85)	58.41(49.84)	57.47(49.30)	56.36(48.66)
Triazophos 40 EC(0.08%)	57.03(49.04)	61.93(51.90)	65.59(54.10)	61.52(51.68)
NSKE 5%	60.06 (50.81)	64.43(53.39)	67.55(55.28)	64.01(53.16)
Untreated control	00.00(00.00)	00.00(00.00)	00.00(00.00)	00.00(00.00)
SEm ±	0.99	0.79	0.72	0.83
CD at 5%	3.05	2.44	2.21	2.57

* Figures in parenthesis are arcsine transformed values.

damaged leaves over untreated control. It was ranged from (19.43 to 55.34%). The significantly higher per cent decline in damaged leaves (55.34%) was recorded in abamectin 1.9 EC at (0.00057%) followed by cartap hydrochloride 50 SP at 0.05 percent (48.71%). The next subsequent better insecticide was fipronil 5 SC at 0.0075 percent (44.04%). The rest of the insecticides *viz.*, NSKE (5%), spinosad 45 SC at (0.018%), triazophos 40 EC at (0.08%) and imidacloprid 17.8 SL at 0.0071 percent were (39.76, 34.59, 29.29 and 21.99%) decline in damaged leaves of cucumber, respectively.The lowest percent decline in damaged leaves (19.43%) was observed in carbosulfan 25 EC at 0.025 percent.

The yield data revealed that all the treatments gave significantly higher yield over untreated control. Amongst the insecticides, abamectin 1.9 EC at (0.00057%) recorded significantly higher yield (122.66 q/ha) than the untreated control (52 q/ha). The next subsequent better yield was cartap hydrochloride 50 SP at (0.05%), fipronil 5 SC at (0.0075%), NSKE (5%), spinosad 45 SC at (0.018%), triazophos 40EC at (0.08%), imidacloprid 17.8 SL at (0.0071%) and carbosulfan 25 EC at (0.025%) recorded 114.66, 105.33, 97.33, 93.33, 89.66, 86.66 and 82.66 q/ha, respectively over untreated control.

In the present investigation, the highest benefit cost ratio of 8.72 was recorded with cartap hydrochloride 50 SP at (0.05%) followed by NSKE (5%) (8.67) and abamectin 1.9 EC at 0.00057 percent (7.12). The least cost: benefit ratio (0.40) was obtained from spinosad 45 SC at (0.018%).

The present findings are in conformity with several workers who reported that these insecticides reduce the infestation of *L. trifolii* on various other crops. (Eswarareddy *et al.*(2004) and Ramesh and Ukey (2007) reported efficacy of abamectin 1.9 EC on tomato while Hanumappa (2006) found cartap hydrochloride 50SP (0.05%) most effective on cucumber. Similarly Ganapathy *et al.* (2010) reported NSKE (5%) and triazophos 40 EC (0.04%) on cowpea and Nadagouda *et al.* (2010) reported imidacloprid17.8 SLon cucumber. Saad *et al.* (2007) reported carbosulfan 25 EC on garden beans. These reports lend support to the present findings. The results indicate that NSKE offers an economically viable solution comparable to that of certain new molecules available in the market and could be used in IPM of serpentine leaf miner on cucumber.

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Table 2. Effect of different insecticides on damaged leaves caused by *L. trifolii* on cucumber

Treatment	Damaged leaves			Yield (q/ha)	Benefit/ Cost ratio
	Pre-count	Post count (Mean of 3 sprays)	decline of damaged l eaves (%)		
Abamectin 1.9 EC (0.00057%)	42.35 (40.60)*	18.91 (25.16)	55.34 (38.03)	122.66	7.12
Cartap hydrochloride 50 SP (0.05%)	42.32 (40.55)	21.70 (27.40)	48.71 (32.42)	114.66	8.72
Spinosad 45 SC (0.018%)	44.60 (42.28)	29.17 (32.59)	34.59 (22.90)	93.33	0.40
Fipronil 5 SC (0.0075%)	44.63 (42.79)	24.97 (29.73)	44.04 (30.51)	105.33	4.37
Imidacloprid 17.8 SL (0.0071%)	43.17 (39.08)	33.67 (35.44)	21.99 (9.30)	86.66	3.72
Carbosulfan 25 EC (0.025%)	43.24 (39.10)	34.83 (36.15)	19.43 (7.54)	82.66	4.24
Triazophos 40 EC (0.08%)	43.19 (43.06)	30.54 (33.48)	29.29 (22.25)	89.33	3.15
NSKE 5%	44.94 (42.57)	27.07 (31.18)	39.76 (26.75)	97.33	8.67
Untreated control	44.98 (44.04)	00.00 (00.00)	00.00 (00.00)	52.00	-
SEm ±	1.25	0.32	-	1.48	-
CD at 5%	NS	1.00	-	4.58	-

* Figures in parenthesis are arcsine transformed values.

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RESEARCH NOTE

Bio-efficacy of non-conventional pesticides against *Tetranychus urticae* Koch infesting ashwagandha (*Withania somnifera* Dunal)

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Withania somnifera Dunal, commonly known as *ashwagandha*, a native of Mediterranean region is an important medicinal plant of family Solanaceae grown widely in arid regions of Rajasthan. Due to rapid expansion for drug industry during recent past, commercial cultivation is also picking up. Among different pests reported on *ashwagandha*, two spotted mite, *Tetranychus urticae* Koch is an the economically important one. The mite inflicts damage to crop by sucking cell sap from leaves and result in yellowing of leaves with stunted growth, ultimately deteriorate the medicinal quality (Rolania and Sharma, 2007 and Anonymous, 2012). Since insecticides are not desirable for use on medicinal plants, there is a need to explore alternative means of management. A number of plants is to possess pesticide properties against various insect and mite pests. Besides, the use of cow urine for the control of insect and mite pests of field crop has also been reported from different parts of India. In order to reduce the frequency of chemical pesticides, it is essential to test the efficacy of alternative compounds. We evaluated certain botanicals and cow urine in comparison to standard insecticides for their efficacy against *T. urticae*.

There were eight treatments replicated thrice including cow urine, plant products and two chemical checks besides an untreated check. The pesticides were sprayed in evening hours on the crop with the help of foot sprayer, when the mite population was sufficiently build up. Only one spray was applied in the third week of December, 2011. The observations on mite population in the experimental area were recorded just before spraying (pre-treatment) and one, seven, and fifteen days after treatment on three randomly selected plants per plot. Three leaves each from top, middle and bottom portions were randomly plucked from these selected plants commencing from the mite infestation and were observed with the help of hand lens. From the data obtained, the per cent population reduction in mite population was calculated by applying a correction factor given by Henderson and Tilton (1955). Data were analysed using ANOVA.

The data presented in Table 1 indicate variation among the efficacy of different pesticides in reducing mite population. The NSKE, neem oil and *karanj* seed extract exhibited a reduction in mite population to the tune of 39.67, 35.33 and 35.12%, respectively, after one day of treatment which was followed by cow urine (28.33%), *Calotropis* leaf extract (33.00%) however, statistically remained at par with each other. *Datura* leaf extract (23.05%) stood at lowest order with respect to the reduction in mite population. The chemical checks, dicofol (64.22%) and ethion (80.97%) were the most effective treatments and reduced the mite population significantly. After seven days of treatment also dicofol and ethion resulted in the highest percent reduction in the mite population (88.97 and 87.02 per cent, respectively) and were at par. Among other treatments, NSKE (47.67%), neem oil (39.02%), cow urine (37.97%) and *karanj* seed extract (37.20%) proved moderately effective with non-significant difference. The least effective treatments were *Calotropis* leaf extract (34.33%) and *datura* leaf extract (33.33%). However, non-significant difference was observed between the treatments of neem oil, cow urine, karanj seed extract, *Calotropis* leaf extract and *datura* leaf extract.

After 15 days of spray, NSKE (26.97%), neem oil (24.20%), karanj seed extract (23.33%) and cow urine (21.20%) remained at par with each other and stood in middle order of efficacy. The treatments of *Calotropis* leaf extract (17.02%) and *datura* leaf extract (16.25%) stood at low order with respect to the reduction in the mite population but were comparable with cow urine. dicofol (61.97%) emerged as the most effective treatment and reduced the mite population significantly over the other treatments. The treatment of ethion could reduce the population by 58.67 per cent and was found at par with dicofol. The mean efficacy data indicated that the treatments of NSKE (40.36%), neem oil (35.03%)

Table 1. Comparative efficacy of pesticides against mite, *Tetranychus urticae* Koch infesting *ashwagandha*

Treatment	Conc. (%)	Per cent reduction in mite population at days after treatment				Yield (q ha^{-1})	B/C ration
		One	Seven	15	Mean		
Cow urine	5.0	28.33 (32.16)	37.97 (38.04)	21.20 (27.42)	30.67 (33.63)	2.30	6.25
Neem oil	2.0	35.33 (36.47)	39.02 (38.66)	24.20 (29.47)	35.03 (36.29)	2.70	10.94
NSKE	5.0	39.67 (39.04)	47.67 (43.66)	26.97 (31.29)	40.36 (39.44)	2.80	16.66
Karanj seed extract	5.0	35.12 (36.34)	37.20 (37.58)	23.33 (28.88)	33.55 (35.40)	2.60	12.50
Datura leaf extract	5.0	23.05 (28.69)	33.33 (35.26)	16.25 (23.77)	27.38 (31.55)	2.10	2.08
Calotropis leaf extract	5.0	23.33 (28.88)	34.33 (35.87)	17.02 (24.37)	28.13 (32.03)	2.20	4.17
Dicofol 18.5 EC (Check-1)	0.05	64.22 (53.26)	88.97 (70.60)	61.97 (51.93)	76.24 (60.85)	3.90	16.80
Ethion 50 EC (Check-2)	0.05	57.33 (49.21)	87.02 (68.88)	58.67 (49.99)	71.22 (57.56)	3.50	28.82
Control		0.00	0.00	0.00	0.00	2.00	
S.E.m±		1.79	2.44	1.43	1.73		
CD at 5%		5.44	7.41	4.33	5.26		

*Figures in parentheses are angular transformed values

and *karanj* seed extract (33.55%) were at par though showed significantly less efficacy compared to insecticides. The cow urine (30.67%), *Calotropis* leaf extract (28.13%) and *datura* leaf extract (27.38%) were found in lowest order of efficacy with respect to reduction in mite population. The efficacy of treatments excluding insecticides in ascending order was: *Datura* leaf extract, *Calotropis* leaf extract, cow urine, *karanj* seed extract, neem oil and NSKE. The present results are in agreement with the findings of Ramaraju (2004), Naga (2008) and Kumawat (2011).

The yield was maximum (3.90 q ha^{-1}) in the treatment of dicofol . The yield was minimum (2.10 q ha^{-1}) in *datura* leaf extract treatment. The *ashwagandha* yield drastically reduced in untreated check plots (2.0 q ha^{-1}). ha^{-1}). Though, less effective against mites compared to chemicals, NSKE recorded an impressive benefit cost ration of 16.66 and on par with dicofol. Hence it can be summarized that NSKE and neem oil can be included in IPM package against mites in *ashwagandha* to minimize chemical usage.

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RESEARCH NOTE

Report of a larvi-pupal parasitoid, *Diachasmimorpha* sp. (Hymenoptera: Braconidae) associated with fruit fly, *Bactrocera caryeae* (Kapoor)

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Fruit flies (Diptera : Tephritidae) are important pests of several fruits and vegetables worldwide. *Bactrocera caryeae* is one of several economically important species of fruit flies belonging to the *B. dorsalis* complex. In India its distribution was reported from the Western Ghats region from South India. The species is restricted to a narrow strip along the west coast of South India (Verghese *et al.*, 2006). Eggs of *B. caryeae* hatch in 1-3 days, larvae feed for up to 35 days, pupate in the soil and adult emerges after 1-2 weeks (Christenson and Foote, 1960). The infested fruits show ovipositional punctures and later due larval feeding the fruits become soft and rot. Information on the natural enemy complex of fruit flies in general and *B. caryeae* in particular is lacking. This limits the scope of using the tool of biological control. Studies were conducted at Central Horticultural Experiment Station (CHES) Chettalli, Kodagu to document natural enemies of *B. caryeae*.

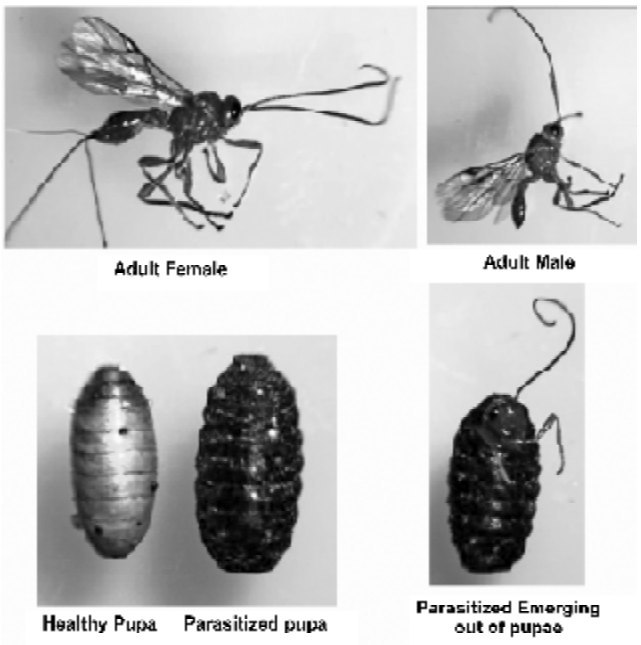

Fig. 1. *Diachasmimorpha* sp. parasitization on pupae of *B. caryae*

Fruit fly infested tropical fruits like *citrus* sp., sapota, avocado, Malayan apple, egg fruit, west Indian cherry and wild fruits like *Spondia dulcis* etc. were collected from the experimental fields and kept for rearing to know the parasitoids associated with fruit flies. A parasitoid, *Diachasmimorpha* sp. (Hymenoptera: Braconidae: Opiinae) was reared from *B.carayeae* infesting the wild host *Spondia dulcis* (Pickle weed). Fruits of *S. dulcis* are known to have food flavouring properties. Around 186 fruits were collected and kept in soil for adult emergence. From these, 250 pupae were collected. It was observed that there was cent per cent parsitization of *B. caryeae* pupae in the month of April 2013 but in the following month, no parasitoids could be recovered. *Diachasmimorpha* sp. was confirmed to be larvi-pupal parasitoid *i.e.* a parasitoid which deposits eggs on the larvae and emerges from the pupae. The parasitized pupae turned black (Fig. 1).

The distribution of *Diachasmimorpha* sp. was reported from Nearctic and Northern neotropical regions, Indo Australian region and Afrotropical region. There are several group species of *Diachasmimorpha* spp.The major species reared from fruit infesting tephritids are *D. Aino, D.carinata, D. fullawayi, D. longicaudata* and *D. Mexicana* (Veireck, 1913a). *Diachasmimorpha* sp. was widely distributed for biological control in Indo-Australia group species namely *D. longicaudata* and *D. tryoni*. These two were described as the most important species for biocontrol by their apical ovipositor (Wharton and Gilstrap 1983, Clausen 1978, Ovurski *et al.*, 2000). X-ray irradiated (that prevented adult fly emergence) larvae of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) was utilized as rearing hosts for the parasitoid, *D. longicaudata* (Ashmead) rearing (Mariana Mabel Viscarret *et al.*, 2012).

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RESEARCH NOTE

Visualization of spread of papaya ring spot virus using "raster" package in r statistical environment

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Papaya (*Carica papaya* L.) is an important tropical fruit crop with an increasing market as a health food. India is the leading papya producer. The Papaya Ring Spot Virus (PRSV) is emerging as a major limiting factor in the production of Papaya. The PRSV causes mosaic on the leaves, leading to malformed leaves. Water soaked lesions appear on the stem, fruit and flower drop would be seen, Malformed fruits appear with characteristic rings on the fruits. Affected plants become unproductive and would die soon after. The PRSV had been extensively described (Singh, 2003 and Tripathi *et al*., 2008) and is vectored in a non-persistent manner, by aphids *Aphis gossypii* Glover, *Myzus persicae* (Sulzer) and *A. craccivora* Koch (Kalleshwaraswamy and Kumar, 2008). The spread of PRSV would be quite rapid in the field as the rate of spread determines the economic loss caused and it is necessary to visualize this spread. An attempt was made to visualize these parameters using R software which is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS, It is available for free download at (http://www.r-project.org). It has the advantage of providing a free alternative to costly commercial software. Geographic Information Systems (GIS) can be used to effectively visualize spatial data. Raster is a package which runs under R and it was used to visualize the spread through charts. raster uses a spatial (geographic) data structure that divides a region into rectangles called as 'cells' (or 'pixels') that can store one or more values for each of these cells. Such a data structure could also referred to as a 'grid' and is often contrasted with 'vector' data that is used to represent points, lines, and polygons. The raster package has functions for creating, reading, manipulating, and writing raster data. The package provides, general raster data manipulation functions that can easily be used to develop more specific functions. It also implements raster algebra and most functions for raster data manipulation that are common in GIS systems.

The PRSV disease incidence was recorded on an increasing scale of 1-9 as was present in an 11x43 plot of papaya field infected with PRSV, at various time points. The data were entered in a Microsoft Excel 2013 worksheet in a r x c matrix. The disease incidence was recorded at seven different time periods *viz* 3 Jan 2012, 18 Jan 2012, 25 Jan 2012, 15 Feb 2012, 23 Feb 2012, 1 March 2012, 8 March 2012. Each time period was recorded in a separate work sheet. The changeover in disease incidence was recorded by subtracting the disease incidence value at the (n^{th}) time point from the ($n\text{-}1^{th}$ time point), this difference was saved in a separate sheet and indicated the changeover of disease incidence at that time point. All the data recorded Microsoft Excel 2013 worksheet files were finally saved as *.csv (coma separated files) as R and the program raster can read *.csv files natively.

Using a Personal Computer with Intel(R) Core(TM) i7-2600K CPU @ 3.40GHz, with 32 GB RAM, a NVIDIA GeForce GTX 560 Ti video card, with 3 x 2 TB drives and running Windows 8.1 64 bits the collected data was analyzed. First "R version 3.02 64 bits" was installed and then the package raster was installed from CRAN. The charts were made by issuing the following commands.

```
x <- read.csv (file.choose()) # helps to choose the data file
require(raster) # raster is the package for the analyses
b <- as.matrix(x) # changes the data to a matrix
r <- raster( b ) # raster computes the figure matrix
  plot( r, axes=F ) # plots and the figure to the screen
```

Command x <- read.csv would assign the csv file values to the variable x, and the command file.choose (), would help to choose the correct csv file using the windows explorer as the file manager.

Require (raster) command would load raster

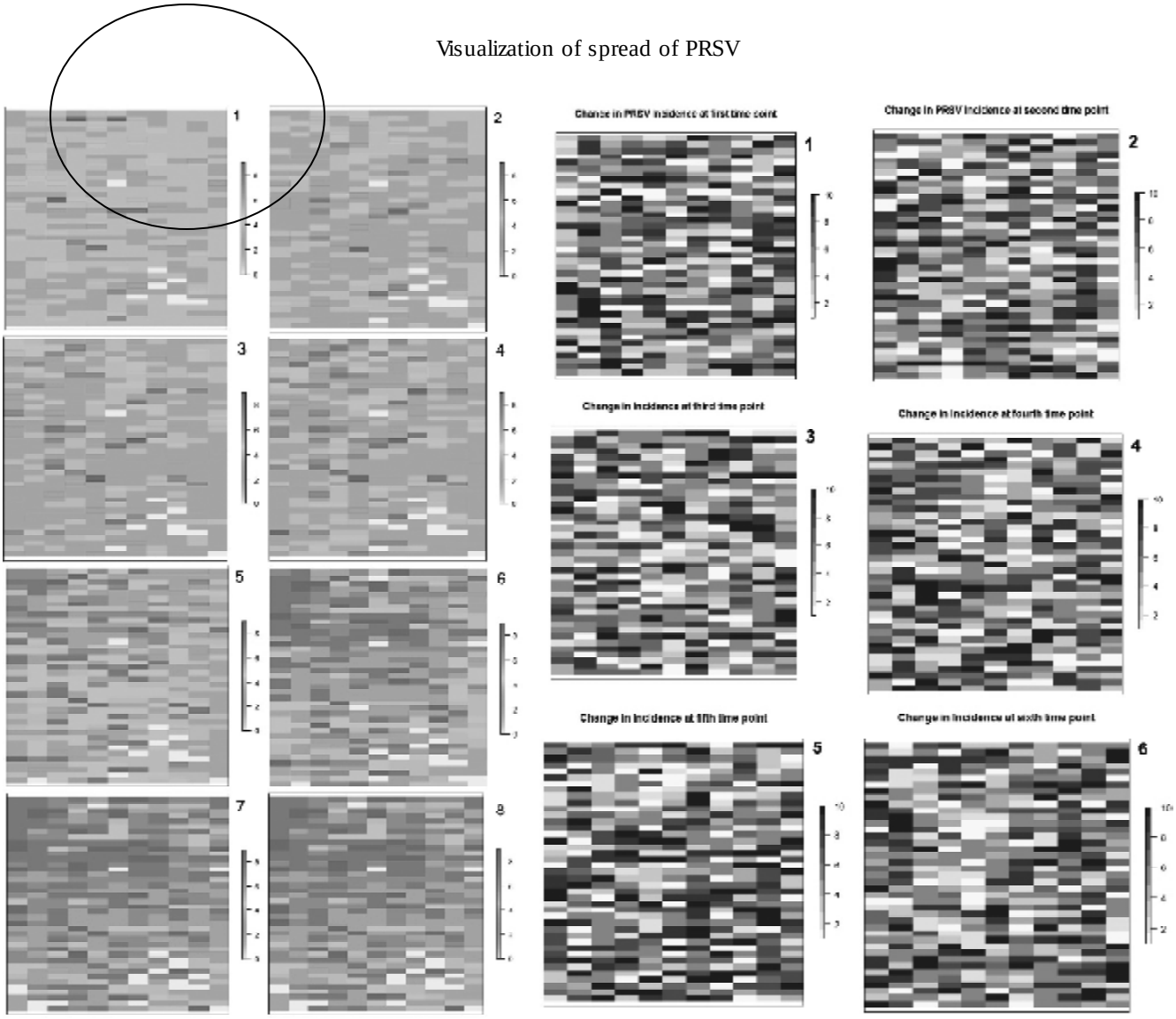

Fig. 1. PRSV infection at 7 time points, (sub charts 7 and 8 are identical and are the final measured values) Right; Rate of change of PRSV infection at 6 time points. Time points are 3 Jan 2012, 18 Jan 2012, 25 Jan 2012, 15 Feb 2012, 23 Feb 2012, 1 March 2012, 8 March 2012 have been indicated by numerals (1-7) at top left position in each sub chart.

package, and the following commands b <- as.matrix(x), would assign the variable (b) as a matrix of (x), and the variable (r) as a raster object of (b) (which itself is a matrix of (x)), Plot (r, axes = F) command would plot the raster object (r), without showing the axes. The above mentioned procedure was repeated for each spread sheet containing the disease incidence and the changeover in disease incidence spread sheets.

The results in the form of color coded graphs revealed that, PRSV spread in the field was non uniform and there was an increase in the rate of spread in certain pockets seen across the field (Fig.1). The PRSV spread was observed to originate from the lower right corner which might have been due to prevailing wind direction. No gradient type of spread as well as the infected plant to non-infected plant spread patterns was also not seen. This in totality meant that PRSV infections were completely random. This randomness could be due to random alignment of aphid vectors and/or PRSV infection was carried over from nursery. In view of the PRSV spread as visualized, to manage PRSV it would be very necessary that initial infections would have to be contained as they were seen to be more important as compared to lateral infections. Hence use of papaya seedlings grown in virus free conditions would be beneficial. The rate of PRSV infection increased after the 3rd time point and stabilized after the 6th time point at which nearly 85 % of the plant got infected. Lecoustre *et al.* (1989) analyzed and mapped the spatial spread of African cassava mosaic virus using geo statistics and the kriging technique and recorded that geostatistics can be

applied to other viral and non-viral plant diseases. Moreno *et al* (2007) analyzed the temporal and spatial patterns of spread of Lettuce Mosaic Virus which is transmitted like PRSV in a semi persistent manner by aphid vectors. Cumulative spatial data for infected plants at different growth stages were analysed using spatial analysis by distance indices. The results that secondary cycle of spread occurred when no colonising aphid species landed on the primary infected plants (probably coming from infected seed) and move to adjacent plants before leaving the crop. The role of weeds growing close to lettuce fields as potential inoculum sources of virus and the aphid species most likely involved in the transmission of LMV were also identified. The nature and the rate of spread determine the economic loss and it is necessary to visualize them. This charting protocol using the free R software environment will help to define the effects of interventions on the spread of a disease in a plant ecosystem. Apart from viral diseases, any character (yield, height, fertilizer response, pest and disease incidence can be similarly mapped using the simple "R: code"

ACKNOWLEDGEMENTS

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RESEARCH NOTE

Infestation of gall thrips, *Corycidothrips inquilinus* A. on *Terminalia chebula* Retz., a medicinally important plant

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Terminalia chebula Retz., (Family: Combretaceae), a native of Indian subcontinent is valued for its fruits rich in several compounds of medicinal importance. It is a deciduous medium tree, known as *Harad* in Hindi and *Haritaki* in Sanskrit, and is regarded as a universal panacea in the Ayur-Vedic medicine reputed to cure several ailments. *Terminalia chebula* is called the "King of Medicines" in the Tibet and is always listed first in the Ayurvedic materia medica because of its extraordinary powers of healing with a wide spectrum of biological activity. The fruit is being used for the treatment of different types of diseases and disorders since antiquity (Chattopadhyay and Bhattacharyya, 2007). The dry nut's peel is used to cure cold-related nagging coughs. The fruits of *T. chebula* also contain terflavin B, a type of tannin and chebulinic acid (Lee *et al.,* 2010). Plant species of traditional medicinal importance are being conserved at Indian Institute of Horticultural Research, Bengaluru. It is a hardy plant and very limited information is available on the status of pests. During the course of our monitoring the *ex-situ* conserved medicinal plants for insect pests, it was observed that the leaves of *T. chebula* were severely infested with galls in July – August 2013. The galls were sac like, subglobose, and were unevenly thick. Some of the leaves almost turned into a mass of gall with hardly any portion of normal lamina left. Galls are those cells, tissues and organs of plants, which are risen mostly by hypertrophy (over-growth) and hyperplassy (Excessive cell division) usually under the influence of parasitic organisms (Mani, 1973). Considering the severity of the infestation an in-depth assessment was done. The leaves with galls were collected, brought to laboratory and cut open to observe the causative agent. The galls were several nymphs and adults inside the galls, which was identified as *Corycidothrips inquilinus* Ananthakrishnan. Thrips are one of the important gall makers in plants besides other organisms like midges and nematodes. To understand the extent of infestation, observations were recorded on the number of leaves per shoot affected, number of galls/leaf, mean diameter of galls and number nymphs and adults per gall.

The extent of infestation ranged from 2-4 leaves per shoot accounting for about 25 per cent of total leaves. The number of galls ranged from 2 to 16 with an average of 7.7 per leaf. The number of thrips per gall ranged from 39 to 376 with an average of 222.6. Of them the number of adults ranged from 28 to 218 with an average of 121.8, constituting to 52.53% in the gall while the rest were nymphs (Table 1and Fig. 1&2). The diameter of the galls varied from 0.8 to 2.3 cm with an average of 1.39 cm. Ananthakrishnan and Raman (1989) provided an ample insight into the bioecology of gal thrips. Over 110 species of 25 genera of haplothripini are known to occur in plant galls Nearly 85% of known gall thrips occur in Peninsular Indian belt (Ananthakrishnan and Raman, 1989). Besides true gall formers, a few other

Table 1. Extent of gall thrips incidence on *T. chebula*

Parameter	Range	Mean ± S.D.
Per cent leaf infestation (n = 60 shoots)	0 - 42.50	25.40 ± 3.35
Mean No. of galls/leaf (n = 100 leaves)	2.0 - 16.0	7.70 ± 1.40
Mean diameter of gall (cm) (n = 30 galls)	0.8 - 2.30	1.39 ± 0.14
Mean No. of thrips/gall (n = 30 galls)	166 - 376	242.60 ± 67.33

Fig. 1. Leaves of *T. chebula* infested with gall thrips

Fig. 2. Cross section of galls showing thrips inside

species are associated with galls of other thrips or insects and known as inquilines. Galls arise in response to the feeding stimulus of thrips and their form range from simple folding, curling and rolling to complex deformation. Pouch galls, which are reported in this paper, are most complex form of galls where the growth of vegetative bud primordium is checked by feeding activities resulting in a pocket-like structure. They are usually crowded, close to petiole with a tendency to fuse with other galls or petiole, thus turning leaf into an abnormal mass. The complexity of galls depends not only on species of thrips and plants but also on the number of thrips inside the gall. The thrips, *C. inquilinus* is reported to be a monophagous inquiline restricted to *T. chebula* often associated with *Dixithrips onerosus,* the primary gall maker (Anathakrishnan and Raman,1989). However in the present study, *D. onerosus* was not found inside the galls. Though many medicinal plants are grown *in-situ,* the trend to cultivate then *ex-situ* is also on the rise to meet the growing demand for plant based medicines. Since medicinal plants are grown free from synthetic insecticides, there is a need to keep a constant vigil on the occurrence of pest species which might affect the yield and quality of economic product. It is very essential to document potential insect pests, as plants like *T. chebula* are grossly understudied from pest point of view.

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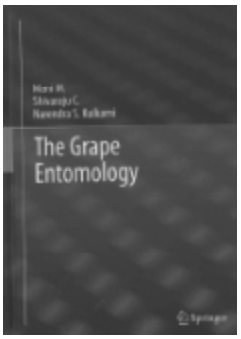
BOOK REVIEW

THE GRAPE ENTOMOLOGY



Title : The Grape Entomology
Authors : M. Mani, C. Shivaraj and N. S. Kulkarni
Publishers : Springer, Newyork
Year of Publication : 2014
Pages : 195
ISBN: 978-81-322-1616-2
978-81-322-1617-9 (e-Book)

The fact that grape cultivation is given a status of an independent branch of fruit science in the name of 'viticulture' exemplifies the uniqueness of grape as a crop. Hence it is understandable that authors preferred to name their compilation on grape pests as 'The Grape Entomology'. The title itself speaks the depth and the importance of the topic. Fully justifying the title, the information provided not just confines to pest management but extends beyond to cover certain basic aspects like taxonomy and bioecology of the species concerned. There are eight sections, starting with Introduction and ending with the most relevant chapter on pesticide residues. The chapter 'Pests', spreading over 160 pages, covers all insect, mite and vertebrate pests. The rich experience of Dr. Mani, a well known biocontrol expert, has been translated as an exclusive chapter on Biopesticides and Biocontrol agents used in vineyards. The authors thoughtfulness is reflected in including as Annexure the list of all chemicals recommended for usage on grapes. This will surely be of high practical value for both farmers and extension personnel.

The book contains around 195 pages of authentic information gathered from vast scientific literature. Provision of references at the end of each group of pests makes it easy to trace the source of particular information. Colour pictures of important pests and damage symptoms enhanced the value of the book. All pages of this book are made of fine quality papers and are very neatly bound in a hard glossy wrap bearing an attractive bright green cover. This publication can serve as a reference book, teaching manual and a field guide thus appealing to researchers, students, extension personnel and progressive farmers. The painstaking efforts of authors will be rewarded when the book has a wider reach and this will be one of the most valuable additions to the libraries of SAUs and ICAR Institutes.

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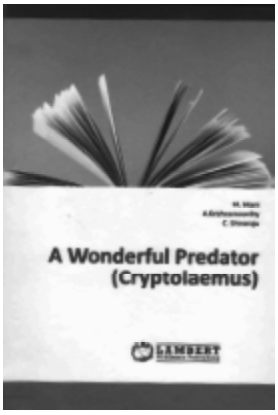
A WONDERFUL PREDATOR (CRYPTOLAEMUS)



Title : A Wonderful Predator (Cryptolaemus)
Authors: M. Mani, A. Krishnamoorthy and C. Shivaraju
Publishers : LAP LAMBERT Academic Publishing
AV Akademikerverlag GmbH & Co. KG
Heinrich-Böcking-Str. 6-8, 66121, Saarbrücken, Germany
Year of Publication: 2013

The Australian ladybird beetle, *Cryptolaemus montrouzieri* Muls. has played an immense role in the management of mealybugs and soft scales. This book is an exhaustive collection of information on *Cryptolaemus*. Initial chapters were devoted to the systematics of *Cryptolaemus* with prominent keys for the identification of *Cryptolaemus montrozieri* with illustrative keys to the various species and subspecies. A comprehensive list is provided on the countries where *C. montrouzieiri* and *Cryptolaemus affinis* were introduced. Chapters covering the host range, biology, life table and feeding potential on various hosts were also included. Mass production techniques and information on storage supported with photographs were also elaborated. Detailed information was provided on the role of ants on the efficiency of *C. montrouzieri*, non-target effects of insecticides on various life stages of *C. montrouzieri*, pheromones/kairomones and interaction with other mealybug parasitoids. More elaborate information is available on the colonization of *C. montrouzieri* against the scales and mealybugs. Crop wise information on the management of mealybugs with *C. montrouzieri* including factors responsible for the success or failure was provided to benefit researchers, extension personnel and farmers. The references covering the period from 1815 to 2011 were provided.

While complementing the authors for the complete coverage of information on Cryptolaemus, I consider this book is a valuable treasure for libraries, graduate and post graduate students and researchers.

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Principal Scientist
National Bureau of Agriculturally Important Insects,
Bangalore

GUIDELINES FOR AUTHORS

Pest Management in Horticultural Ecosystems, an international journal, covers the field of pest (insects, mites, nematodes, pathogens, slugs, snails, birds, mammals) management in its broadest sense. It includes bioecology, population modelling, biocontrol, host plant resistance, use of plant products, semiochemicals, insecticide resistance, insect biotechnology and IPM in fruits, vegetables, ornamental, medicinal and aromatic crops, plantation crops, tuber crops, spices, etc. The journal is published twice in a year, in June and December.

Two copies of the manuscript and an electronic form (3 ½" floppy or CD, MS Word) should be submitted to the **Chief Editor.** The manuscript should preferably pertain to the research work carried out during the last five years. Author(s) must certify that the manuscript has not been submitted elsewhere. All papers will be refereed. Short communications on significant research findings or new records are welcome. Only invited review papers will be considered. Decision of the Chief Editor is final. Authors are permitted to photocopy their articles for non-commercial and scientific purposes.

TITLE : Title of the article should be bold. Scientific names should be in running and italicized. Author name(s) should be in capital and bold, with full address in running with left alignment.

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TEXT : The text should be typed in double space on one side of bond paper (21 x 29 cm) with 3 cm margin on all sides and in clear and concise English. The title should be brief, specific and describe the scope of the paper. Below the title, author(s) name(s), and address of the first author should be given. Address(es) of the other author(s) should be given as footnotes and indicated by consecutive superscript numbers. The paper should be divided into subheadings such as Abstract, Key Words, Introduction, Materials and Methods, Results and Discussion, Acknowledgements, and References. Subheadings should be on the left margin. *It is desirable that authors get their papers edited for english grammar prior to submission.*

NOMENCLATURE : Common names of organisms should be qualified at the first mention by full Latin name and authority, followed by the order and family in parenthesis. Pesticides should be referred by their accepted common names. The trade name may be used with the chemical formula given in parenthesis.

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Morris, R. F. 1954. Single factor analysis in population dynamics. *Ecology,* **40** : 580-589

Takabayashi, J., Dicke, M. and Posthumus, M. A. 1991. Introduction of indirect defence against spider mites in uninfested Lima bean leaves. *Phytochemistry,* **30** : 1459-1462.

Litsinger, J. A. and Moody, K. 1986. Integrated pest management in multiple cropping systems. In *Multiple Cropping* Ed. Solly, M., American Society of Agronomy, Wisconsin, pp. 293-316.

Singh, D. P. 1986. *Breeding for resistance to diseases and insect pests.* Springer Verlag. New York.

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