

From the Managing Editor's Desk

I am very happy to introduce a new journal namely “**JOURNAL OF BIOPETICIDES**”. Agriculture is the back-bone of the human life. Its production is destroyed by pests. Chemical means of plant protection occupy the leading place in agriculture. But pesticides cause toxicity to humans and warm-blooded animals. Therefore, there is a need to develop biopesticides. Moreover, the synthetic pesticides market is expected to show declining trend. At the same time Biopesticides market is slowly growing, due to the benefits of biopesticides including effective control of insects, plant diseases and weeds, as well as human and environmental safety. Biopesticides are pest management tools which are based on beneficial microorganisms, nematodes, natural enemies, and other safer, and biologically-based active ingredients. To bring out more information and usage value of the biopesticides to the scientific community we proposed to start this new journal. On this gracious occasion, I would like to thank the management of St. Xavier's College (Autonomous), including **Rev. Fr. Britto Vincent, S.J., Rector; Rev. Fr. Leo Antony Tagore, S. J., Secretary and Rev. Fr. Alphonse Manickam, S.J., Principal** in encouraging me to start this new journal. I wholeheartedly thank **Prof. M. Thomas Punithan**, Head, and all the staff members of my department for their co-operation, encouragement and support for this Herculean task. I express my sincere gratitude to the members of the **Editorial Advisory Board and Editorial Board** for accepting invitations and their willingness to support in all the way to bring this journal a reputed one. Last but not least I express my thanks to **Dr. V. S. Joseph Albert**, Head of the Department of English of our college for doing the wonderful job of rectifying the typographical and grammatical errors of the manuscript.

Dear biopesticides scientists, specialists, research scholars and students, I assure you that you will be benefited from this journal and I expect your fullest co-operations by the way of your valuable contribution to basic and applied aspects of ecofriendly crop protection.

सुखदेव थोरात

अध्यक्ष

Sukhadeo Thorat

Chairman



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April 23, 2008

MESSAGE

25 APR 2008

I am very happy to know that the Crop Protection Research Centre, Department of Advanced Zoology and Biotechnology, St. Xavier's College, Palayamkottai is bringing out a new journal "*Journal of Biopesticides at the end of April or beginning of May, 2008*". The aim, objective and purpose of the Journal is interdisciplinary in approach bringing together scientists and researchers in the field of ecofriendly crop protection encompassing pests, diseases, weeds, rodents and molluscan pests management. I send my best wishes and greetings to the editorial team and wish the publication good luck and grand success.


(Sukhadeo Thorat)

Dr. K. Sahayaraj
Managing Editor
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Dr.S.VINCENT
Member Secretary
Tamilnadu State Council
for Science and Technology
Chennai- 25

21.4.2008

I am happy to note that the Crop Protection Research Centre (CPRC), Department of Advanced Zoology and Biotechnology, St.Xavier's College, Palayamkottai is bring out a Journal of Biopesticides.

The idea of bring out the journal for exchange of information and interaction between industry, researchers, academicians, students extension workers and the farmers is quite impressive.

I appreciate Dr.K.Sahayaraj, Managing Editor of the Journal for his great effort.

I wish the journal of Biopesticides to come out well.


21/4/08
Member Secretary.

Dr.K.Sahayaraj
Managing Editor
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Biotechnological approaches in IPM and their impact on environment

B. Vasantharaj David

ABSTRACT

The use of chemical pesticide and other agro chemicals are getting reduced /being banned globally because of their toxic effects on human beings and his live stock, residual toxicity, environmental problems, pest out-breaks and drastic effects on beneficial insects. Therefore, now it is imperative to develop a holistic system of tackling pests to make it more eco-friendly, economically viable and socially acceptable for the farmers. In the WTO regime, it is absolutely necessary to limit the usage of chemicals, to remain in the world market and sustain the competition. In this regard to tackle the major pests and diseases of major crops biotechnological approaches are gaining momentum. Compared with usage of chemical pesticides biopesticides constitute around 2% in the country. The biotechnological approaches of pest control such as use of botanical pesticides, use of microbial pesticides, augmentative biocontrol by inundative releases, pheromones and attractants in pest management and plant incorporated protectants (PIPs) / GM crops which are discussed in detail. Advantages and limitations of biopesticides have been outlined and the future approaches are highlighted.

INTRODUCTION

The use of chemical pesticide and other agro chemicals are getting reduced / being banned globally because of their toxic effects on human beings and his live stock, residual toxicity, environmental problems, pest outbreaks and drastic effects on beneficial insects. Therefore, now it is imperative to develop a holistic system of tackling pests to make it more eco-friendly, economically viable and socially acceptable for the farmers. In the WTO regime, it is absolutely necessary to limit the usage of chemicals, to remain in the world market and sustain the competition. In this regard to tackle the major pests and diseases of major crops biotechnological approaches are gaining momentum. Crop-wise market share of pesticide usage in India indicates highest use pattern to the extent of 45% in cotton followed by 22% in rice, 9% in vegetables, 7% in plantations, 4% each in wheat and pulses, and 9% constituting others. Compared with usage of chemical pesticides biopesticides constitute around 2% in the country.

Domestic Market of Organic Products

The current consumption of organically produced fruits and vegetables at the global level is valued at 12,150 crores. To a large extent this sale is also based on individual initiative of the farmers, Non Governmental Organizations and some entrepreneurial traders etc. The Agricultural Products Export Development Agency (APEDA) had proposed to export organically produced fruits and vegetables to a value of Rs. 5,500 crores annually during the Tenth Five Year Plan period. This would require

enormous efforts to produce and use biopesticides in the context of IPM.

Biopesticides: Global Demand/Projection

Biopesticides are likely to have a greater impact on insecticide sector. Some analysts believe that biopesticides would account for 15% of the total insecticide market by the year 2010. Presently, biopesticides represent approximately 4.5% of the world insecticide sales. The growth rate for biopesticides over the next ten years has been forecast at 10-15% per annum in contrast to 2.5% for chemical pesticides.

Availability of Biopesticides in India

About 700 products of different microbials are currently available worldwide. In India about 16 commercial preparations of *Bacillus thuringiensis*, 38 fungal formulations based on *Trichoderma*, *Metarhizium*, *Beauveria*, *Verticillium* and about 45 baculovirus based formulations of *Helicoverpa* and *Spodoptera* are available.

The biotechnological approaches include:

- I. Use of botanical pesticides
- II. Use of microbial pesticides
- III. Augmentative Biocontrol by Inundative Releases
- IV. Pheromones and attractants in pests management
- V. Plant Incorporated Protectants (PIPs) / GM crops

I. USE OF BOTANICAL PESTICIDES

In general use of neem formulations has been limited as they are moderately effective against a few pests like plant

III. AUGMENTATIVE BIOCONTROL BY INUNDATIVE RELEASES	
Biocontrol agents	
Parasitoids	
<i>Trichogramma chilonis</i> <i>T. japonicum</i>	<i>S. incertulas</i> <i>Cnaphalocrocis, medinalis, Pectinophora, gossypiella, Chilo infuscatellus</i> <i>Helicoverpa armigera</i> and other lepidopteran pests
<i>Goniozus nephantidis</i> <i>Trichospilus pupivora</i>	Coconut black headed caterpillars
<i>Bracon brevicornis</i>	Coconut black headed caterpillars
Predators	
<i>Cryptolaemus montrouzieri</i>	Mealy bugs
<i>Crysoperla carnea</i>	Soft bodied insects
Organism	Targetpests
Virus	
<i>Nuclear Polyhedrosis Virus</i>	<i>Helicoverpa armigera, Spodoptera litura</i>
<i>Granulosis virus</i>	<i>Helicoverpa armigera</i>
Bacteria	
<i>Bacillus thuringiensis</i>	<i>Helicoverpa armigera</i>
Fungi	
<i>Beauveria bassiana</i>	Lepidopteran and Coleopteran pests
<i>Metarhizium anisopliae</i>	Lepidopteran and Coleopteran pests, soft-bodied insects like Scales Aphids and Thrips
<i>Verticillium lecanii</i>	Soft-bodied insects like Scales Aphids and Thrips
<i>Paecilomyces fumosoroseus</i>	Whiteflies on cotton, mites
Disease control	
Bacteria	
<i>Pseudomonas fluorescens</i>	Sheath blight in rice, Root rot disease in sugarcane Foot rot and slow decline in black pepper, Capsule rot and clump rot in cardamom, Rhizome rot in ginger and turmeric, Fusarial wilt in coriander, Leaf spot in cucumber, Psudostem and leaf spot in banana
Fungi	
<i>Trichoderma viride</i>	Quick wilt, Slow wilt, Leaf blight, Anthrocanose, stem rot and root wilt in black pepper, fungal diseases of Cardamom, Ginger and Turmeric
Nematode control	
Bacteria	
<i>P. fluorescens</i>	Inhibits early root penetration of cyst nematode in sugar beet
Fungus	
<i>Paecilomyces lilacinus</i>	<i>Meloidogyne</i> spp., <i>R. similis</i> and <i>Heterodera</i> spp.
<i>Myrothecium</i> sp.	<i>Meloidogyne</i> spp., <i>R. similis</i> and <i>Heterodera</i> spp.

IV. PHEROMONES IN PESTS MANAGEMENT

Pheromones and attractants	
Species specific sex pheromones	<i>Helicoverpa armigera, Spodoptera litura, Earias vittella, Rhynchophorus ferrugineus, Pectinophora gossypiella, Cnaphalocrocis medinalis, Scirpophaga incertulus</i>
Methyl eugenol	Fruitflies - <i>Bactrocera</i> spp.
Cuelure	Cucurbit fruitfly – <i>B. cucurbitae</i>

and leaf hoppers, leaf folders and bollworms. These have limited use due to their lack of quick knock down effect. Thus farmers resort to mixing neem formulations with chemicals and the purpose of reducing insecticides load is defeated. Though a large number of plant products are reported to possess insecticidal, fungicidal and nematocidal effects, still there is lack of proper development of commercial products. It is opined by many that the plant products are safer. It must be kept in mind that the active compounds are chemicals and it is necessary to evaluate the safety of such plant derived chemicals. Another inherent problem is plant extracts if applied as such will not be stable and the stability of the commercial products needs to be looked into.

II. USE OF MICROBIAL PESTICIDES

The beneficial and ecofriendly microorganisms of fungi, bacteria, virus and protozoans capable of killing specific disease causing microbes, nematodes and insect pests and also those promoting plant growth are being considered as potential biological alternatives in ecofriendly agriculture. Entomopathogenic virus, bacteria, fungi and protozoans are widely used against lepidopteran pests and specific success has been achieved in case of white grub, stalk borer, sugarcane black bug etc. Similarly the viral pathogens like NPV and GV also viable in controlling *Spilosoma*, *Amsacta*, *Spodoptera*, *Helicoverpa* etc. and bacteria like *Bacillus thuringiensis*, become popular in controlling *Plutella* and *Helicoverpa*. The fungi like *Trichoderma* and the bacteria like *Pseudomonas* are being used as disease control agent of various fungal and bacterial plant diseases (Ramarethinam, 2003). These organisms limit the growth of pathogenic fungi and bacteria by mycoparasitism or by the production of antibiotics. Use of nematophagous fungus and bacteria *P. lilacinus* and *P. fluorescens* are reported as potential nematode control agents of parasitic nematodes in many crops.

Seed treatment with *P. fluorescens* alone or in combination with other microbial and botanical agents such as neem cake has reduced pest and disease problems (Swarnakumari and Lakshmanan, 1999; Swarnakumari *et al.*, 1999). Root knot nematodes belonging to the genus *Meloidogyne* viz., *M. incognita*, *M. graminicola* and *M. javanica* can be controlled by the fungus *P. lilacinus*. Though presently they appear to have less scope in pest management suitable formulations, cost effective production and application technology may lead to success. However the production and marketability of such bio agents are not sufficient even to cover 2% of total consumption of the pesticides in the country.

III. Augmentative Biocontrol by Inundative Releases

In India, inundative releases of natural enemies have been restricted to only egg parasitoids, particularly *T. japonicum* and *T. chilonis*, mainly because of their amenability. These parasitoids may be useful against the yellow stem borer (YSB) *S. incertulas* and leaf folder *C. medinalis* of rice, sugarcane shoot borer *C. infuscatellus* and other lepidopteran pests of vegetables. There are several successful reports of the inundative releases of *T. japonicum* and *T. chilonis* against stem borer and leaf folder in rice and sugarcane (Bentur *et al.*, 1994; Shrike and Bade, 1997). Studies carried out to test the effectiveness of inundative releases of *T. japonicum* and *T. chilonis* as a component of rice IPM have also been promising (Balasubramanian *et al.*, 1994). Gururaj Katti *et al.* (2001), reported that large scale on farm evaluation of *T. chilonis* revealed both the leaf folder as well as stem borer pest could be effectively managed through integration of two non insecticidal components such as parasitoids and pheromones resulting in higher net returns. Weekly release of *T. chilonis* at 2,50,000 adults per hectare from flowering season till the ripening of the boll showed a progressive decline in the infestation of pink bollworm *P. gossypiella* and *H. armigera* on cotton (David, 2003).

IV. Pheromones and attractants in pests management

Pheromones in pests management aims at mating disruption by treating the crop with appropriate pheromone to prevent male moths from locating "calling females" and thus suppresses mating. The principle is development of slow release formulations, which maintain relatively high concentration of pheromone for several weeks and thus disrupt mating. Trapping the male moths of the target pest species utilizing the species-specific pheromones facilitates early detection of the pest occurrence. Determination of moth population dynamics through crop seasons helps in formulating pest management strategies and justifying or timing insecticide application. Krishnaiah (1995) reported that the pheromones of the rice the leaf folder *C. medinalis* viz., Z,11- Hexadecenyl acetate and Z,13- Octadecenyl acetate dispersed from either rubber septa or polythene vials in the ratio of 1:10 were pheromonally active.

Series of attempts made under DRR- National Research Institute, UK project utilizing a controlled release formulation with polyvinyl chloride matrix as base for control of *S. incertulus* the pheromone blend of Z, 9-hexadecenal and Z,11-hexadecenal in 1:10 ratio applied at 40 g a.i./ ha suppressed 98% communication disruption during Rabi 1992 (Ganeswar *et al.*, 1994) in on farm trials.

Pheromones applied at 10 DAT equally effective as that applied at 40 DAT in bringing down white ear heads. Similarly species specific lures developed are promising and getting momentum.

V. PLANT INCORPORATED PROTECTANTS (PIPS) / GM CROPS

The genetic material, which is responsible for the production of pesticidal substance, is being incorporated into the genome of the target crop plant thus making the plant capable of destroying the pest. For example the gene producing Bt pesticidal protein was introduced in cotton and Brinjal making them resistant to pest attack.

Advantages of using Biopesticides

1. Maintains the health of the soil and sustain its life by increasing soil organic matter
2. Generally species specific and are safe to the natural enemies and non target organisms
3. Biopesticides are less toxic than chemical pesticides and safer to the environment
4. Microbial pesticides rely upon the potential biochemicals synthesized by the microbes
5. It requires in small quantities often decompose rapidly, there by avoids pollution problems

LIMITATIONS

- Beneficial effects not seen immediately
- Lack of awareness – farmers, dealers etc.
- No standard recommendations
- Short shelf-life
- CIB registration- Expensive & time consuming
- Slow flow of latest research findings
- Problem in price/demand/supply

FUTURE APPROACHES

I. Botanical pesticides

Large-scale control of agricultural pests and stored grain pests are even today depends mainly on chemical pesticides. There is need to develop new technologies using to minimize the usage of chemicals. Biotechnology based approaches can be tried using available research data.

II. Biopesticides

The use of species-specific organisms need to be properly identified, production technology and suitable formulation developed and commercialized. The use of *P. fluorescens* and *P. lilacinus* in nematode management must be given due attention as no safer nematicide is available in the country. There is need for research on nematode problems of major crops and assess the utility of technology.

III. Biocontrol agents

Augmentation of biocontrol agents like *Trichogramma* is a proven technology and farmers' response is on the increase. More biocontrol laboratories must be encouraged to popularize the benefits and ensure supply. Though the natural enemy complex of many pest species of rice is known their utility in biocontrol programme by way of mass multiplication and supply to farmers needs a practical approach.

IV. PHEROMONES

Though several other natural enemies of many pest species are known their utility in biocontrol programme limited. The concern is tackling when two or three species occur simultaneously. The technology of integration of pheromones with use of biocontrol agents needs to be developed for different agro climatic zones and popularized. Pheromone trap data may be useful for timing the mass multiplication and inundative release of egg parasitoids.

V. PLANT INCORPORATED PROTECTANTS (PIPS)

Though biotechnology laboratories because of the environmental concern develop a number of GM crops these crops are not yet popularized and supported legally. In such case the GM crops should be studied thoroughly for the environmental, biodiversity problems etc and those crops found suitable can be encouraged. Small-scale farm trials can be done for satisfactorily long period to ensure the biosafety.

CONCLUSION

In fact farmers are now evincing interest in organic farming as pesticide free produce grown under conditions free of pesticide and fertilizer application is the most preferred and fetches premium price. Certifying agencies are now identified and they are certifying the farms and their produce. Though biotechnological approaches are not very popular due to constraints enumerated above in the near future it is likely to have impact in crop protection in the country, creating situation conducive for a safer environment.

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Prospects and Promises of Endocrine Biopesticides

D. Muraleedharan and U. Gayathri Elayidam

ABSTRACT

The environment hazards resulting from intensive use of synthetic organic crop protection agents, demands that the pest management studies should be biointensive. Environment friendly, safe and compatible approaches paved the way to develop biopesticides. The multiple functional capacities of insect neuropeptides, based on intervention on this system at any level provide opportunities for new insect control strategies. Potential biochemical for insecticidal action like Juvenile hormone, Juvenile hormone esterase, ecdysone, acetyl choline, ion channels and new peptides are discussed in detail.

INTRODUCTION

Biological control of agricultural pests has gained importance in recent years primarily due to increased pressure to reduce the use of agrochemicals and their residues in the environment and food. Publication of *Silent Spring* (Carson, 1962) evoked an increase in public concern of toxic chemicals over the welfare of the environment, especially the detrimental effects associated with pesticide use that heightened in the 1960's. The environmental hazards resulting from intensive use of synthetic organic crop protection agents make it imperative to consider alternative or complementary approaches to sustainable agricultural development and integrated pest management. The current millennium demands that the pest management studies should be bio-intensive. One of the methods that emerged in recent years gaining increased attention is the use of biopesticides in order to develop environment friendly, safe and compatible approaches and tactics for pest management. Most of these attributes do not harm the natural enemies and play a significant role in pest management systems that seek to reduce pesticide inputs and conserve our natural fauna.

Biopesticides are certain types of pesticides derived from natural materials as animals, plants, bacteria, or certain minerals. The different classes of biopesticide include biochemical pesticides, microbial pesticides and plant-incorporated protectants. They are usually inherently less toxic than the conventional pesticides and generally affect only the target pest and closely related organisms, in contrast to a broad spectrum, conventional pesticides that may affect other organisms as well. Biopesticides are often effective in very small quantities and often decompose quickly (Ware, 1994), thereby resulting in lower exposures and largely avoiding pollution problems caused by the conventional pesticides. When used as a component of Integrated Pest Management (IPM) programs, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high.

In the recent decades a variety of biocontrol methods employing peptidic or proteinaceous insect-specific toxins

derived from microbes, plants and animals have been examined both in the laboratory and field with varying results. Interdependent factors involved with biopesticides are the production of a cost-effective, pesticide-production expense, kill efficiency, environmental persistence, pest-specificity, pest resistance-development, public perception and ease of delivery-sprayable biopesticides. Several approaches are being investigated for the design of new (bio) pesticides. These include the development of transgenic plants and recombinant baculoviruses as delivery systems for a variety of insect-selective toxins. The killing efficiency of baculoviruses may be augmented by genetic modifications of the baculovirus genome with the genes of another natural pathogen (Szewczyk, 2006). Baculovirus-insect cell system (BICS), with increased insect toxicity is established by universal gene silencing (UGS) system using RNA interference (RNAi). Bicistronic RNA seems an efficient way to lower both cost and effort of gene silencing approach while maintaining the biological activity of the protein of interest when the RNAi is not in use (Salem and Maruniak, 2007). Additional approaches for the development of foliar sprays include the rational design of peptidomimetics based on the key residues of these toxins that interact with the insect target (Nicholson, 2007).

Multi-level endocrine systems control a wide range of physiological processes like moulting, metamorphosis, diapause and reproduction. The multiple functional capacities of insect neuropeptides, based on intervention on this system at any level provide opportunities for new insect control strategies (Muraleedharan and Devi, 1992). Insect specific applications aiming at the disruption of neuroendocrine dependent processes include interference with neurosecretion, inhibition or induction of biosynthesis (transcription, processing, post-translational modifications), release, transport, and binding, activation of the receptors on the target cell, signal transduction and degradation (Couillaud and Peypelut, 1995). Biochemical sites such as chitin formation, Juvenile hormone, Juvenile hormone esterase, ecdysone,

acetylcholine and GABA receptors, ion channels and neuropeptides are all potential targets for insecticide action (Ishaaya, 2001).

The last decades have brought remarkable advances in the field of comparative neuropeptide research and this comparative approach paved the way for the discovery of many novel peptide structures, as well as a few of their receptors. Exposure of insects to genetically-engineered agonists or antagonists is generated either by transgenic plants or insect control enhanced by insect specific vectors by a combination of biological control contributed by microorganisms and neurochemical control contributed by neuropeptides.

Disruption of metamorphosis

Endocrine approaches to insect control have focused largely on hormones involved in regulating insect growth and metamorphosis. The two principal players towards this are the sesquiterpenoid Juvenile hormone (JH) and the steroid, molting hormone Ecdysone. Ecdysteroids are secreted from the prothoracic glands (PTG) that lies on the dorsal surface of the lateral tracheal trunks in the prothoracic segment. Upon stimulation by prothoracicotropic hormone (PTTH) the glands secrete ecdysone into hemolymph, which is hydroxylated to the active hormone, 20-hydroxy ecdysone.

Juvenile hormone is being secreted by a pair of tiny glands the corpora allata (CA), attached to the base of brain, and is controlled by the neuropeptides, allatostatins and allatotropins secreted by the neurosecretory cells in the brain (Sindhu *et al.*, 2001). Several hemolymph, cytosolic and nuclear JH binding proteins have been identified and characterized from insects (Mohan *et al.*, 2005). JH receptors, enzymes involved in biosynthesis and degradation of JH have also been characterized (Gilbert *et al.*, 2000).

JH Endocrine System

The first attempt to utilize JH endocrine system as a pesticide was to develop Juvenoids or JH analogues. These are substances which elicit hormonal imbalance by disrupting vital functions of the insect resulting in arrested development, suppression of reproduction or other lethal morphogenetic effects (Muraleedharan and Devi, 1992). As a biopesticide, they induce adult sterility, physical body changes, water loss and premature death in insects. Methoprene, Hydroprene, Kinoprene, Juvocimen, Juvabione, R-394 are glaring examples of JH analogues that attracted the attention as insecticidal agents. Juvenogen, a fatty acid ester of a juvenoid alcohol, induced greatest soldier differentiation in representatives of three *Reticulitermes* species tested, showing the potential of juvenile hormone analogues in termite control (Hrdý *et al.*, 2006). The biological activity of the juvenoids was also studied against the red firebug *Pyrrhocoris*

apterus, termites *Reticulitermes santonensis* and *Prorhinotermes simplex*, and the blowfly *Neobellieria bullata* (Wimmer *et al.*, 2007). Methoprene acts on *Aedes aegypti* by interfering with the expression of genes involved in 20E action, resulting in a block in midgut remodeling and death during pupal stage (Wu *et al.*, 2006). Pyriproxyfen and Fenoxycab are the most important components in IRM strategy in cotton fields (Horowitz *et al.*, 1999).

The discovery and subsequent development of effective Juvanoids, functional mimics of endogenous juvenile hormones, inspired that the reverse principle, Anti-Juvenile hormone action could be explored to complement the use of Juvanoids. Precocene II (anti-JH) showed a very strong antifeedant effect against *Tribolium confusum*, the granary weevil beetle *Sitophilus granarius*, and the khapra beetle *Trogoderma granarium*, larvae and also against the herbivorous pest insects, Colorado potato beetle *Leptinotarsa decemlineata* and aphid *Myzus persicae* (Szczepanik *et al.*, 2005). Alternatively, because many juvenoids including JH III itself have been isolated from plants (Bede *et al.*, 2001), genetic engineering of plants could force them to produce Juvenoids for phytophagous insects. Common limitation of Juvenoids as pesticides is that they prolong the destructive instar of many pest insects, acting at specific periods of development.

Changes in the rate of biosynthesis are of considerable importance in determining the physiology of insect. Identification of allatomodulatory neuropeptides (Sheng *et al.*, 2007; Noriega *et al.* 2006; Sindhu *et al.*, 2001) together with their cDNA (Abdel-latif *et al.*, 2003) opens new avenues for the practical use of these neuropeptides to disrupt JH biosynthesis. Furthermore, identification of allatomodulatory peptide receptors at the level of the allatal cells, elucidation of the mechanisms of signal transduction and identification of their mode of action on the activity of enzymes of the JH biosynthetic pathway will certainly provide new targets for the design of JH biosynthesis inhibitors.

There has been considerable interest in the discovery of chemical inhibitors of JH biosynthesis. Juvenile hormone acid methyl transferase (JHAMT) is an enzyme that converts JH acids or inactive precursors of JH to active JHs at the final step of JH biosynthesis pathway in insects. It has been indicated that JHAMT enzyme is developmentally regulated in a few lepidopteran insect species (Bhaskaran *et al.*, 1990). Correlation of JHAMT gene expression and the biosynthetic activity in the CA suggests that the transcriptional suppression of the JHAMT gene is crucial for the termination of JH biosynthesis in the CA, which is a prerequisite for the initiation of metamorphosis (Shinoda and Itoyama, 2003). Deeper insight into insect metamorphosis and its endocrine mechanism at

the molecular level especially in the field of developmental Insect Endocrinology should be of fundamental value in developing such newer strategies for disrupting insect life cycle and reproductive potential that are destructive to crops and hence of immense economic importance.

The reduction in JH titer is a key event in insect development that leads ultimately to inhibition of JH biosynthesis, causing premature metamorphosis, termination of feeding and adult sterilization (Kamita *et al.*, 2003). This reduction is associated with dramatic increase in the levels of very active Juvenile Hormone esterase (JHE) (Gilbert *et al.*, 2000, Wogulis *et al.*, 2006). Because of its pivotal role in insect development, JHE have been targeted for use as a biopesticide. Hammock *et al.* (1990) constructed a homology based molecular model of JHE from *Heliothis virescens*. This model is being used as a predictive basis to design such biopesticides. The expressed recombinant JHE acting as an anti-JH is potentially used for Mosquito control (Harshman *et al.*, 1991). Thomas *et al.* (1999) have constructed a homology-based molecular model of JHE from the agricultural crop pest, *Heliothis virescens* and also have identified a site on the protein surface that is suggestive of a recognition site for the putative JHE receptor. A fast acting recombinant baculovirus, that expresses a modified form of JHE, which is inactive with respect to its function of JH catalysis, was developed by Bonning *et al.* (1995). The alteration of specific residues of JHE that disrupted lysosomal targeting dramatically increased the insecticidal activity of this enzyme. The insect growth regulator (IGR) imidazole KK-42 induces hemolymph juvenile hormone esterase activity and precocious metamorphosis in *Bombyx mori* (Hirai *et al.*, 2002)

Ecdysone Endocrine System

Ecdysteroids do not apparently play physiological roles in vertebrates and hence offer a complete panel of potential targets for insect pest management strategies which have the advantage of being unlikely to affect vertebrates. The heterodimer of the ecdysone receptor (EcR) and ultraspiracle protein (Usp), members of the nuclear receptors superfamily, is considered the functional receptor for ecdysteroids initiating molting and metamorphosis in insects. The ligand binding domains of EcR and Usp from insects belonging to different orders fall into two separate groups and this can be exploited to discover order-specific insecticides (Palli and Retnakaren, 2001). Two compounds, tebufenozide and methoxyfenoxide, which bind to ecdysone receptor have been commercialized and used to control lepidopteran pests. These compounds are considered highly selective doing no harm to parasitoids and predators and fit well in IPM and insect resistance management programs (Dhadialla *et al.*, 1998). Enzymes involved in biosynthesis and degradation of ecdysteroids as well as proteins that are critical

for secretion and uptake of ecdysteroids into cells can be used as targets for developing newer insecticides.

Over the years, several attempts have been made to use ecdysone analogs for insect control. Diacyl hydrazines (RH) compounds bind to ecdysone receptor/ultraspiracle heterodimer that leads to incomplete precocious molt resulting in the mortality of the larva due to persistence of the RH compounds (Dhadialla, 1998). Characterization of a non-steroidal, ecdysone agonist, bisacylhydrazine compound, RH-131039 was carried out by Dhadialla *et al.* (2007).

Genetic engineering of plants using constitutive promoters are presently the primary means used to express transgenes in plants. The ecdysone receptor gene switch is one of the best inducible gene regulation systems available, because the chemical, methoxyfenoxide, required for its regulation is registered for field use. An EcR gene switch with a potential for use in large-scale field applications has been developed by adopting a two-hybrid format CfEcR:LmRXR for regulating the expression of a Superman-like single zinc finger protein 11 (ZFP11) gene in both Arabidopsis and tobacco transgenic plants (Tavva *et al.*, 2007). Kojima *et al.* (2007) is the first to report of an ecdysone response element in a baculoviral gene promoter. These results also suggested that the regulation of the immediate-early gene baculovirus *iel* by ecdysone may militate viral replication at least under certain conditions during natural infections *in vivo*.

Neuropeptide antagonists

A new integrated approach is the generation of a novel type of insect neuropeptide (Np) antagonists and putative insect control agents based on conformationally-constrained compounds. Antagonists which are selective inhibitors of the neuropeptide receptors may disrupt and interfere with the normal growth, development, homeostasis and behavior of the insect by blocking the receptor of the neuropeptide; therefore, they can form receptor-selective, insect-specific insecticides.

The approach, termed "Insect Np-based Antagonist insecticide (INAI)", was applied to the insect pyrokinin (PK)/pheromone biosynthesis-activating Np (PBAN) family as a model (Altstein and Gilon, 2001). In the noctuid moth, sex-pheromone biosynthesis follows a circadian cycle, which is cued by the release of the neurohormone pyrokinin/pheromone biosynthesis activating neuropeptide (PK/PBAN family) to the hemolymph, which also mediates a variety of other functions in moths and other insects (Ajitha and Muraleedharan, 2005). These neuropeptides exert their functions through activation of the G protein-coupled receptor PBAN receptor (PBAN-R), in pheromone glands (Zheng *et al.*, 2007).

This new approach led to the discovery of a potent linear lead antagonist and several highly potent, metabolically

stable backbone cyclic (BBC) conformationally constrained antagonists (BBC antagonists), devoid of agonistic activity, which inhibited PBAN-mediated activities in moths *in vivo* antagonists and acted as the basis for the design of insect control agents (Altstein 2001,2004). Ben-Aziz *et al.*, (2006) showed that Backbone cyclic pheromone biosynthesis activating neuropeptide (PBAN) antagonists inhibit melanization in the moth *Spodoptera littoralis*. Beyond the immediate benefits introduced by the cyclic peptides as selective antagonists, the information on the bioactive conformations may serve as a basis for the design of improved nonpeptide, mimetic antagonists. Such compounds are potential candidates for agrochemical applications and could serve as prototypes for the development of a novel group of highly effective, insect-specific and environmentally friendly insecticides.

Systematic replacement of the naturally occurring L-amino acids by their non natural D-isomers or replacement of amino acid residues, such as D-Phe or D-Trp will convert agonist to antagonist (Altstein and Gilon, 2001). The D-Phe approach to proctolin resulted in the discovery of a few peptides with antagonistic activity (Kuczer *et al.*, 1999).

Chitin synthesis inhibitors

The insect cuticle serves as an interface between the living animal and its environment forming the exoskeleton and supporting the lining of the gut, respiratory systems, reproductive ducts and some gland ducts (Tunaz and Uygun, 2004). The first chitin synthesis inhibitor was benzoylphenylurea, diflubenuron (Miyamoto *et al.*, 1993). Benzoyl phenyl ureas affect ecdysone-dependent biochemical sites which lead to chitin inhibition. The chitin synthesis inhibitors were quite effective against multi resistant *Musca domestica* strains (Pospiscil *et al.*, 1997). The chitin synthesis inhibition site has proved to be important for the development of control agents which act selectively on important groups of insect pests.

Acetyl Choline Receptors.

Efforts have been made to develop nicotinyl insecticides with high affinity to insect nicotinic acetyl choline receptors (nAChR) resulting in the development of a new group of neonicotinoid insecticides (Elbert *et al.*, 1996). Imidacloprid, acetamiprid and thiamethoxam are potential neonicotinoids used as agro-chemicals. Acetamiprid has been introduced in Israel as a component in the IRM program to control *B. tabaci* in cotton fields, while imidacloprid is used systemically through the soil to control white flies and aphids in vegetable and ornamentals. The neonicotinoids act specifically on sucking pests and have no effect on parasitoids and predators, and as such fit in various IPM programme.

GABA and Glutamate Receptors and Ion Channels.

The γ -aminobutyric acid (GABA) receptor/chloride ionophore complex has been the focus of intense interest as a site of insecticidal action. A Voltage-dependant sodium channel and γ -aminobutyric acid (GABA)-gated chloride channels are primary sites of a number of established insecticides. Abamectin, emamectin, milbemectin and spinosad, which act on GABA receptors and ion channels, have been developed to control mites and other agricultural pests (Bloomquist, 2001).

Insect Gut as a Site for Insecticide Action

Naturally occurring protease inhibitors have been explored since they interact and block the active center of the digestive enzymes, both proteases and amylases, in the gut system. Commonly used inhibitors include crystalline soybean trypsin inhibitor of Kunitz, Bowman-Birk soybean trypsin inhibitor, lima bean inhibitor, chickpea trypsin inhibitor, chymotrypsin inhibitor and ovomucoid (Reeck *et al.*, 1997). These proteins bind tightly to the active site of the enzyme in the midgut preventing access to normal substrates. Failure by the pest insect to overcome this inhibition of digestion results in death by starvation. This principle of digestive enzyme inhibitors can be exploited with the recombinant DNA-technology in transgenic plants for crop protection (Nathan *et al.*, 2006).

CONCLUSIONS

Several of the developed botanical pesticides to-date inculcate repellency, anti-feedancy or often interfere with either growth or reproduction in the specific pest species. Invertebrate hormones like Juvenile hormone, Ecdysone and so many related regulatory neuropeptides; all control several vital physiological processes including morphogenesis or development and reproduction in arthropods. Compounds that interact with JH and ecdysone receptors such as fenoxycarb and pyriproxifen (JHA) and tebufenocide and methoxy fenozide (ecdysone agonists) are being developed as selective insecticides especially for the control of scale insects, white flied or lepidopteran caterpillars. Inhibitors of acetylcholine receptors like Neonicotinoids and imidacloprid acetamiprid and theomethoxam that are more specific to aphids and white flies. Abamecvtin, emamectin, milbermectin and spinosad are inhibitors of GABA receptors and ion channels specific to mires and related agricultural pests. Rational design and selection methods to develop antagonistic cyclic peptides based on their specific insect neuropeptide sequences like PBAN or allatomodulatory neuropeptides is another attractive mode of approach to combat specific arthropod pests. Signal transduction mechanisms of many of these hormones and neuropeptide ligands to their respective target cell and

also their respective gene have been poorly understood. Some of the key enzymes involved in the synthetic cycle of these hormone molecules and even their genes also need to be understood at the nanoscale as manipulations of these are being projected as strategy for effective management of arthropod pests of both agricultural and medicinal importance. Novel approaches to develop arthropod pesticidal agents much more specific to insect biochemical sites that are not active to mammal need to be explored in future for developing safer and efficient pesticidal agents as a principal component of Integrated Pest Management program.

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Induction of Specific Biochemical Pathways in Plants for Pest Management

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ABSTRACT

The biochemical pathways encompassing induced resistance involve oxidizing enzymes such as PPO (Polyphenol Oxidase), PO (Peroxidase) and LOX (Lipoxygenase). Increased activities of these enzymes in plants serve as markers for visualizing induced resistance. Induced resistance, being an active defense mechanism, results from the expression of PR-proteins, low molecular weight compounds like phytoalexins and proteinase inhibitors. This paper highlights on the spatial induction, variability and persistence of induced defenses responses in *Lycopersicon esculentum* Mill. using lepidopterans, aphids and mechanical wounding as elicitors. The early signally events upon wounding by an insect resulting in the release of elicitors JA, Ethylene, Glycans and Absisic acid and the transmission of signals through unwounded sites is described. The practical utility of induced resistance is discussed based on the results of the systematic small scale laboratory essays.

INTRODUCTION

Plants have developed and optimized a considerable diversity of defense mechanisms against adverse environmental conditions caused by either biotic or abiotic factors. These defenses are commonly divided into constitutive or passive and induced or active defenses. An understanding of induced resistance, a non-heritable qualitative and quantitative enhancement of a plant's defensive mechanisms against pest in response to external physical or chemical stimuli (Heinrichs, 1998, Dilawaria and Dhaliwal, 1993, Panda and Kush, 1995) is a key element in insect pest management programs. There is great scope for using chemical elicitors of plant resistance to protect plants against insect and pathogen attack. Work in the area of plant resistance to pathogens has advanced more rapidly than studies of plant resistance to herbivores and has resulted in the production and marketing of elicitors of salicylate-dependent plant resistance to pathogens (Lyon and Newton 1999, Tally *et al.*, 1999). However, the role of insects in induced resistance build up in plants is still not fully understood. Insect feeding has been reported to elicit local, as well as systemic responses in more than 100 plant species (Karban and Baldwin 1997). These responses might function either as direct resistance (physical or chemical traits that act directly against further attack or reduce herbivore performance) or as indirect resistance. The latter is based on the attraction of 'enemies of the plant's enemies' (Price *et al.*, 1980).

Induction of defense enzymes might be either a local or a systemic event (Edwards and Wratten, 1983). Occurrence of biochemical and physical changes confined at the site of injury represent localized defense response (Mauch-Mani *et al.*, 1998; Stout and Bostock, 1999; Norman *et al.*,

1999). Systemic induction is represented as molecular, chemical or morphological changes that occur in distant undamaged leaves (Zhang and Baldwin, 1997; Stratmann, 2003; Orians, 2005). Systemic induction can be uniform throughout the plant or vary between different plant parts (Jones *et al.*, 1993; Stout *et al.*, 1996b; Sanjayan, 2005). Induced responses can selectively affect the performance of herbivores and the behavior of natural enemies (Thaler, 1999a, 1999b; 2002; Agrawal, 2000; De Moraes *et al.*, 2001; van Poecke *et al.*, 2001).

Induced plant responses, especially different responses in different stages of development, will produce variability within individual plants (Baldwin and Karb, 1995, Zangerl and Rutledge 1996). Also, the responses of the plant may vary in relation to the species of insect attacking it. Against this background, the present study attempts to examine the response of the tomato plant, *Lycopersicon esculentum*, to different induction treatments *viz.*, 1) feeding by *Spodoptera litura*, *Aphis gossypii*, as well as chemical and mechanical injury, 2) the spatial mapping of chemical induction and 3) the degree and persistence of polyphenol oxidase enzyme activity against the exposure of tomato plants to *S. litura* larval feeding for 24 hours.

MATERIALS AND METHODS

Tomato plants were grown in pots in a green house for about 30 days. They were subjected to damage on the third leaf from the cotyledon. Plants of similar age and size were left undamaged as a control. Four types of damage induction was tested namely 1) feeding by *S. litura* representing biting and chewing damage, 2) feeding by *A. gossypii* representing sucking type, 3) Mechanical damage and 4) Insecticidal soap treatment.

One second instar larva of *S. litura* was confined to the third leaf (position LI) using the clip cage and allowed to feed for up to 24 h. Plants with a clip cage without larva served as the control. After 24 h, cages and insects were removed and the plants left for an additional 24 h. Similarly 45-50 *A. gossypii* (nymphs and adults) were confined to the same position and the same procedure was followed. For mechanical damage, two wounds were made perpendicular to the mid-vein of the terminal leaflet of the third leaf and for chemical damage the entire third leaf was immersed for 5-10 seconds in a 5% (v: v) soap solution. After two days, plants were transported to the laboratory and the leaflets from several positions on control and damaged plants were then excised at the petiole with a razor blade and assayed for Polyphenol oxidase (PPO), Lipoxygenase (LOX) and Peroxidase (POD). The activities of these proteins in leaflets from damaged plants were compared to activities in corresponding leaflets from control plants (Stout *et al*, 1996a). In order to quantify the persistence of resistance induction, induced leaves were sampled at 1, 3, 5 and 7 days after feeding and PPO and POD analysis carried out. Leaflets from the four positions were sampled: damaged leaflets (terminal leaflet of the third leaf, designated position LI), undamaged leaflets from the damaged leaf, adjacent to the damaged leaflets (position A), leaflets from the leaf immediately below the damaged leaf (position L) and leaflets from the leaf above the damaged leaf (position U). This method is to assess induction at three spatial levels – leaflet-local induction (induction confined to the damaged leaflet), leaf-systemic induction and plant-systemic induction.

RESULTS AND DISCUSSION

Plants have a generalized defensive response to wounding that can be divided into two phases- activation and induction. Activation represents the immediate response to cellular damage wherein cell integrity is lost and a variety of hydrolytic and oxidative enzymes are released from compartmentalization. This release results in the generation of chemical signals that trigger the systemic and/or local induction of defenses, and in the generation of chemically reactive products that lead to cell death through destruction of membranes and polymerization of cellular components. This polymerization is mediated by PPO, POD and LOX.

PPO and POD oxidizes phenolics to quinones which alkylates nucleophilic functional groups of proteins, peptides and amino acids (-SH, -NH₂, -HN and OH). The amino acid becomes nutritionally inert and reduces the digestibility of the protein by tryptic and chymotryptic enzymes. Further nutritive value of the protein can be

lost via polymerization and subsequent denaturation and precipitation. POD is also capable of decarboxylating and deaminating free and bound amino acids to aldehydes. The aldehyde facilitates polymerization by forming Schiff's bases with -NH₂ of the protein molecules. POD can also initiate free radicle formation on -SH and tyrosinyl functions of proteins which leads to polymerization of proteins. LOX converts polyunsaturated fatty acids (Linolenic and linoleic acid) into lipid hydroperoxides. The lipid hydroperoxides then form hydroperoxide radicals, epoxides and are degraded to form malondialdehyde. These products are strongly electrophilic and can 1) destroy amino acids by decarboxylative deamination 2) cause free radicle mediated cross linking of proteins and 3) cause Schiff's base formation.

Table 1 indicate that the spatial pattern of protein induction varied with the damage type. Comparison of the grand means of the damage treatments through two factor ANOVA, indicate that an increased induction of PPO activity in *S. litura* (biting and chewing type), which was significantly different from the *A. gossypii* (sucking type), as well as the mechanical and chemical damages. At the spatial level, a higher induction in position D and A was found to be a general tendency for all treatments followed by positions U and L, the levels being primarily different significantly. Therefore PPO induction could be regarded as a plant systemic phenomenon. The induction of POD was maximum in position D for both *S. litura* and *A. gossypii* damage treatments. Maximum induction was in *S. litura* damaged plants followed by *A. gossypii* and mechanically damaged plants. Spatial difference in activity for POD as a whole for all the treatments was more or less the same with no significant difference for positions A, U and L indicating a localized induction.

Feeding by *S. litura* resulted in the enhancement of induction of both POD and PPO. While the sucking pest, *A. gossypii* showed less induction when compared to *S. litura* damage but significant increase in induction was observed over the control plants. There are several mechanisms for this increase. Aphids may come into contact with inducible compounds via several routes, for example, mechanical damage induces PPO in tomato trichomes and phloem (Thipyapang *et al.*, 1997). Aphids walking on the surface break trichomes and may be entrapped by phenolics polymerized by PPO and POD (Duffey, 1986). The difference in induction for different pests *viz.*, *S. litura* and *A. gossypii* may be due to their feeding mechanisms and elicitor variability. Stout *et al.*, (1996a) worked out the spatial mapping for the induction of four foliar proteins against *Heliothis zea* and russet mite *Aeulops lycopersici* Masee and indicated that for *H. zea*, PI, PPO and POD were induced leaf systemically and

Table 1. Induction of PPO and POD activity in tomato against different damage treatments and leaf positions (LSD)*

Damage/Location	PPO activity (OD increase/min/g sample)				Grand Mean
	A	U	D	L	
<i>S. litura</i>	0.6820 ^a	0.5720 ^b	0.6560 ^a	0.5780 ^b	0.6220 ^A
<i>A. gossypii</i>	0.5400 ^c	0.4860 ^c	0.5840 ^b	0.4760 ^c	0.5215 ^B
Soap solution	0.3400 ^{de}	0.3120 ^{ef}	0.4460 ^c	0.2920 ^f	0.3475 ^D
Mechanical damage	0.3740 ^d	0.3520 ^{de}	0.4800 ^c	0.3580 ^d	0.3910 ^C
Grand Mean	0.4840 ^B	0.4305 ^C	0.5415 ^A	0.4260 ^C	
Damage/Location	POD activity (OD increase/min/g sample)				Grand Mean
	A	U	D	L	
<i>S. litura</i>	0.7800 ^b	0.716 ^{bcd}	0.9760 ^a	0.7580 ^b	0.8075 ^A
<i>A. gossypii</i>	0.706 ^{bcd}	0.654 ^{cdef}	0.7720 ^b	0.636 ^{efg}	0.6920 ^B
Soap solution	0.5200 ⁱ	0.5500 ^{hi}	0.5700 ^{ghi}	0.5140 ⁱ	0.5385 ^C
Mechanical damage	0.626 ^{fgh}	0.640 ^{defg}	0.7300 ^{bc}	0.632 ^{efg}	0.6570 ^B
Grand Mean	0.6580 ^B	0.6400 ^B	0.7620 ^A	0.6350 ^B	

Rows and columns followed by the same letter(s) are not significantly different at P<0.05. * Values represent difference in OD (activity) between the experiment and control leaves

induction of PPO and PI was also plant systemic. Results for *S. litura* feeding damage in the present study corroborate with their observation. However the results for the insects with sucking type of mouthparts varied. Stout *et al* (1996b) showed that mite feeding brought about a plant systemic induction of PPO and POD in contrary to our results with *A. gossypii* damage (a sucking pest) which showed POD induction only up to leaf systemic level. Different pest damages in different stages of development will produce variability within individual plants (Baldwin and Karb, 1995, Zangerl and Rutledge, 1996). Sucking insects in particular cause only very local damage and seem to be recognized by the plants as 'pathogens' rather than as 'classical herbivores', thus eliciting ISR (Walling, 2000). Bostok *et al.*, (2001) reported strong induction of proteinase inhibitor and polyphenol oxidase for *Spodoptera exigua* and *Heliothis zea* feeding, but in contrast, aphid feeding damage (*Macrosiphum persicae* and *M. euphorbia*) induced PR proteins but did not elicit proteinase inhibitors (Fidantsef *et al.*, 1999, Stout *et al.*, 1999).

There is a complex temporal and spatial array of signalling events in wounded plants. The earliest known events detected in wounded leaves include ion fluxes across the plasma membrane, changes in cytoplasmic calcium concentration, the generation of active oxygen species and changes in protein phosphorylation patterns. These early events occur in the first few minutes following damage, and are probably not directly responsible for inducing defense gene expression. Instead defense gene

expression is mediated primarily through the synthesis and action of Jasmonic acid. Other hormones with roles in regulating wound gene expression are ethylene and abscisic acid (ABA). The synthesis of JA and ethylene is well characterized, with many of the genes encoding their biosynthetic enzymes being up-regulated within 30-40 minutes of wounding, leading to peaks in hormones synthesis in wounded leaves at 1-2hours. Other elicitors of wound responses have also been identified, The most important include cell wall glycans, such as oligogalacturonides (OGAs) and systemin. These elicitors of wound response may either be primary signals released upon cellular damage, or may function to amplify the response in the wounded leaf. In addition, they may also perform a key role in systemic signaling. Proposed mechanisms for the transmission of signals to unwounded sites include electrical activity, the active transport of elicitors in the phloem and the passive transport of elicitors via hydraulic mass flow in the xylem. Wound inflicted by insect herbivory also results in signaling beyond the plant itself to mediate an indirect form of defense. Plants under attack from herbivores produce characteristic blends of volatiles that serve to attract predators and parasitoids of those herbivores.

An important criteria for practical usage of induced resistance techniques is to evaluate the persistence of induction in the plant. The PPO activity was persistent in tomato plants at levels significantly different from the controls even on the seventh day after a single feeding induction schedule. The degree of resistance was highest

with *S. litura* damage treatment. Very meager information is available on the persistence and magnitude of PPO induction in tomato due to herbivory. Many researchers reported that the external spray of JA mimics the herbivore damage in the induction of plant defense phenolics. Thaler *et al.*, (2001) reported increased activities of PI and PPO in JA (1.5mM=0.315mg of JA/plant) induced plants and was maximum at 13 days after spray. But for the herbivore damage (*S. litura* and *A. gossypii*) difference between control and induced plants, the PPO induction was maximum during 3rd day after damage treatment and then significant reduction in induction was observed, but still there was an increase in induction when compared to control. We may not yet fully understand the persistence of induced responses to herbivory. It is obviously important to determine the pattern of persistence for different types and combinations of herbivory.

A central signalling molecule in induced responses against herbivores is Jasmonic Acid (JA). In response to wounding or insect feeding, linolenic acid is released from membrane lipids and then converted enzymatically into JA. JA in turn causes the transcriptional activation of genes encoding proteinase inhibitors (PIs) and of enzymes involved in the production of volatile compounds or of secondary compounds such as nicotine, numerous phenolics and other defence related compounds.

Oligosaccharides and oligogalacturonides released from damaged cell walls bring about the general wound response and also some specific elicitors such as systemin. Systemin is an 18-amino acid polypeptide released upon wounding from a 200- amino acid precursor-pro-systemin, and leads to the release of linolenic acid. This activates the octadecanoid signalling cascade. Both JA and Systemin can be transported in the phloem and this may act as systemic signals. Besides systemin, cellulysin, a mixture of several cell wall-degrading enzymes can also induce JA responsive volatile.

It is therefore evident that the diversity of feeding types among insect pests plays a central role in the plant's response, which is frequently altered by insect-specific elicitors, giving plants to optimize their defenses. Both the plant variety and herbivore species affect the composition of induced volatiles, and it is becoming clear that both the predators and parasitoids are able to differentiate between various blends of herbivore induced volatiles to an amazing degree. It is to be noted that under the natural environmental conditions plants are exposed to a wide array of predators, either simultaneously or periodically. With each predator attack the plant shows characteristic biochemical induction. With numerous biochemical pathways underway, there is a lot of interactions between the molecules and their response to the predator.

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Host and non-host plant volatiles on oviposition and orientation behaviour of *Trichogramma chilonis* Ishii.

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ABSTRACT

In the process of host location and selection several cues associated with host's play a major role. Volatile compounds emitted by plants as a consequence of herbivore activities are often attractive to insect natural enemies including the Hymenopteran egg parasitoids, *Trichogramma* species. We studied the plant - parasitoid interactions where the plant surface chemicals act as infochemicals that attract or arrest the parasitoids for egg parasitization and strategically help in preventing the pest infestation. The leaf surface chemicals of *R. communis*, damaged due to the feeding of the host, *Achaea janata* (L) (castor semilooper) (Lepidoptera: Noctuidae), and a non - host, Serpentine leaf miner *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) were evaluated for their influence on host location, host acceptance and ovipositional behavior against the egg parasitoid, *T. chilonis* in laboratory bioassays. The *A. janata* damaged leaf emissions had synomonal effects on the parasitoid and induced orientation and oviposition, whereas, the surface chemicals from the plant infested with non-host *L. trifolii* ceased to produce any such effects. The maximum egg parasitization was observed in *A. janata* infested *castor* leaf extracts compared to the leaf miner infested or normal healthy castor leaf extracts. The results are interesting in the context of tritrophic interactions between the pest, parasite and the host plant and are useful in biological control of insect pests.

Keywords: *Ricinus communis*, *Trichogramma chilonis*, Host and Non -host synomones.

INTRODUCTION

Plant infochemicals play a major role in the tritrophic interactions of the plant -host - parasitoid interactions, especially during host selection process by parasitic wasps in general, including *Trichogramma* (Vinson, 1976; Nordlund, 1994). Host-habitat location, host location, host recognition and host acceptance are the important aspects of prey/host location (Nordlund *et al.*, 1988). Plant-derived stimuli are assumed to be more detectable from a distance (Jacqueline *et al.*, 1994). The volatiles emitted from the plants due to induction of herbivore feeding act as synomones, which are involved in indirect defense, because they attract their natural enemies (Dicke, 1999; Dicke and van Loon, 2000).

Plants produce and emit volatile organic chemicals in response to attract by feeding herbivores and often organisms. This area of research has become important in various contexts, primarily the utilization of plant generated volatiles for the attraction of the pest's natural enemies. The herbivore attack induce the plant to generate and emit organic compounds particularly terpenoids which are used as host location cues by wandering parasites or predators (Turlings *et al.*, 1990, 1995). *Trichogramma* the common egg parasitoid use the plant volatiles as host location cues from the damaged plant parts (Vet and Dicke, 1992).

The egg parasitoids are known to respond to the plants on herbivory by insects (Karan and Baldwin, 1997). The volatile signals used by foraging parasitoids can originate from the plant, the host, or from an interaction between the two. In a few plants (Dicke *et al.*, 1990a; Turlings *et al.*, 1990; Dicke, 1994; McCall *et al.*, 1994; Takabayashi *et al.*, 1991) the latter comprises passive release volatiles as well as induced volatiles. These volatiles can be plant species specific and/or herbivore species specific (De Moraes *et al.*, 1998). Studies with plants like lima beans (Dicke *et al.*, 1990b, 1993), corn (Turlings and Tumlinson, 1992), and recently cotton (Turlings *et al.*, 1995; Rose *et al.*, 1996) has shown that induced volatiles were released not only locally by the damaged leaf, but also systemically in undamaged parts of the plant.

Trichogramma wasps are minute egg parasitoids of lepidopteran species having widespread application as biological control agents. They are widely used in an inundative release programme for lepidopterous pest control (Romeis *et al.*, 1999; Wang *et al.*, 1999). Different *Trichogramma* species/strains show different preferences for certain host eggs (Zhou, 1985; Ballal and Singh, 2003). Parasitism by *Trichogramma* spp. is also affected by host egg location on plants, including both leaf surface and plant height (Burbutis *et al.*, 1977; Wang *et al.*, 1997; Romeis *et al.*, 1999; Ballal and Singh, 2003). Additionally,

different levels of parasitism by *Trichogramma* spp. can be found on the same host on different plants (Zhou *et al.*, 1997; Kuhar *et al.*, 2004). Quite a few reports indicate that within an ecosystem *Trichogramma* spp. are able to distinguish between host-infested and uninfested areas (Vet and Dicke, 1992). It is interesting to know the behavioral responses of *Trichogramma* egg parasitoids towards the plant/leaf surface chemicals from the host and non-host infested plants

The Castor semi-looper, *Achaea janata* L. (Lepidoptera : Noctuidae) is a noctuid moth, which feeds on Castor *Ricinus communis* L. leaves. During population outbreaks, larvae consume most of the foliage leaving just the veins and petioles. As measure to control the infestation caused by these insect-*Trichogramma* species are used as biocontrol agents. They have been reported to parasitize 50-83% of castor semilooper eggs (Salvador *et al.*, 1986). The serpentine leaf miner *Liriomyza trifolii* (Burgess) (Insecta: Diptera: Agromyzidae) damage by mining of leaves by larvae, which results in destruction of leaf mesophyll. It is a non-host for the *Trichogramma* spp. In this paper, we demonstrate the ovipositional and orientation behavior of *Trichogramma chilonis* towards the surface chemicals originating from plants infested with the host *Achaea janata* L and non-host *Liriomyza trifolii* Burgess.

MATERIAL AND METHODS

Insect material

T. chilonis reared on eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) at $28 \pm 2^\circ\text{C}$, $50 \pm 10\%$ r.h., L16:D8. The female parasitoids were 1–2 d old, mated females, inexperienced, and fed with honey were used in the experiments. Only fertile eggs, recognized by a horizontal brown ring, which develops after approximately 12 h, were used. Eggs were 24 h old and killed with UV-irradiation (60W, 50 min, 30 cm distance) or untreated. This short UV-irradiation does not affect the acceptance and suitability of the eggs for *T. chilonis*. UV-irradiated eggs have the advantage that no larvae emerge from unparasitized eggs and destroy the experiment. The castor semilooper, *A. janata* insects were reared on the castor leaves at $28 \pm 2^\circ\text{C}$, $60 \pm 10\%$ RH in the laboratory. Only third instar larvae were used for the experiments.

Plant material

The experimental plants, castor was grown in the laboratory green house under controlled conditions. Potted castor plants about 40cm in height, were selected for the experiments. The third instar larvae of *A. janata* were released on to the experimental plants at the rate of 1 larva/leaf and were allowed to feed for about 1hr. During

this period almost 50% of the leaf the larvae have consumed area. Another set of castor plants were infested with the larvae of leaf miner 1larva/leaf and the leaves having the mines all over the surface (10 days after infestation) was collected for extraction.

Extraction

The plant material was collected in the morning hours and was extracted using the organic solvent Dichloromethane (DCM) by leaf dip method (Varela and Bernays, 1988). 10 leaves of each *A. janata*, *L. trifolii* infested and healthy leaves were dipped in DCM solvent for 45seconds. The plant surface extracts obtained were filtered, concentrated by rotary evaporation and stored in refrigerator at -20°C .

Ovipositional Bioassay

All the bioassays on oviposition was carried out under laboratory conditions at $28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ relative humidity and under a light bank with overhead fluorescent tubes (1200 Lux) as source of light. The procedure adopted for oviposition bioassay was similar to the one described by Jones *et al.* (1973) with suitable modifications Usha Rani *et al.*, (2007). Clean, healthy, 0 to 24 h old eggs of *Corcyra cephalonica* Stainton sterilized under UV lamp were washed twice in hexane to remove any traces of scales or kairomones present on the surface of eggs. They were then pasted on 1cm^2 white cardboard pieces at 50 eggs per piece (hereafter referred to as egg cards). The plant surface extracts were applied at $0.2\text{mg}/\mu\text{l}$ concentrations on the egg cards at the rate of $10\ \mu\text{l}$ of sample on each egg card. A control was maintained in which only DCM was used. Five such treated egg cards with surface extracts along with one control card were arranged equidistantly in the experimental arena, which consisted of a 150 mm diameter Petri dish, the base of which was covered with Whatman No. 1 filter paper, each piece of egg card containing 50 eggs was considered as one replication and each extract was replicated four times. Five healthy, fed 0 to 24-h-old *Trichogramma* mated females were released at the centre of each petri dish containing egg cards. The percentage parasitism was recorded on the fifth day based on the number of eggs that turned black due to parasitization.

Orientation Behavior

The response of parasitoids to the volatile components of the plant surface extracts were recorded in the laboratory using small glass tubes. Behavioral observations were conducted in small flat-bottomed culture tubes (9 cm long; 2.5 cm diameter). Sample material ($20\ \mu\text{l}$) was applied with a micropipette to a localized spot on a piece of absorbent paper ($4 \times 0.7\text{ cm}$). After evaporation

of solvent for about 2 min, paper strips were placed onto the inner side of the lid of the tube. For each treatment, controls were maintained in a separate tube with the same amount of DCM One single adult-mated female was released into the glass tube before closing the lid. Observations on landing behavior, time spent on each treated and control patch, probing, and antennation were recorded visually. Landing and antennation of the treated spot by the parasitoid denoted a positive response and assigned a highest activity. The observations were terminated after 20 min.

Behaviors were classified 0–4, with 0 as no reaction, no movement; 1 as moved upwards towards the paper strip; 2 as made circular movements around the paper strip; 3 as entered/ landed on the paper strip; and 4 as antennated and probed on the chemical spot. Parasitoids almost always responded quickly, either positively or negatively, to a treatment. Twenty females were tested for each treatment, and controls were tested at the same time. All treatments were repeated on three different days with 20 insects. Almost all parasitoids showed a response to a treatment, so hardly any nonresponders were observed. All experiments and observations were conducted under laboratory conditions.

Statistical Analysis

The data obtained by the ovipositional bioassay of the *T. chilonis* parasitisation was analysed by the paired t-test for comparison between the treatments (Sigma stat 3.5). Bars represent the % mean eggs parasitized ± SE.

RESULTS

Ovipositional behaviour

In the ovipositional assays *T. chilonis* highly preferred the host plant leaf surface extracts infested with *A. janata* over the surface extracts from the non host infested castor

Table 1. Effect of normal, host and non-host infested Castor Plant surface extracts on the Orientation behavior of *T. chilonis* .

Leaf surface extracts	Type of Response /Score	Initial time of response (in min)
Normal plant	2	10
<i>A. janata</i> infested plant	4	3
<i>L. trifolii</i> infested plant	1	15

The type of response shown by 20 individual parasitoids is presented. 0=No response, 1= moved upwards, 2=landed on the paper strip, 3=moving around the patch, and 4=probing on the patch.

plants or solvent treated controls. The host eggs parasitized by *T. chilonis* in all the three treatments were significantly different at $P < 0.001$ (Figure 1). Interestingly leaf miner infested castor leaf extracts were significantly less attractive to the parasitoid than the normal healthy castor leaf extract. In choice bioassays too parasitoids preference for *A. janata* infested plant extracts was high (Figure 1).

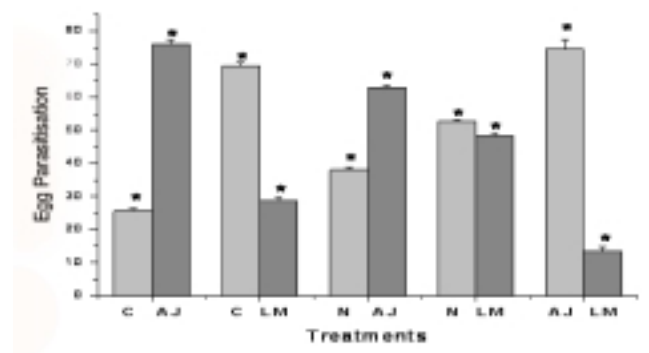


Figure 1. Effect of pest infested and uninfested Castor plant surface extract on parasitisation rates of *Trichogramma chilonis* on *C. cephalonica* eggs.

C-Control (Solvent), N- Normal Plant surface extract AJ- *Achaea janata* L. infested plant surface extract, LM- *Liriomyza trifolii* B. infested surface extract.

Orientation Behavior

In orientation assays also parasitoids response towards the extracts of castor plants infested with the host insect is very high. There was an immediate and quick movement of *T.chilonis* in the glass tube containing the extracts of host insect infested castor leaf extracts soon after their release. The parasitoid wasps had shown the probing and antennation on the strip having the host- plant surface extract. Parasitoids were highly active in these treatments, i.e. strips treated with *L. trifolii* infested castor leaf extracts and normal healthy castor leaf extracts there was only a slight upward movements by the parasitoids, *T. chilonis* did not even reach the paper strip on the treated patch (Table 1). However, the behavior of *T. chilonis* in solvent treated control treatments was entirely different. Parasitoids after their release into the glass tubes remained at the bottom of the tube without making any efforts to move or fly.

DISCUSSION

The observations on *T. chilonis*, behavior towards various host, non-host infested castor plant extracts indicated

the importance of type of herbivore feeding on a single host plant. It appears that the parasitoids were able to recognize certain chemicals or chemical combinations existing in the castor leaves infested with the host insect's *A. janata*. Presence of these chemicals in the host eggs increased their attractiveness/preference for the oviposition by *T. chilonis*. We presume these extracts contain some volatile as well as contact chemicals, which guide and stimulate the parasitoids to oviposit on the treated surface. A small number of host eggs treated with the extracts of non host (*L. trifolii*) infested leaves as well as Uninfested (Healthy) castor leaves were parasitized by *T. chilonis*. In orientation assays too parasitoids exhibited an upward movement (score-2) in the glass tube, thus acknowledging the presence of a few synomonal compounds in the extracts.

Herbivore feeding stimulates the production of volatile organic compounds and this has been demonstrated by several plant herbivore systems (Kessler and Baldwin, 2001; De Moraes *et al.*, 1998). Our experiments show that castor plants under the attack by *A. janata* larvae (which cause severe damage to the leaf by their voracious feeding), emit volatile and also contact chemicals that enhance the location/ acceptability of host eggs by parasitoid. These induced emissions of chemicals had occurred only in castor plant infested with *A. janata* larvae and not the *L. trifolii*.

We suggest certain organic volatile compounds might have been emitted by the *A. janata* damaged castor leaves which drew the parasitoids towards their source and certain contact chemicals that are also present in the extracts could have stimulated the oviposition. Because, though in less percentage, the host eggs treated with non-host insect infested as well as normal healthy castor plant extracts also has considerably stimulated parasitisation by *T. chilonis* in comparison with the solvent treated host eggs.

Our studies showed that the attractiveness of *A. janata* herbivore-induced synomones towards *T. chilonis* were specific at the herbivore level. The synomones induced by *L. trifolii* could not attract the egg parasitoids though they are from the same plant. Some specialist parasitoids of lepidopteran larvae and aphids could differentiate between volatiles emitted from host-infested plants and odor from plants damaged by non-hosts (De Moraes *et al.*, 1998; Powell *et al.*, 1998). Learning of distinct stimuli, which indicate the presence of suitable host species, has been shown to be a tool for parasitoids to cope with the variability of chemical signals available (Vet and Groenewold, 1990; Turlings *et al.*, 1992; Vet and Dicke, 1992; Dicke and Vet, 1999). Thus the parasitoids use the

plant synomones. Investigations of the preference of *T. chilonis* for oviposition on castor semilooper infested leaves are necessary for a comprehensive elucidation of the role of host specificity for host location of this parasitoid.

Here we found that the surface extracts from the same host plant infested with different insects had shown varied responses. Parasitoids, in particular, are highly susceptible to very small changes in the quality of the host's internal biochemical environment (Harvey, 2005). Other studies have shown that parasitoids can be more affected than their hosts by the quality of the host plant (Harvey *et al.*, 2003; Soler *et al.*, 2005). In plant both host and non-host larvae induce the plant to emit qualitatively and quantitatively the same synomones (Röse *et al.*, 1998). But in the case of castor *R. communis*, we found that the egg parasitoids preferred host insect infested surface extracts when compared to the non-infested plant surface extracts. At present there is not enough experimental evidence available to discern a general pattern of herbivore specificity of induced plant synomones. A better knowledge of the surface chemistry will help to understand interactions between plant parasitoid, insect-parasitoid interactions.

In conclusion, the specialist *A. janata* appears to be host that induces plant synomones which are the indicators for the *T. chilonis* during the entire host selection process from location of host habitats to location of hosts and host acceptance/recognition. The tritrophic system studied here is described the relationship of the oligophagous and monophagous on the second and the third trophic level by little variation of chemical cues for the parasitoid. These built in characteristics of the tritrophic system might be a prerequisite for the development of such selective responses of a parasitoid towards specific infochemicals.

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Induction of resistance through organic amendments for the management of spotted leaf beetle, *Epilachna vigintioctopunctata* Fab. on Ashwagandha

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ABSTRACT

Ashwagandha or Asgandh (*Withania somnifera* Dunal.), is an important medicinal plant which is attacked by several insect pests including spotted leaf beetle, *Epilachna vigintioctopunctata* Fab. The present investigation was carried out to under field conditions. Results revealed that farmyard manure (FYM) (12.5 t/ha) + Azophos (2 kg/ha) + neem cake (1000 kg/ha) was found to be very effective in reducing the damage of spotted leaf beetle by 69.79 per cent. FYM + Azophos + neem cake combination was less preferred for oviposition which recorded 62.00 eggs/plants, coupled with a minimum feeding area of 6.75 cm².

Key words: *Withania somnifera*, *Epilachna vigintioctopunctata*, eco-friendly pest management, biology studies

INTRODUCTION

In India, the use of several medicinal plants to cure specific ailments is in vogue from ancient times. The WHO has estimated that over 80 per cent of the world population meets their primary health care needs through traditional medicine (Lambert, 1997). In India Ashwagandha is cultivated in about 4000 ha in marginal lands of Madhya Pradesh and Rajasthan (Nigam *et al.*, 1984). In Tamil Nadu, the commercial cultivation of this plant is gaining momentum in certain areas *viz.*, Erode, Namakkal, Salem and Coimbatore districts. It is ravaged by a few major insect pests like, *Epilachna vigintioctopunctata* Fab. (Coccinellidae: Hemiptera) has been reported as a serious defoliator of some solanaceous medicinal plants. *Epilachna vigintioctopunctata* F. damage the foliage and is considered a major pest of Ashwagandha (Mathur and Srivastava, 1964; Parjhar *et al.*, 1997; Patra *et al.*, 2004). The inorganic fertilizers provide the nutrients in appreciable quantities for a shorter period to the plants. Thereby the plants are endowed with luxuriant growth which offers adequate food to the insects leading to heavy insect population build up. The organic manures act as a slow release fertilizers providing balanced nutrition to the plants and ensure balanced growth, thereby making them less prone to pest incidence. Induced resistance is the qualitative and quantitative enhancement of plant's defence mechanisms which is a non heritable resistance where host plants are induced to impart resistance to tide over pest infestation (Heinrichs, 1988; Dilawari and Dhaliwal, 1993; Panda and Khush, 1995). Through the addition of organic sources of nutrients and amendments the production of defensive chemicals in plant increases. So, organic farming provides an eco-technological stability in pest management and is a vital component of

sustainable agriculture. The integration of organic nutrients can play a vital role in creating unfavorable environment to the herbivores by the mechanism of induced resistance either by antibiosis or antixenosis. Ashwagandha, being a medicinal herb, frequent and large scale application of insecticides for the control of these pests often leads to the endangerment of ecosystem. Keeping these aspects in view, the present investigations were carried out to test the efficacy of organic components in order to combat the incidence of insect pests of Ashwagandha.

MATERIALS AND METHODS

The field trial was conducted at Agricultural College and Research Institute, Madurai. All the Agronomic practices were adopted uniformly for all the treatments. The details of the treatments are as follows: FYM (12.5 t/ha) alone (T₁), FYM (12.5 t/ha) + Azophos (2 kg/ha) + Neem cake (1000 kg/ha) (T₂), FYM (12.5 t/ha) + Azophos (2 kg/ha) + Pungam cake (1000 kg/ha) (T₃), FYM (12.5 t/ha) + Azophos (2 kg/ha) + Mahua cake (1000 kg/ha) (T₄), NPK + Botanicals (3 %) (T₅), FYM (50%) + Azophos (2 kg/ha) + NPK (50%) + Neem cake (50%) (T₆), FYM (50%) + Azophos (2 kg/ha) + NPK (50%) + Pungam cake (50%) (T₇), FYM (50%) + Azophos (2 kg/ha) + NPK (50%) + Mahua cake (50%) (T₈), NPK + Malathion (2 ml/lit) (T₉), NPK as inorganic form (90:50:50 kg/ha) (T₁₀) and Untreated control (T₁₁). The treatments were replicated thrice in randomized block design. The variety Jawahar was used. Farmyard manure (FYM) with computed quantity was applied basally at the time of main field preparation in the respective treatments. The biofertilizer *viz.*, Azophos @ 2 kg/ha was incorporated with soil in the respective treatments. Half of the dose of the total requirements of other organic amendments *viz.*,

Table 1. Effect of organic sources of nutrients on *Epilachna* beetle damage

Treatments	Days After Transplanting															
	15		25		35		45		55		65		75		Mean	
	% Damage	% reduction over NPK	% Damage	% reduction over NPK	% Damage	% reduction over NPK	% Damage	% reduction over NPK	% Damage	% reduction over NPK	% Damage	% reduction over NPK	% Damage	% reduction over NPK	% Damage	% reduction over NPK
T ₁	2.46 (9.02) ^d	37.08	5.57 (13.65) ^f	32.97	8.24 (16.68) ^g	33.81	9.05 (17.50) ^g	36.97	11.53 (19.85) ^{gh}	31.49	8.65 (17.10) ^{ef}	29.15	5.73 (13.85) ^f	21.29	7.31 (15.68) ^f	32.06
T ₂	0.97 (5.65) ^a	75.19	2.26 (8.64) ^a	72.80	3.45 (10.70) ^b	72.28	3.9 (11.39) ^{bc}	72.84	4.98 (12.89) ^b	70.40	4.22 (11.85) ^b	64.43	3.02 (10.00) ^b	58.51	3.25 (10.38) ^a	69.79
T ₃	1.74 (7.58) ^b	55.49	3.82 (11.27) ^c	54.03	5.58 (13.66) ^{cd}	55.18	6.02 (14.20) ^d	58.07	7.32 (15.69) ^{cd}	56.50	5.94 (14.10) ^c	51.35	3.74 (11.15) ^c	48.62	4.88 (12.76) ^c	54.64
T ₄	1.22 (6.34) ^a	68.79	3.08 (10.10) ^b	62.93	4.55 (12.31) ^c	63.45	4.82 (12.68) ^c	66.43	6.23 (14.45) ^{bc}	62.93	4.83 (12.69) ^b	60.44	3.38 (10.59) ^{bc}	53.57	4.01 (11.55) ^b	62.73
T ₅	3.7 (11.09) ^f	5.37	8.1 (16.53) ^h	2.52	2.98 (9.94) ^{ab}	76.06	3.68 (11.06) ^b	74.37	3.75 (11.16) ^a	77.71	2.82 (9.66) ^a	76.90	2.22 (8.56) ^a	69.50	3.89 (11.37) ^{ab}	63.84
T ₆	1.79 (7.68) ^b	54.21	4.2 (11.82) ^{cd}	49.45	6.07 (14.26) ^{de}	51.24	6.82 (15.13) ^{de}	52.50	8.32 (16.76) ^{de}	50.56	6.83 (15.15) ^{cd}	44.06	4.36 (12.05) ^d	40.10	5.48 (13.53) ^{cd}	49.07
T ₇	2.36 (8.83) ^{cd}	39.64	5.32 (13.33) ^{ef}	35.98	7.86 (16.28) ^{fg}	36.86	8.74 (17.19) ^{fg}	39.13	10.66 (19.05) ^{fg}	36.66	7.92 (16.34) ^{de}	35.13	5.02 (12.94) ^e	31.04	6.84 (15.16) ^{ef}	36.43
T ₈	2.02 (8.17) ^{bc}	48.33	4.58 (12.35) ^{de}	44.88	6.82 (15.13) ^{ef}	45.22	7.45 (15.84) ^{ef}	48.11	9.2 (17.65) ^{ef}	45.00	7.54 (15.93) ^{de}	38.24	4.72 (12.54) ^{de}	35.16	6.04 (14.22) ^{de}	43.86
T ₉	3.89 (11.37) ^f	0.51	8.18 (16.61) ^h	1.56	2.45 (9.00) ^a	80.32	2.7 (9.45) ^a	81.19	2.82 (9.66) ^a	83.24	2.34 (8.79) ^a	80.83	1.98 (8.08) ^a	72.80	3.48 (10.75) ^{ab}	67.65
T ₁₀	3.91 (11.40) ^f	-	8.31 (16.75) ^h	-	12.45 (20.66) ⁱ	-	14.36 (22.26) ^h	-	16.83 (24.22) ^j	-	(24.22) (20.45) ^g	-	7.28 (15.65) ^g	-	10.76 (19.14) ^h	-
T ₁₁	3.22 (10.33) ^e	17.64	6.94 (15.27) ^g	16.48	10.12 (18.54) ^h	18.71	10.89 (19.26) ⁱ	24.06	12.84 (20.99) ^h	23.70	9.85 (18.29) ^f	19.32	6.06 (14.25) ^f	16.75	8.56 (17.0) ^g	20.44

Figures in parentheses are arc sine transformed values, in a column, means followed by same letter(s) are not significantly different at P=0.05 DMRT

neem cake, mahua cake and pungam cake were applied as basal and the remaining half was applied as top dressing in two equal splits at 20 days interval. Inorganic fertilizers in the form of urea, single super phosphate and muriate of potash were applied at recommended doses. Fifty per cent of total N and entire P and K were applied as basal and the rest of the 50 per cent N was applied in two equal splits as top dressing at 20 days interval. Neem oil @ 3 per cent and malathion 0.1 per cent were sprayed in the respective treatments on 30th, 45th and 60th day after transplanting (DAT). Pest damage was assessed on ten randomly selected plants from each plot. The total number of leaves and the number of scraped leaves were counted to compute the per cent leaf damage. The observations were recorded at ten days interval commencing from 15th to 75th DAT besides a pretreatment count. Root yield was recorded in kg / plot and converted for hectares.

Screen house and laboratory experiments were conducted with the following treatments in completely randomized block design with three replications. The material and methods, the treatment details were the same as adopted for field trial. A free choice test using 60 days old potted plants of various treatments in an insect rearing cage of the size 2 x 1.5 x 2 m was conducted. Ten pairs (male and female in 1:1 ratio) of freshly emerged beetles were released into the cage for oviposition. Ten days later, the number of eggs laid on each plant was observed. Leaf discs of 5 cm diameter from respective treatments were collected and placed in a petridish over a moist filter paper. Two prestarved (4 hours) adult *Epilachna* beetles were released in each petridish and treatment setup was replicated thrice. After 24 hours, the feeding rate was assessed in terms of the total leaf area scraped by adult beetles through graphical method.

RESULTS AND DISCUSSION

The present study revealed that the overall mean values of the results revealed that per cent damage in various treatments was FYM + Azophos + NC (69.79 %) was superior to all other treatments except NPK + malathion (3 sprays) and NPK + botanical (3 sprays) in reducing the damage with corresponding per cent reduction of 67.65 and 63.84 per cent respectively (table 1). This is in close agreement with the earlier findings of Rajendran and Chandramani (2002) who reported that application of FYM along with neem cake and biofertilizers was effective in reducing the incidence of aphid (*Myzus persicae*) and thrips (*Scirtothrips dorsalis*) on chillies. The efficacy of these treatments is also in conformity with the findings of Suresh (2003) and Kavitha (2004) who demonstrated the reduction of *Epilachna* beetle in brinjal by the application of neem cake combined with FYM and biofertilizers. Further

Mohan *et al.* (1987) also proved that the combination of FYM + biofertilizers either with neem cake or mahua cake significantly reduced the *Epilachna* beetle infestation on brinjal.

The data on fresh root yield (table 2) revealed that FYM + Azophos + neem cake registered the yield of 991.16 kg / ha with corresponding per cent increase of 34.88 over NPK as inorganic form. This was followed by NPK + malathion (3 sprays) (926.13 kg / ha) and NPK + botanical sprays (902.13 kg / ha) with 30.31 and 28.45 per cent increase of fresh root yield over NPK. Further, the treatments with yield increase over NPK were high in organics, FYM + Azophos + mahua cake (24.48%) and NPK + Azophos + pungam cake (21.36 %). This is in conformity with the findings of Swarnapriya *et al.* (2006) who reported that the application of FYM + neem cake recorded the highest root yield in ashwagandha. Earlier Subbaiah *et al.* (1982) explained that the yield increase with organic manure was due to solubilization of nutrients in the soil and increased availability to the plants.

Ovipositional preference

FYM + Azophos + neem cake (62.00 eggs/plants) and FYM + Azophos + mahua cake (67.33 eggs/plants) treated plants were least preferred for oviposition (Table 3). The present investigation revealed that the least ovipositional preference might be associated with the low level of leaf nitrogen which was in agreement with the findings of Waghray and Shivaraj Singh (1965). Kavitha (2004) also has reported that ovipositional preference of *Epilachna*

Table 2. Effect of organic sources of nutrients on fresh root yield

Treatments	Yield of fresh roots (kg/ha)	Increase over NPK (%)
T ₁	602.29 ^f	-
T ₂	991.16 ^a	34.88
T ₃	820.71 ^c	21.36
T ₄	854.65 ^c	24.48
T ₅	902.10 ^b	28.45
T ₆	765.12 ^d	15.64
T ₇	724.32 ^d	10.89
T ₈	740.00 ^d	12.78
T ₉	926.13 ^b	30.31
T ₁₀	645.38 ^e	-
T ₁₁	464.42 ^g	-

In a column, means followed by same letter(s) are not significantly different at P=0.05 by DMRT

Table 3. Ovipositional preference of *E.pilachna* beetle

Treatments	Eggs / Plant	Oviposition (%)
T ₁	183.67 (15.33) ^f	14.92 ^c
T ₂	62.00 (7.87) ^a	5.03 ^a
T ₃	95.67 (9.78) ^b	7.77 ^b
T ₄	67.33 (8.20) ^a	5.47 ^a
T ₅	112.67 (10.61) ^c	9.15 ^c
T ₇	151.67 (12.31) ^e	12.32 ^d
T ₈	126.66 (11.25) ^d	10.29 ^c
T ₁₀	235.67 (15.35) ^h	19.15 ^f
T ₁₁	195.00 (13.96) ^g	15.84 ^e

Figures in parentheses are square root transformed values. In a column, means followed by a same letter are not significantly different at P=0.05 (DMRT).

beetle was least when treated with FYM + biofertilizers + neem cake and FYM + biofertilizers + mahua cake as compared to NPK as inorganic form.

Feeding deterency

The treatment with FYM + Azophos + neem cake recorded a minimum feeding area of 6.75 cm² and on a par with FYM + Azophos + mahua cake (8.25 cm²) as against 18.75 cm² in NPK as inorganic form. The corresponding per cent reduction of feeding over NPK was 64.00 and 56.00 respectively (table 4). The result obtained from the laboratory study shows that the feeding preference

Table 4. Feeding deterency of different manure and fertilizer treatments to *Epilachna* beetle on

Treatments	Feeding area	
	cm ²	% reduction over NPK
T ₁	16.75 (4.09) ^{ef}	10.67
T ₂	6.75 (2.59) ^a	64.00
T ₃	9.50 (3.08) ^b	49.33
T ₄	8.25 (2.87) ^{ab}	56.00
T ₅	12.50 (3.53) ^c	33.33
T ₇	15.25 (3.90) ^{de}	18.67
T ₈	14.00 (3.74) ^{cd}	25.33
T ₁₀	18.75 (4.33) ^g	-
T ₁₁	17.50 (4.18) ^{fg}	6.67

Figures in parentheses are square root transformed values. In a column, means followed by a same letter are not significantly different at P=0.05 (DMRT)

increased with the increased levels of nitrogenous fertilizers. The feeding area of *Epilachna* beetle was more on leaves treated with NPK as inorganic form. Adverse effect of organics on feeding activity of BPH and WBPH in rice was earlier been observed by Chandramani (2003). Kavitha (2004) has also reported that the feeding area by *Epilachna* beetle was less in the combination of FYM + biofertilizers + neem cake. The increase in the feeding preference of brinjal leafhopper with increased fertilizer application is also in agreement with the findings of Godase and Patel (2001); Yein and Singh (1982) in green gram and Rote and Puri (1992) in cotton.

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Evaluation of certain organic nutrient sources against mealy bug, *Coccidohystrix insolitus* (Green.) and the spotted leaf beetle, *Epilachna vigintioctopunctata* Fab. on Ashwagandha, *Withania somnifera* Dunal.

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ABSTRACT

Ashwagandha is considered as an important medicinal crop in Indian system of medicine. Ashwagandha suffers attack by several insect pests. The mealy bug, *Coccidohystrix insolitus* (Green.) and spotted leaf beetle, *Epilachna vigintioctopunctata* Fab. are found to be the key pests. Since Ashwagandha is a herbal medicine, application of synthetic chemicals leads accumulation of toxic residues. Field experiments were conducted with application of farmyard manure (FYM) (12.5 t/ha) + Azophos (2 kg/ha) + neem cake (1000 kg/ha) and need based foliar application of neem oil (3%) were found to be very effective in reducing the incidence of mealy bug and the damage of spotted leaf beetle.

Key words: Ashwagandha, *Epilachna vigintioctopunctata*, *Coccidohystrix insolitus*, eco-friendly pest management.

INTRODUCTION

Ashwagandha or Asgandh (*Withania somnifera* Dunal.) is considered an important medicinal plant. It is extensively exploited in Indian system of medicine. This solanaceous plant belongs to the genus *Withania*, which includes 23 species, of which *Withania somnifera* and *Withania coagulans* are the two species found in India that have high medicinal value. It is a household remedy and is commonly known by various local names as Ashwagandha in Sanskrit, Asgandh in Hindi, Amukkiran or kizhangu in Tamil and Winter cherry in English. It is used as a tonic in geriatrics, being efficacious in relieving hand and limb tremors of people at old age (Atal *et al.*, 1975). It has been equated to ginseng (*Panax ginseng*) of China and is popularly known as the "Indian Ginseng". The most important pharmacological use of Ashwagandha is as adaptogen with antistress antioxidant, antitumor, anti-inflammatory, mind boosting and has rejuvenating properties (Singh *et al.*, 1990).

Ashwagandha is devastated by an array of insect pests, of which *Epilachna vigintioctopunctata* F. damages the foliage heavily (Mathur and Srivastava, 1964; Parjhar *et al.*, 1997; Patra *et al.*, 2004). A study conducted in 2003 in Gurah Bramana, Kotgarhi, Rakh and Tanda, Jammu and Kashmir, India on Ashwagandha (*W. somnifera*) revealed the infestation of mango mealy bug, *Drosicha mangiferae* (Margarodidae : Hemiptera) on the foliage (Bhagat, 2004). White and waxy-coated nymphs and adults of *Coccidohystrix insolitus* (Pseudococcidae: Hemiptera) infest lower side of leaves in colonies and desap profusely (Jhansi Rani, 2001). Hence, considering its medicinal

property and commercial value with rapid expansion of drug industries there is an urgent need to develop eco-friendly management practices to control the various pests devastating Ashwagandha to obtain insecticidal residue free herbal products.

MATERIAL AND METHODS

The field trial was conducted at the orchard of Agricultural College and Research Institute, Madurai with Jawahar variety. All the Agronomic practices were adopted uniformly for all the treatments, the details of treatments are as follows: T₁ - FYM (12.5 t/ha) + Azophos (2 kg/ha) + Neem cake (1000 kg/ha), T₂ - FYM (12.5 t/ha) + Azophos (2 kg/ha) + Mahua cake (1000 kg/ha), T₃ - FYM (12.5 t/ha) + Azophos (2 kg/ha) + Neem cake (1000 kg/ha) + Neem oil (3%), T₄ - FYM (12.5 t/ha) + Azophos (2 kg/ha) + Mahua cake (1000 kg/ha) + Neem oil (3%), T₅ - FYM (50%) + Azophos (2 kg/ha) + NPK (50%) + Neem cake (50%), T₆ - FYM (50%) + Azophos (2 kg/ha) + NPK (50%) + Mahua cake (50%), T₇ - FYM (50%) + Azophos (2 kg/ha) + NPK (50%) + Neem cake (50%) + Neem oil (3%), T₈ - FYM (50%) + Azophos (2 kg/ha) + NPK (50%) + Mahua cake (50%) + Neem oil (3%), T₉ - NPK + Botanical (neem oil 3%), T₁₀ - NPK + Malathion (2ml / litre), T₁₁ - NPK alone (90:50:50 kg/ha), and T₁₂ - Untreated control.

The treatment were replicated in randomized block design, the variety being Jawahar. Farmyard manure (FYM) with computed quantity was applied basally at the time of main field preparation in the respective treatments. The biofertilizer *viz.*, Azophos @ 2 kg/ha was incorporated to the soil in the respective treatments. Half of the dose of

Table 1. Effect of organic sources of nutrients and botanical on *Epilachna* beetle damage (%) (DA) and per cent reduction over NPK (PR) on Ashwagandha

Treatments	Days After Transplanting															
	15		25		35		45		55		65		75		Mean	
	DA	PR	DA	PR	DA	PR	DA	PR	DA	PR	DA	PR	DA	PR	DA	PR
T ₁	1.25 (6.41) ^a	72.40	2.34 (8.79) ^a	73.79	3.75 (11.16) ^d	71.04	4.40 (12.10) ^{cd}	70.37	5.01 (12.93) ^c	71.33	3.74 (11.15) ^c	70.43	2.94 (9.87) ^d	70.12	3.35 (10.54) ^{cd}	71.12
T ₂	1.39 (6.77) ^a	69.31	2.76 (9.56) ^a	69.09	3.91 (11.40) ^d	69.80	4.58 (12.35) ^d	69.15	5.12 (13.07) ^c	70.72	3.92 (11.41) ^{cd}	69.01	3.10 (10.14) ^d	68.49	3.54 (10.84) ^{cd}	69.48
T ₃	1.28 (6.49) ^a	71.74	2.35 (8.81) ^a	73.68	1.98 (8.08) ^a	84.71	2.28 (8.68) ^a	84.46	2.65 (9.36) ^a	84.83	2.10 (8.33) ^a	83.39	1.72 (7.53) ^a	82.52	2.05 (8.23) ^a	82.32
T ₄	1.43 (6.86) ^a	68.43	2.78 (9.59) ^a	68.86	2.32 (8.76) ^{ab}	82.08	2.64 (9.35) ^{ab}	82.22	3.22 (10.33) ^{ab}	81.57	2.31 (8.74) ^{ab}	81.73	1.96 (8.04) ^{ab}	80.08	2.38 (8.81) ^{ab}	79.48
T ₅	2.18 (8.49) ^b	51.87	4.26 (11.91) ^b	52.29	5.62 (13.71) ^e	56.60	6.37 (14.61) ^e	57.10	6.75 (15.05) ^d	61.38	4.87 (12.74) ^{de}	61.50	3.93 (11.43) ^e	60.06	4.85 (12.72) ^{ef}	58.18
T ₆	2.40 (8.91) ^b	47.01	4.65 (12.45) ^b	47.92	6.59 (14.87) ^e	49.11	6.58 (14.86) ^e	55.69	7.16 (15.52) ^d	59.03	5.26 (13.25) ^e	58.41	4.12 (11.71) ^e	58.13	5.25 (13.24) ^f	54.74
T ₇	2.29 (8.70) ^b	49.44	4.29 (11.95) ^b	51.95	3.20 (10.30) ^{bcd}	75.28	3.22 (10.33) ^{ab}	78.31	3.82 (11.27) ^{abc}	78.14	2.74 (9.52) ^{ab}	77.94	2.30 (8.72) ^{abc}	76.62	3.12 (10.17) ^{bc}	73.10
T ₈	2.42 (8.94) ^b	47.03	4.60 (12.38) ^b	48.48	3.28 (10.43) ^{cd}	74.67	3.36 (10.56) ^{bc}	77.37	4.10 (11.68) ^{bc}	76.54	3.68 (10.10) ^{bc}	75.62	2.47 (9.04) ^{bcd}	74.89	3.32 (10.49) ^c	71.37
T ₉	4.29 (11.95) ^{cd}	5.29	8.82 (17.27) ^c	1.23	3.34 (10.53) ^d	74.20	3.42 (10.65) ^{bcd}	76.96	4.18 (11.79) ^{bc}	76.08	3.14 (10.20) ^{bc}	75.17	2.49 (9.07) ^{bcd}	74.69	4.24 (11.88) ^{de}	63.44
T ₁₀	4.24 (11.88) ^{cd}	6.40	8.65 (17.10) ^c	3.13	2.39 (8.84) ^{abc}	81.54	2.72 (9.49) ^{ab}	81.68	3.32 (10.49) ^{ab}	81.00	2.39 (8.89) ^{ab}	81.10	2.04 (8.21) ^{ab}	74.26	3.67 (11.04) ^{cd}	68.36
T ₁₁	4.53 (12.28) ^d	-	8.93 (17.38) ^c	-	12.95 (21.09) ^f	-	14.85 (22.66) ^f	-	17.48 (24.71) ^e	-	12.65 (20.83) ^e	-	9.84 (18.28) ^e	-	11.60 (19.91) ^e	-
T ₁₂	3.74 (11.15) ^c	17.43	8.08 (16.51) ^c	9.51	12.26 (20.49) ^f	5.32	13.48 (21.54) ^f	9.22	16.28 (23.79) ^e	6.86	10.78 (19.16) ^f	14.78	8.18 (16.61) ^f	16.86	10.40 (18.81) ^e	10.34

Figures in parentheses are arc sine transformed values

In a column, means followed by same letter(s) are not significantly different at P=0.05 (DMRT)

the total requirements of other organic amendments viz., neem cake, mahua cake and pungam cake were applied as basal and the remaining half was applied as top dressing in two equal splits at 20 days interval. Inorganic fertilizers in the form of urea, single super phosphate and muriate of potash at recommended were applied. Fifty per cent of total N and entire P and K were applied as basal and the rest of 50 per cent N in two equal splits as top dressing at 20 days interval. The neem oil @ 3 per cent and Malathion (2ml / litre) were sprayed in the respective treatments at 30, 45 and 60 days after transplanting (DAT).

Pest damage was assessed from ten randomly selected plants/plot. The total number of leaves and the number of scraped leaves/plant were counted to work out the per cent leaf damage. The observations were recorded at ten

days interval commencing from 15th to 75th DAT besides a pretreatment count. In each plant, three leaves representing top, middle and bottom portions were selected. The total number of nymphs and adults on each leaf was recorded and the mean was worked out to express the population as mean number per three leaves. The population was recorded from 35 to 95 DAT at an interval of ten days. Root yield was recorded in kg / ha.

RESULTS AND DISCUSSION

The results of the investigation on the effect of organic sources of nutrients against the pests of Ashwagandha are presented in tables 1-3. The overall per cent damage recorded throughout the period of observation revealed that among organics imposed, FYM + Azophos + NC with

Table 2. Effect of organic sources of nutrients and botanical on Mealy bug population on Ashwagandha

Treatments	Days After Transplanting															
	35		45		55		65		75		85		95		Mean	
	DA	PR	DA	PR	DA	PR	DA	PR	DA	PR	DA	PR	DA	PR	DA	PR
T ₁	0.43 (0.65) ^{cd}	70.94	0.54 (0.73) ^d	69.31	0.61 (0.78) ^e	70.67	0.66 (0.81) ^e	71.55	0.69 (0.83) ^{de}	74.34	0.83 (0.91) ^{de}	72.69	0.47 (0.68) ^{ef}	67.13	0.60 (0.77) ^{de}	71.56
T ₂	0.47 (0.68) ^d	68.24	0.57 (0.75) ^d	67.61	0.66 (0.81) ^e	68.26	0.71 (0.84) ^e	69.39	0.76 (0.87) ^e	71.74	0.91 (0.95) ^e	70.06	0.51 (0.71) ^f	64.33 (0.80) ^f	0.65	69.19
T ₃	0.20 (0.47) ^a	86.48	0.26 (0.50) ^a	85.22	0.30 (0.54) ^a	85.57	0.32 (0.56) ^a	86.20	0.35 (0.59) ^a	86.98	0.44 (0.66) ^a	85.52	0.24 (0.48) ^a	83.21	0.30 (0.54) ^a	85.78
T ₄	0.23 (0.47) ^a	84.45	0.29 (0.53) ^{ab}	83.52	0.34 (0.58) ^{ab}	83.65	0.37 (0.60) ^{ab}	84.05	0.42 (0.64) ^{ab}	84.38	0.51 (0.71) ^{ab}	83.22	0.29 (0.53) ^{ab}	79.72	0.35 (0.59) ^{abc}	57.34
T ₅	0.56 (0.81) ^e	55.40	0.76 (0.87) ^e	56.81	0.89 (0.94) ^f	57.21	0.99 (0.99) ^f	57.32	1.12 (1.05) ^f	58.36	1.26 (1.12) ^f	58.55	0.64 (0.80) ^g	55.24	0.90 (0.94) ^f	57.34
T ₆	0.71 (0.84) ^e	52.02	0.81 (0.90) ^e	53.97	0.93 (0.96) ^f	55.28	1.04 (1.01) ^f	55.17	1.16 (1.07) ^f	56.87	1.46 (1.20) ^f	51.97	0.68 (1.82) ^f	52.44 (1.98) ^f	0.97	54.02
T ₇	0.34 (0.58) ^{bc}	77.02	0.38 (0.61) ^{bc}	78.40	0.46 (0.67) ^{bcd}	77.88	0.49 (0.70) ^{bcd}	78.87	0.54 (0.73) ^{bcd}	79.92	0.64 (0.80) ^{bcd}	78.94	0.37 (0.60) ^{bcd}	74.12	0.46 (0.67) ^{bd}	78.19
T ₈	0.38 (0.61) ^{cd}	74.32	0.49 (0.70) ^{cd}	72.15	0.52 (0.72) ^{cde}	75.00	0.55 (0.74) ^{cde}	76.29	0.57 (0.76) ^{cde}	78.06	0.70 (0.83) ^{cde}	76.97	0.40 (0.63) ^{cde}	72.02	0.51 (0.71) ^{cde}	75.82
T ₉	0.41 (0.64) ^{cd}	72.29	0.52 (0.72) ^d	70.45	0.58 (0.76) ^{de}	72.11	0.61 (0.78) ^{de}	73.70	0.63 (0.79) ^{cde}	76.57	0.75 (0.86) ^{cde}	75.32	0.45 (0.67) ^{def}	68.53	0.56 (0.74) ^{de}	73.45
T ₁₀	0.25 (0.50) ^{ab}	83.10	0.32 (0.56) ^{ab}	81.82	0.39 (0.62) ^{abc}	81.25	0.42 (0.66) ^{abcd}	81.03	0.49 (0.70) ^{abc}	81.78	0.59 (0.76) ^{abc}	80.59	0.33 (0.57) ^{bc}	76.92	0.40 (0.63) ^{abc}	81.04
T ₁₁	1.48 (1.21) ^g	-	1.76 (1.32) ^g	-	2.08 (1.44) ^g	-	2.32 (1.52) ^g	-	2.69 (1.64) ^g	-	3.04 (1.74) ^g	-	1.43 (1.19) ⁱ	-	2.11 (1.45) ^g	-
T ₁₂	1.26 (1.12) ^f	14.86	1.52 (1.23) ^f	13.63	1.86 (1.36) ^g	10.57	2.04 (1.42) ^g	12.06	2.43 (1.55) ^g	9.66	2.81 (1.67) ^g	7.56	1.23 (1.10) ^h	13.98	1.87 (1.36) ^g	11.37

Figures in parentheses are square root transformed values

In a column, means followed by same letter(s) are not significantly different at P=0.05 (DMRT)

need based application of neem oil (3%) and FYM + Azophos + MC + neem oil (3%) were found significantly effective in reducing the damage due to *Epilachna* beetle by recording 82.32 and 79.48 per cent reduction over NPK (Table 1). The results of the present study is in accordance with the findings of Dhandapani *et al.* (1985) who reported 63 per cent reduction in the *Epilachna* beetle population due to neem oil spray. Kavitha (2004) also reported that *Epilachna* beetle damage could be significantly reduced by the application of FYM + Biofertilizer + neem cake + neem oil sprays and FYM + Biofertilizers + mahua cake + neem oil sprays. The reports on the efficacy of neem extracts and its formulations against the *Epilachna* beetle, *H. vigintioctopunctata* was reported by other workers (Jayarajan and Sundarababu, 1990; Mishra *et al.*, 1990; Rao *et al.*, 1992 and Reddy Venkataraimi *et al.*, 1993) were also in consonance with the present investigation.

While computing through all the periods of observation revealed that FYM + Azophos + neem cake + neem oil recorded less population of mealy bug (0.30/3 leaves) which was closely followed by FYM + Azophos + mahua cake + neem oil (0.35 / 3 leaves) as against 2.11 in NPK as inorganic form treated plots. The corresponding over all percent reduction over NPK was 85.78 and 83.41 (Table 2). This is in line with the findings of Saminathan and Jayaraj (2001) who reported that neem oil and pungam oil at 3 % effectively controlled the Mealy bug *Ferrisia virgata* Cockrell on cotton. Varghese and Tandon (1990) proved that Indian beech oil reduced the percent survival of grape vine mealy bug *Maconellicoccus hirsutus* (Green) from 90.44% to 56.87%. Further, Gahukar and Balbande (1997) also confirmed the efficacy of neem oil based formulation containing 300 ppm azadiractin against sucking pest viz., aphid *A. gossypii*; Jassid, *Empoasca spp* and whitefly *B. tabaci*. Venkateshan *et al.*, (1987)

also reported the efficacy of neem oil and neem leaf extract against brinjal aphid, *Aphis gossypii*.

The data on fresh root yield revealed a significant increase of fresh root yield as 1095.16 kg/ha in T₃ (1095.16) followed by T₄ (1055.24 kg/ha), T₁₀ (960.10 kg/ha), T₇ (940.12 kg/ha), T₈ (926.08 kg/ha), T₉ (880 kg/ha), T₁ (874.56 kg/ha), T₂ (850.32 kg/ha), T₅ (788.20 kg/ha), T₆ (746 kg/ha), T₁₁ (642 kg/ha) and T₁₂ (625.04 kg/ha). These results are in line with the findings of Kavitha (2004). They reported that application of FYM along with neem cake and bio fertilizers recorded higher yield in brinjal.

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Microbial Management of Crop - Pest

Hem Saxena

ABSTRACT

In India most of the farmers depends upon synthetic pesticides for protecting their crops from pest attack. These pesticides not only caused environmental pollution, but also causing health hazardous to human being and domestic animals. This could be prevented by using bio-intensive integrated pest management (BIPM) where microbial insecticides play an important role. Here I have discussed about the important bacterial, fungal, viral, protozoan and nematode - based insecticides.

INTRODUCTION

With the globalization and liberalization of agriculture the microbial management of the crop pest has attained immense importance. All the microbial insecticides can be intensively used without any possibility of development of resistance. In view of their specificity and safety, there will be least environmental, ecological and health hazards. The use of microbes is highly sustainable and can contribute to increase the productivity and profitability in agriculture. Providing safe, economical and reliable control of crop pests is not only a contribution to sustainable agriculture but also a question of mere survival of a huge number of poor farmers in India. Microbial pesticide would offer a new method to control insecticide resistant pest population of *Helicoverpa armigera*, could prevent the rise of secondary pest problems, extend the life span of chemical insecticides, while protected the efforts and investment of the farmers and help in organic – production of vegetables, fruits and pulses etc. for domestic consumption and export (Pawar and Borikar, 2005). India is endowed with a rich biodiversity of several viral, bacterial, fungal, protozoan and entomopathogenic nematodes of crop pests, which offer great scope in the microbial control of crop pests. Microbial pesticides are safe to mankind and animals, do not pollute the environment, do not kill beneficial parasites and predators and generally pests do not develop resistance to these microbes. In view of these, several organisms are currently being developed in India as eco-friendly biopesticides. In microbial management pathogens are utilized which may be virus, bacteria, fungi, protozoans and nematodes. Use of these pathogens may vary considerably between crops and locations depending upon climate, symptomatology and economic threshold of crop damage. In general the pathogens function naturally in the environment as population suppressors. Microbial management of *H. armigera* in different crops has been very extensively reviewed by Pawar and Borikar (2005).

RESULTS AND DISCUSSION

Insect viruses

Work on insect viruses in India was initiated as early as 1968 with the report of nuclear polyhedrosis virus (NPV) from *Helicoverpa armigera* (Patel *et al.*, 1968), a pest of national importance and *Spodoptera litura* (Dhandapani *et al.*, 1992), a polyphagous pest attacking several crops. Since then studies on insect viruses have progressed rapidly and several viruses were reported to occur in insect pests, most of them from the order Lepidoptera. These comprises of Nuclear Polyhedrosis Virus (NPV), granulosis virus (GV) and cytoplasmic polyhedrosis virus (CPV).

Development of NPV

Of the different types of viruses, the NPV has the greatest potential since they are more virulent, killing the insects much faster than the GV and CPV. The NPV of *Helicoverpa armigera* (HaNPV) has been studied very extensively to evaluate its efficacy as a viral pesticide. HaNPV, a single embedded virion type has a high virulence against *Helicoverpa armigera* and the techniques of mass production, formulation (Chandel *et al.*, 2001, Grzywacz *et al.*, 2005, Rabindra *et al.*, 2005 and Saxena and Ahmad, 2003, 2005) and field use have been developed. The virus at a dose of $1.5-3 \times 10^{12}$ polyhedral occlusion bodies (POB)/ha effectively controlled *Helicoverpa armigera* on crops like chickpea (Pal *et al.*, 1998), pigeonpea (Ahmad and Saxena, 2001), groundnut (Muthuswami *et al.*, 1993), sunflower (Ahmad and Saxena, 2001), sorghum (Dhandapani *et al.*, 1993), cotton (Dhandapani *et al.*, 1987) and tomato (Mistry *et al.*, 1984). Rabindra and Jayaraj (1988) used several adjuvants to increase the efficacy of the virus. Aqueous leaf extract of *Vitex negundo* increase the efficacy of the virus in the field (Rabindra *et al.*, 1991). Several new strains of NPV of *Helicoverpa armigera* (Ahmad *et al.*, 2001), *S. litura* and *Amsacta albistriga* with increased virulence have been isolated. *S. litura* a polyphagous defoliator pest has been controlled with NPV on cotton, tobacco, banana, black gram and cauliflower. A wettable powder formulation of

Table 1. Transgenic plants with pest resistance

Crop	Gene	Pest
Cotton	<i>B.t.</i> toxin Cry IA(b)	Boll worm
Maize	Syn Cry 1A (b)	<i>Ostrinia nubilalis</i>
Tomato	Cry 1 A (c)	Fruit borer
Rice (Japonica)	Modified Cry 1A (b)	Striped stem borer + <i>C. medinalis</i>
Tobacco	Cry 1 A (a)	—
Cauliflower	Cry 1 A (a)	—
Rice (Indica)	Syn Cry 1 A (b)	<i>Scirpophaga incertulas</i> , <i>Cnaphalocrosis medinalis</i> <i>Chilo suppressalis</i> , <i>Marasmia patnalis</i>

the virus was found effective in reducing the damage to groundnut plants (Rabindra *et al.*, 2001). The red hairy caterpillar *A. albistriga*, a gregarious seasonal pest causing extensive damage to groundnut can be controlled with NPV could induce and epizootic of the viral disease in field populations of the pest resulting in long term control of the pest (Rabindra *et al.*, 2001). The safety of NPV to several beneficial organisms like the silk worm *Bombyx mori*, honey bees *Apis cerana indica* and parasitoids and predators have been established.

Development of granulosis virus

A granulosis virus was found to control *Chilo infuscatellus* effectively (Easwaramoorthy and Santhalaxmi, 1988). The virus was found to be safe to the egg parasitoids *Trichogramma chilonis* and *Trichogramma japonicum*. New strains of granulosis virus was also reported from *C. infuscatellus* (Easwaramoorthy and Cory, 1990). A granulosis virus was reported from the rice leaf folder *Cnaphalocrocis medinalis* from Kerala. Recently a GV from the diamondback moth *Plutella xylostella* was reported.

Baculovirus of *Oryctes rhinoceros* L.

Attempts were made to utilize the baculovirus for the suppression of the coconut rhinoceros beetle *O. rhinoceros* and the results are encouraging (Mohan *et al.*, 1993). Release of infected beetles spread the disease to subsequent generation of adults and larvae in breeding sites.

Bacillus thuringiensis

The spore forming, crystalliferous and ubiquitous bacterium *B. thuringiensis* (B.t.) is one of the earliest microbial insecticides to be commercially produced world wide. In India, this bacterial insecticide has been used in the management of several insect pests notably the diamond back moth *P. xylostella* larvae on cruciferous vegetable (Ashokan *et al.*, 1996). Other insect pest controlled by *Bt* are *H. armigera* on pigeonpea and

chickpea (Saxena *et al.*, 1992 and 1995), *Autographa nigrisigna* in chickpea (Saxena and Ahmad, 1997), *Diacrisia obliqua* (Saxena *et al.*, 1992), *S. litura* on cabbage (Dutta and Sharma, 1997) and fruit borers of okra (Sathpathy and Panda, 1997). There are several reports however indicating the ineffectiveness of *Bt* preparations particularly against the noctuids. Delfin was ineffective in controlling *H. armigera* on chickpea (Saxena *et al.*, 1995). Therefore, it is that new strains of *Bt* with increased host spectrum and efficacy are developed. A few indigenously prepared *Bt* formulations are not as effective as the imported preparations. There is an urgent need to isolate indigenous *Bt* strains with high pesticidal activity, develop techniques of mass production and stable formulations and patent them. Since, insects can develop resistance to the *Bt* toxin, this bacterial insecticide should be used judiciously. Commercial formulations of *Bacillus thuringiensis* (Bt.) such as thuricide, dipel, delfin etc., have provided high mortality of *H. armigera* in laboratory as well as in field conditions (Saxena and Ahmad, 1998).

FUNGAL PATHOGENS

Fungal pathogen particularly *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Nomuraea rileyi* have been found to be promising in the control of several agricultural pests (Lingappa *et al.*, 2005). *B. bassiana* and *M. anisopliae* were found effective against *H. armigera* and other pest of pulses (Saxena, *et al.*, 1989 and Saxena and Ahmad, 2002) and *S. litura* (Gopalakrishnan and Narayanan, 1989), the sweet potato weevil *Cylas formicarius*, the termite *Odontotermis brunneus* and *O. obesus* (Khader Khan *et al.*, 1993). *M. anisopliae* when applied to manure pit suppressed the population of *O. rhinoceros* larvae. The white halo fungus *V. lecanii* effectively controlled the coffee green bug *Coccus viridis* in coffee plantations (Easwaramoorthy and Jayaraj, 1978) and addition of glycerol as a humectant increased the efficacy of the fungus.

N. rileyi a fungal pathogen active against several lepidopteran insects was found to occur in epizootic form on *H. armigera* (Gopalakrishnan and Narayanan, 1989),

S. litura and *Spodoptera exigua* (Phadke *et al.*, 1978) suppressing the pest under field conditions. While *N. rileyi* killed *Achaea janata* larvae, it was not pathogenic to *Telenomus proditor* an egg parasitoid of *A. janata* (Phadke and Rao, 1978). Saxena and Ahmad (1997a) reported *Beauveria bassiana* is effective against *H. armigera* infesting chickpea. Saxena and Chaudhary (2002) recorded two new entomogenous fungi infesting larvae of *H. armigera* in fields. They are identified as *Aspergillus flavus* and *Aspergillus niger*. *A. flavus* was found 80% pathogenic to 3rd and 4th instar larvae of *H. armigera* in laboratory (Saxena, *et al.*, 2005 and Pandey *et al.*, 2007). *Nomuraea rileyi* has been established as a viable pathogen of *H. armigera* which is suitable for commercialization, as it is readily amenable to the mass production (Vimala devi, 2001). These reports clearly indicate the scope of entomopathogenic fungus for pest control in India.

ENTOMOPATHOGENIC NEMATODES (EPN)

Entomopathogenic nematodes popularly called as EPNs offer great scope for the management of many lepidopteran insect pests (Hussaini, 2001). In India, the work on EPN started in 1960 with the use of DD-136 for the control of pests of rice, sugarcane and apple. In 1970 other workers studied in the laboratory and field, the life cycle and compatibility of DD-136 with insecticides and fertilizers. Our eco-system is rich in diversity of EPN. A field survey in several locations in Gujarat during 1997 revealed 4 species of *Heterorhabditids* and 11 of *Steinernematids*. A survey in vertisols revealed abundance of *Steinernema feltiae*. Recently two new species of entomopathogenic nematodes (EPN) *i.e.*, *Steinernema masoodi* and *S. seemae* have been reported from the larvae of *H. armigera* at Kanpur (U.P.), which is causing 67% mortality of *H. armigera* larvae in the laboratory.

PROTOZOA .

Two protozoans *Nosema* sp. and *Vairiormorpha* sp. are reported on *H. armigera*.

BIOTECHNOLOGY AND BIOPESTICIDES

There are tremendous opportunities for genetic improvement of microbial pathogens for virulence, persistence, host range, speed of kill and stability in storage. The nuclear polyhedrosis viruses have been prime targets for genetic improvement of microbial pathogens for virulence, persistence, host range, speed of kill and stability in storage. Recently, a strain of *S. litura* NPV has been isolated with a characteristic restriction profile which was more virulent than the standard Coimbatore

strain by over 100 times. Development of *in vitro* systems for replication of baculoviruses in insect cells has opened new avenues for genetic improvement as well as genetic engineering of NPV with foreign genes to enhance its virulence.

Bt in Transgenics

The advantages of insect resistant transgenic plants are well known. The first results of insect control by plants engineered with the crystal protein gene were demonstrated in tomato, cotton and tobacco. Subsequently, several other *Bt* crops have been constructed (Table 1). In 1997, over 70 transgenic crops have been approved for commercialization in nine countries plus the E.U. Many are approved for growing and human consumption particularly in the U.S.A. and Canada, whereas some are for import and human consumption of the product and over 10 crops were pending approval. Of the 80 crops or so approved or pending approval in eight countries, 21 are *B.t.* transgenic crops dominated by corn and followed by potato and cotton. The developing countries that commercialized transgenic crops are Mexico and South Africa.

Bt. expressing transgenic corn, cotton and potatoes were grown in over 2 million acres in the U.S. during 1996 with excellent results. It is estimated that by 1997 the area would have increased to 2.4 million acres. However, there are two major concerns: a. Outbreak of less susceptible secondary pests and b. Possibility of development of resistance.

Genetic Improvement of Entomopathogenic Fungi

Culture conditions can influence the characteristics of fungal spores and can be manipulated to increase the efficiency. Blastospores of *B. bassiana* from nitrogen limited culture had higher concentration of carbohydrate and lipid and were significantly more virulent (lower LT_{50}) towards the rice green leaf hopper than blastospores from carbon limited culture (Lane *et al.*, 1991). Growth of *B. bassiana*, *M. anisopliae* and *Paecilomyces farinosus* on agar-based media with low water activity or with high concentration of glycerol encouraged accumulation of polyols in conidia that are more pathogenic at lower RH than produced on control media. Chemical mutagenesis, parasexual cycle, protoplast fusion and direct genetic manipulation could be used. Mutants of *M. anisopliae* and *P. farinosus* have been generated which are significantly more virulent (reduced LT_{50}) at low RH than parentals.

FUTURE OF MICROBIAL PESTICIDES IN INDIA

The baculoviruses particularly NPVs have tremendous scope for development as microbial pesticides for the

management of *H. armigera* (Saxena and Ahmad, 2005) and *S. litura* on crops like cotton, gram and groundnut. The GV of *P. xylostella* is another promising candidate. However, commercial availability of quality formulations is the immediate need. Multinational companies who have the production capabilities are not interested in view of the limited market potential. A few indigenous small producers who ventured into commercial production of NPV of *H. armigera* and *S. litura* did not succeed in producing quality virus. Most of the samples tested had extremely low virus content if not no virus at all. Many had unacceptable levels of spores of microsporidians. NPV production is done *in vivo* in respective host larvae and hence producers should employ properly trained manpower for production of host insects as well as the virus.

Most of the *B.t.* products now being sold in India are very expensive since they are imported. One or two products developed indigenously in India are not as effective as the imported ones. A few institutions in India including the TNAU and the BARC have isolated indigenous *B.t.* strains which are as potent as the standard HD 1. There is ample scope for development of techniques of indigenous production and formulation of native *Bts*. Species of entomopathogenic fungi like *Metarhizium*, *Beauveria*, *Verticillium* and *Nomuraea* isolated from different agroecosystems in India have shown promise in pest control. We should genetically improve these strains and develop techniques of mass production and stable formulations. India with the rich biodiversity of organisms, has tremendous potential for development of native strains of microbes well adapted to the Indian subcontinent.

There has been some success in the use of pathogens such as *Bacillus thuringiensis*, *N. rileyi* and *Helicoverpa armigera* nuclear polyhedrosis virus (*HaNPV*). However, the relatively high cost and rapid inactivation by ultraviolet light often lead to poor performance under field conditions. In the case of *HaNPV*, the difficulty in obtaining consistently high levels of purity and virulence necessary to achieve satisfactory control have limited its usefulness. The increasing prevalence of resistance to insecticides and awareness of environmental concerns has given a new impetus to the development of suitable microbial insecticides for use in IPM of *H. armigera*.

Educational programmes in institutions should give special emphasis on development of biorational pest control technologies like microbial control. There is a need to develop inter-institutional collaborations in order to pool all the resources available in different institutions and develop microbial pest control technologies which

would ultimately reduce the pressure on chemical insecticide use.

In view of the new patent laws, it is very important that we take special care to preserve our entomopathogenic microbial wealth. We should characterise all indigenous microbial species with pest control potential and develop a suitable mechanism to patent them in due course of time. Microbial management is a very broad concept. A number of strategies and techniques are involved. Many pathogens operate unsuspected and it is amazing how complex bio-ecological interaction is going on. For the promotion of microbial pesticides, their intensive commercial production and formulation with strict enforcement of quality control measures are necessary.

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Use of Carpovirusine for Control of Codling Moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), in Bulgaria Progress Report

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ABSTRACT

The trials were carried out in Central-South Bulgaria in 2006 and 2007. In a conventionally managed reference orchard, sixteen chemical treatments were applied during the season. Fifteen of them could act against codling moth larvae. In spite of that, fruit damage by CM reached at harvest 6.8 in 2006 and 18.7 in 2007. At the same time damage in the plot, treated twelve times with GpGV, was only 0.05% in 2006 and 0.4% in 2007. The overwintering population in the plot treated with GpGV was 0.125 larvae per tree in autumn 2006 and 0.175 larvae per tree in 2007, whereas in the reference orchard 3.32 larvae per tree in autumn 2006 and 7.97 in 2007. The high fruit damage and overwintering CM indicate the population to be resistant to the organophosphates used. Positive results obtained with Carpovirusine show that it may be effective as a means of biocontrol of CM in Bulgaria.

Keywords: codling moth, flight-dynamics, overwintering-larvae, granulosis-virus, CpGV

INTRODUCTION

The codling moth (CM), *Cydia pomonella* L. (Lepidoptera: Tortricidae) is a major pest of fruit orchards worldwide. In Bulgaria it is currently controlled in apple orchards by conventional spray applications, mainly with organophosphates. Intensive use of chemical pesticides, especially of the same group, leads to development of resistant CM strains (Charmillot *et al.*, 1999). Such resistance is successively increasing and even cross-resistance to new pesticides may appear (Charmillot and Pasquier, 2002). Recently strong resistance to organophosphates and pyrethroids has been revealed in the strains of codling moth from Bulgarian apple orchards (Charmillot *et al.*, 2007).

In order to overcome the problems of resistance and to avoid contamination of fruit products with pesticide residues, novel methods of biological control of codling moth have to be elaborated. A possible solution of this problem represents the codling moth granulosis virus, CpGV (Charmillot and Pasquier, 2003). CpGV was first isolated in Mexico 44 years ago (Tanada, 1964). Numerous experiments have shown that CpGV is efficient against codling moth (Glen and Payne, 1984). A commercial product, Carpovirusine, produced by Natural Plant Protection unit (N.P.P.) a subsidiary of Arysta Life Science, has been registered in France since 1993 (Biache *et al.*, 1998a). Soon after registration the product exhibited positive effects in control of codling moth (e.g. Biache *et al.*, 1998b, Pluciennik *et al.*, 1999). Regarding the difficult insecticide resistance situation in Bulgaria the tests of

Carpovirusine for control of codling moth were undertaken in this study.

MATERIAL AND METHODS

The 2-ha commercial orchard, used for testing Carpovirusine is located in the village Kalekovee, Plovdiv region, Central-South Bulgaria. It was established in 1994 with the cultivars Jonagold, Florina and Granny Smith. Carpovirusine was applied, there at the dose of 1 litre per ha (1.10¹³ granules per ha), twelve times during the season, five times against the first and seven times against the second generation of codling moth, at 10-12 day intervals, as suggested by Stara and Kocourek (2003).

Another orchard of the Plovdiv region, with an area of 1.8 ha, served as a reference with conventional management in 2006 and 2007. Sixteen conventional pesticide treatments, including fenitrothion, triflumuron, cipermetrin and clorpyrifos-ethyl, were applied there during each season to control CM, leaf miners, leaf rollers, aphids and mites; fifteen of them were supposed to have an action against CM larvae.

In both years of study, for monitoring of CM flights, two standard pheromone traps were installed in the centre of the plot treated with Carpovirusine as well as in the reference orchard, before the flight started: The triangular delta traps baited with a standard pheromone dispenser capsule (Pheronet OP-72-T1-01), containing 1 mg codlemone. All traps were checked twice a week. For evaluation of damage caused by CM, samples of 1000 or 2000 fruits were examined in the trial plot and in the reference orchard during season. Preharvest evaluation of damage was carried out on 3000 fruits in each orchard. In June of each year of study, corrugated cardboard band

traps were placed on tree trunks in the trial plot (4 at the border and 16 inside) as well as 40 bands in the reference orchard. They were recovered in autumn, after harvest, and the diapausing CM larvae were counted.

RESULTS AND DISCUSSION

In 2006, the first flight in the reference orchard began on 26 April and reached its maximum by the second decade of May (Figure 1a). The flight of the second generation, which overlapped the first one, started at the end of June, reached its maximum in the third decade of July, continued with varying intensity in August and finished on the 17th of September. The traps in the reference orchard caught 146 moths during the whole season. In the virus treated plot at Kalekovec followed a similar trend, albeit was less intense. Two standard pheromone traps caught in total only 32 moths there.

In 2007, the first catches of CM males were noted on April 10, then the flight successively intensified to reach its maximum by the second decade of the month (Figure 1b). Later on, a considerable flight peak appeared in the second decade of May. The flight of the second generation, which did not overlap the first one in 2007, started at the beginning of July, reached its maximum in the second decade of the month and then declined. However, additional flight peak appeared in the third decade of August. The CM flight finished on 19 September. Traps in the reference orchard caught 291 moths during the

whole season. In the virus treated plot at Kalekovec CM flight was very weak and standard pheromone traps caught only 36 males in total; 11 of them before the first application of Carpovirusine.

In 2006 no fruit damage by codling moth larvae was noted in the Carpovirusine treated plot till the beginning of August. Later on very few damaged fruits were found at harvest the damage rate was still only 0.05% (Table 1). In the reference orchard, treated with conventional insecticides, some fruit damage appeared as early as in June and successively progressed, reaching 6.9% at harvest. Infestations rates were significantly different between the treated plot and the reference orchard from July on until harvest (Chi-square test, $P < 0.001$).

In 2007 the damage in the Carpo virusine treated plot also appeared quite late in season and at a very low rate, reaching 0.4% at harvest. Fruit damage in the reference orchard was higher in the second year of study than in the first year. In spite of numerous pesticide treatments applied, damaged by CM amounted to 18.7% at harvest, thus causing serious economic losses. Except for the sampling at 2nd June and 5th July 2007, infestation rates were significantly different between the the treated plot and the reference orchard from July on until harvest (Chi-square test, $P < 0.001$).

In the Carpovirusine treated plot at Kalekovec only very few diapausing codling moth larvae were found under corrugated paper bands after harvest in 2006 (0.125 larvae per tree) as well as in 2007 (0.175 larvae per tree). This result matches very well both the low mid - season damage rates as the few infested fruit at harvest. In contrast, in the reference orchard, located in the same Plovdiv region, treated fifteen times with chemical insecticides against codling moth per year, showed a considerable overwintering population in 2006 (3.32 larvae per tree) and even increasingly high number of CM in 2007 (7.97 larvae per tree).

Such population increase in only one year and under intensive insecticide treatment applied pinpoints the considerable resistance problems in the region (Charmillot *et al.*, 2007). Increasing resistance of codling moths result ineffectiveness of classical control programs and in increasing population of the pest in the conventionally treated apple orchards. Granulosis virus (CpGV), applied in form of carpovirusine 2000 may be helpful in overcoming the problem of resistance of CM to pesticides. As shown in the present study, this product, applied at short intervals of about 10-12 days (Stara and Kocourek, 2003), may almost completely prevent fruit damage by CM and may reduce its population considerably. Use of the virus products instead of chemical pesticides should therefore favour protection of the environment in the orchards and

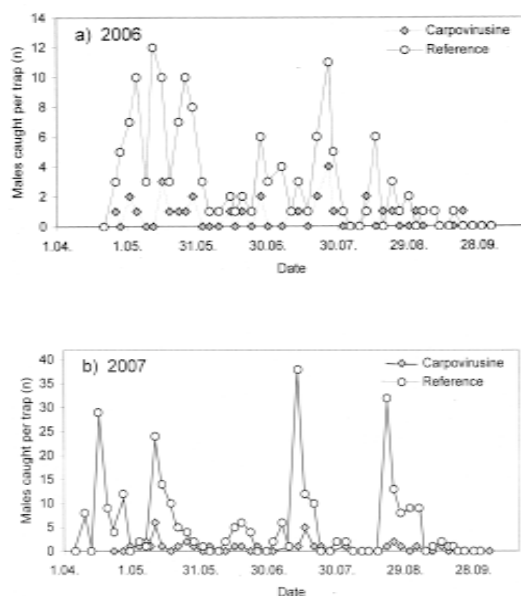


Figure 1. Seasonal flight of CM as males caught per pheromone trap at half-week intervals in the Carpovirusine treated plot and in the reference orchard in 2006 and 2007

Table 1. Percentage of fruits damaged by codling moth larvae in the Carpovirusine trial plot and in the reference orchard at successive dates in two seasons

2006			2007		
Date	Carpovirusine trial plot	Reference orchard	Date	Carpovirusine trial plot	Reference orchard
June 1	0	0.2	June 2	0	0.2
June 19	0	0.8	June 23	0	21.3
July 31	0	2.3	July 5	0	0
August 8	0	2.5	July 27	0.05	2.7
August 22	0.2	3.3	August 10	0.05	4.7
September 28	0.05	5.9	August 31	0.1	11.2
-----	-----	-----	September 27	0.4	17.4
at harvest	0.05	6.8	at harvest	0.4	18.7

their surroundings both as prevent contamination of fruit products and thereby improving human health conditions.

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Tri-tropic Interaction of Cotton, Red Cotton Bug and Green Muscardine Fungi under *In-Vitro* Condition

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ABSTRACT

Laboratory bioassay was conducted to find out the impact of a green muscardine fungus, *Metarhizium anisopliae* (Metsch.), Sorokin (Deuteromycotina: Hyphomycetes) on an economically important cotton pest, red cotton bug *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae) under laboratory condition using cotton saplings, *Gossypium hirsutum* Linn. (Malvaceae). Two fungal isolates of *M. anisopliae* (CPRC 16 and CPRC 18) and also a standard *M. anisopliae* MTCC 892 were used for the present study. The present experimental results revealed that the tested strains were effective against the red cotton bug on the cotton saplings. Highest mortality (86.67 %) was observed in CPRC 18 at 1.9×10^8 spores/ml, followed by CPRC 16 (80.64 %) at 2.8×10^8 spores/ml. Subsequently, the LC_{50} values were also lower (2.27×10^5) and higher 2.83×10^6 for CPRC 18 and CPRC 16 respectively. Percentage of insect infected with *M. anisopliae* of the former category was highest among live and dead cadavers at 80.00 and 92.30 respectively. The infected dead cadavers were attached on the upper surface of the cotton leaves, which grew very healthy. In future *M. anisopliae* CPRC 18 can be used to manage this sucking pest in cotton field.

Keywords: Biological control potential, *Dysdercus cingulatus*, *Gossypium hirsutum*, *Metarhizium anisopliae*, Tri-tropic interaction

INTRODUCTION

In India, cotton *Gossypium hirsutum* Linn. (Malvaceae) is grown on large scale in Maharashtra, Gujarat, Karnataka, Madhya Pradesh, Punjab, Rajasthan, Haryana, Tamil Nadu and Uttar Pradesh. This crop is severely attacked by a number of pests (David and Ananthkrishnan, 2004). Red cotton bug or cotton stainer or red seed bug, *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae) is a serious pest of cotton (Leakey and Peery, 1955; David and Kumaraswami, 1978; Waterhouse, 1998; Sahayaraj, 2007), which infests cotton in all the cotton growing regions of India (Waterhouse, 1998; David and Ananthkrishnan, 2004). Chemical pesticides like benzophenylureas (Chakraborti, and Chatterjee, 1999), monocrotopas and DDT (David and Ananthkrishnan, 2004) were used to manage this pest, but these chemicals are phytotoxic (Miranpuri, and Khachatourians, 1996), caused resistance, outbreak and resurgence of the insect-pest, pollution hazards and disruption of the eco-balance (Chakraborti, and Chatterjee, 1999, Yadav *et al.*, 2006). Hence the search for less disruptive and environmental friendly control strategies has increased over the recent years (Gauchan *et al.*, 1998; Ericsson *et al.*, 2007; Dong *et al.*, 2007; Balaji and Hemavathi, 2007; Gao *et al.*, 2007; Guo *et al.*, 2007; Jarrold *et al.*, 2007; Kunimi, 2007; Sahayaraj, 2007; Velasquez *et al.*, 2007).

Metarhizium anisopliae (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) is a filamentous entomopathogenic fungus, which is commercially used as a biological control agent against many insect pests (Gopalakrishnan and Narayanan, 1988; Ramanujam *et al.*, 2002; Nahar *et al.*, 2004; Abed *et al.*, 2005; Anonymous, 2004; Balaji and Hemavathi, 2007; Gao *et al.*, 2007; Guo *et al.*, 2007; Jarrold *et al.*, 2007; Kunimi, 2007; Velasquez *et al.*, 2007).

al., 2007; Jarrold *et al.*, 2007; Kunimi, 2007; Velasquez *et al.*, 2007) including sucking pests like aphids in general (Butt, 1995; Chandler, 1997) and *A. craccivora* in particular (Ibrahim, and Nugroho, 2005; Nirmala *et al.*, 2006), green stink bug *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Borges *et al.*, 1993; Sosa-Gomez *et al.*, 1997; Daniel *et al.*, 1998). No report was available for the impact of *M. anisopliae* isolate against this pest. The present study was undertaken to evaluate the virulence of two isolates of *M. anisopliae* (CPRC 16 and CPRC 18) and also a standard *M. anisopliae* MTCC 892 against *D. cingulatus* adults under laboratory with cotton sapling conditions.

MATERIALS AND METHODS

Source of *Metarhizium anisopliae*

Both *M. anisopliae* CPRC 16 and CPRC 18 were obtained from our pure stock culture. Standard *M. anisopliae* MTCC 892 was obtained from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. Isolates were subcultured using Potato Dextrose Agar (PDA) (HiMedia, Mumbai, India). Inoculated test tubes were maintained at $26 \pm 2^\circ\text{C}$ in an incubator (Kemi, Mumbai, India) till sporulation and the slope cultures were then maintained at 4°C . More than 15 days old working cultures have not been utilized for the study.

Preparation of conidial suspension

The fungal conidia were collected from 7 days old cultures. Stock cultures at concentrations of 1.9×10^8 , 2.8×10^8 , and 4.4×10^8 conidia/ml were prepared for CPRC 16 and CPRC 18 and MTCC 892 respectively. Four spore concentrations such as 10^5 , 10^6 , 10^7 and 10^8 spores/ml were prepared for

all the strains from the respective stocks and water was used as control. Conidial concentrations of the suspensions were determined using an improved Neubauer Haemocytometer (Pohem, Mumbai, India). A homogeneous conidial suspension was prepared in sterile distilled water by adding few drops of castor oil (0.1 %) as surfactant.

***Dysdercus cingulatus* sources**

D. cingulatus of various life stages were collected from the cotton field from different parts of Tirunelveli District in South India and were maintained in the cotton plants and also with soaked cotton seeds. Laboratory emerged adults (>6 hrs) were used for the present study.

Cotton saplings

Cotton, *Gossypium hirsutum* RC4 seeds were purchased from Agriculture Office, Palayamkottai, Tamil Nadu and cultivated in plots (15 x 20 x 10cm) under organic substrate farming (cow dung). 15 days old healthy plants were selected and used for the present study

Experimentation

Five *D. cingulatus* adults were released into the cotton saplings. After six hours of acclimatization, 5.8×10^5 spores/ml of fungal conidial suspension of CPRC 16 was sprayed on the pests and the saplings using hand sprayer (Amway product, U.S.A). Three replicates were maintained. Similar procedure was adapted for the remaining concentrations. Water was used as control. The mycelial growth on the pests and mortality were recorded for every 24 hours till 96 hours. The percentage of infection among the live and dead insects were calculated using the following formulae

Infection followed by mycelial growth among total live insects (%) = $\frac{\text{Number of infected insect}}{\text{Total insect released}} \times 100$

Infection followed by mycelial growth among dead cadavers (%) = $\frac{\text{Number of infected insect}}{\text{Total number of insect dead}} \times 100$

Statistical analysis

Finney's formula was used to calculate corrected mortality (Finney, 1971). From the corrected mortality data, the

probability integral of the chi-square distribution and LC_{50} were calculated in order to find out the efficacy of *M. anisopliae* on *D. cingulatus*. The results of the three strains were compared by correlation analysis using STATISTICA / w 5.0. software.

RESULT

In-vitro biological control potential evaluation of two *M. anisopliae* isolates such as CPRC16 and CPRC18 and the standard MTCC892 under cotton saplings revealed that all the three tested conidial suspensions caused mortality in *D. cingulatus* adults. Spore concentration dependent mortality was recorded. The highest (86.67 %) mortality was recorded in CPRC18 (1.9×10^8 spore/ml), followed by CPRC16 (80.64%) at 2.8×10^8 spore/ml (Figure 1). Lowest mortality (76.67%) at high concentration (4.4×10^8 spore/ml) was observed in MTCC892. Highest mortality (63.33 %) at the lowest (1.4×10^5) spore concentrations was observed in *M. anisopliae* CPRC18. Obviously no mortality was observed in all the controls. Among the three strains tested the mortality in CPRC18 and MTCC892 are highly correlated (0.97). Least correlation (0.89) was observed between CPRC16 and CPRC18. The mortality was subjected to LC_{50} analysis and the results are also presented in table 1. The LC_{50} values were also lower (2.27×10^5) and higher (2.83×10^6) for CPRC18 and CPRC16 respectively. The χ^2 values were also highest in CPRC18 (19.3413). Fiducial limits (FL) were also lower in CPRC18 [lower FL and higher FL = 2.04×10^4 and 2.16×10^4 (Table 1)].

The percentage of insects infected followed by fungal growth on the surface of *D. cingulatus* by *M. anisopliae* standard and CPRC18 was the highest among the live and dead insects with 80.00 and 92.30 respectively. The percentage of insect infected followed by fungal growth on the surface of *D. cingulatus* by *M. anisopliae* was in increasing order when the spore concentration increased. Table 2 represents the percentage of fungal growth on *D. cingulatus* surface among the total live and dead cadavers. The results clearly indicate that direct relationship was observed between the concentration of spore and percentage of their growth. Here, we have not observed any fungal growth on the surface of control insects. The highest (57.14%) percentage of infection among the dead

Table 1. Impact of *Metarhizium anisopliae* on *Dysdercus cingulatus* adults LC_{50} parameters

Isolate name	LC_{50}	Fiducial limits		Slope	Intercept	Chi ²
		Lower	Higher			
CPRC 16	2.83×10^6	2.25×10^5	2.52×10^7	16.07004	8.862925	8.028951
CPRC 18	2.27×10^5	2.16×10^4	2.04×10^6	15.142878	8.532675	19.34137
MTCC 892	2.40×10^5	2.26×10^4	2.55×10^6	14.39237	8.142397	12.43774
Mean	1.09×10^6	8.97×10^5	9.93×10^5	15.202	8.513	13.269
SEM	8.65×10^5	6.76×10^5	7.63×10^5	0.485	0.208	3.292
Variance	2.24×10^{12}	1.37×10^{12}	1.74×10^{12}	0.706	0.130	32.511
COEFF. OF VARIA	1.364	1.305	1.332	0.055	0.042	0.430

Table 2. Percentage of infection among the total insects and the dead cadavers of *Dysdercus cingulatus* adults treated with *Metarhizium anisopliae* under sapling condition

Spores /ml	Percentage of infection					
	Among the total insects			Among the dead cadavers		
	CPRC16	CPRC18	MTCC892	CPRC16	CPRC18	MTCC892
10 ⁵	26.66	26.66	13.33	57.14	44.45	20.00
10 ⁶	40.00	33.33	13.33	60.00	50.00	25.00
10 ⁷	53.33	40.00	13.33	72.73	54.55	28.57
10 ⁸	60.00	80.00	36.66	75.00	92.30	36.37
Control	0.00	0.00	0.00	0.00	0.00	0.00

cadavers was observed in CPRC16 at 5.8×10^5 conidia/ml. Subsequently the same result (60.00 and 72.73%) was observed for 4.3×10^6 and 2.1×10^7 concentrations respectively. The lowest percentage (36.67%) of fungal growth the highest (4.4×10^8) spore concentration was observed in MTCC 892. Correlation coefficient of fungal growth among the three fungi revealed that percentage of infection among dead insects positively correlated. High and least correlations (0.77 and 0.19) were found between CPRC16 and CPRC18 and CPRC16 and MTCC892 respectively in dead cadavers percentage. The highest and the lowest correlations of percentage of infection among the live *D. cingulatus* were 0.97 and 0.68 between CPRC18 and MTCC892 and CPRC16 and MTCC892 respectively.



Figure 2. Infected dead cadavers attached on the upper surface of the cotton leaves (a) or fall down in the soil surface (b).

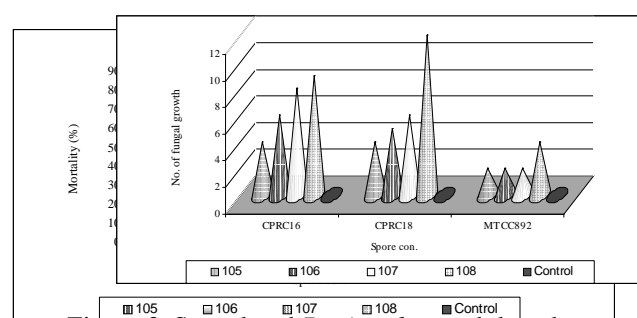


Figure 3. Sporulated *D. cingulatus* adult cadavers treated with *Metarhizium anisopliae* isolates under sapling condition.

Figure 1. Corrected mortality (%) of *Metarhizium anisopliae* on *Dysdercus cingulatus* adults under sapling condition.

Most of the infected dead cadavers were attached on the upper surface of the cotton leaves (Figure 2). Some of the cadavers fell down on the soil surface (Figure 2). Through out the experiment, the cotton saplings were normal and healthier. No color change was observed in the cotton leaves, all were growing as normal ones. The highest (12) sporulation on *D. cingulatus* cadavers were observed in CPRC18 (Figure3). Amazingly we observed a live insect infected followed by fungal growth on the dorsal surface, which died with in 12 hours.

DISCUSSION

Both the pesticide-related and non-pesticides suicides among farmers are growing annually at 3.9% in worldwide. In India, farmer suicide cases are higher in Andhra Pradesh, Maharashtra, Madhya Pradesh, Goa, Tamil Nadu, Kerala, West Bengal and Puducherry. Almost two-third of the suicides and nearly 60 percent of the suicides by pesticide consumption (Anonymous, 2007). It is clear that there was no single and simple solution for this problem in the present scenario. Thus the availability of safer products should be increased. Among the pesticides, the market

share for microbial pesticides is about 3%, while in India only 1% biopesticides of the total agrochemical scenario are being used (Dubey *et al.*, 2002). Though chemical control is the spinal cord of the insect pest management, it causes numerous problems, such as pest resistance, secondary pest outbreak and environmental hazards including human poisoning. There is, thus, a strong pressure to modify the present approach of insect pest management. The deleterious effects of chemical molecules on ecological sustainability has furthered the research for alternatives to pesticides (Dhaliwal and Arora, 2001). Entomopathogens can be used as an alternative of chemical pesticides (Balaji and Hemavathi, 2007; Gao *et al.*, 2007; Guo *et al.*, 2007; Jarrold *et al.*, 2007; Kunimi, 2007; Velasquez *et al.*, 2007). Recently Purwar and Sachan (2006) also reported the harmless nature of the entomopathogens to man, mammals and plants.

Successful biological control with entomopathogenic fungi is based on the strain with high rate of infection (Gindin *et al.*, 2001, Zimmerman, 1993). Our study expresses variation among the mortality. This is due to heterokaryotic and saprobic growth of the fungi has undergone in the environment prior to its interaction with the insects reported by Roberts and Yendol (1997). Virulence in the microbes in nature depends on the ecological situation (Ignoffo *et al.*, 1977). The same variation in virulence of strains was also reported by Zimmerman (1993) and Gindin *et al.* (2001). Very recently, Borgio and Sahayaraj (2007) reported the ovicidal effect of *M. anisopliae* on *D. cingulatus*. They were also reported the fungal growth on the surface of *D. cingulatus* eggs at higher concentration in four strains, but only two strains showed fungal growth on the surface of egg in all concentrations. In our present study, we have observed fungal growth in all the strains at all concentrations on the body of *D. cingulatus*.

In determining whether the use of entomopathogenic fungi has been successful in particular pest management, it is necessary to consider each case individually (Shah and Pell, 2003). Gelernter and Lomer (2000) suggested that for any microbial agent to be successful, efficacy was essential, but had to combine with at least two other criteria. The safety of entomopathogenic fungi towards human and plants is clearly an important criteria. With this view in mind, the present experiment was carried out to find out the impact in tritropic interaction of cotton sapling, red cotton bug and green muscardine fungi. The study fulfilled the safety criteria for plant, which is already reported by Purwar and Sachan (2006).

The fungus emerges from the dead host and sporulation or conidiogenesis usually occurs on the outside of the cadavers (Shah and Pell, 2003). We found the same in our present investigation. The conidia of *M. anisopliae* is hydrophobic and are passively dispersed from infected cadavers (Shah and Pell, 2003). Secondary infection from the conidia on the cadavers occurs when they are carried on wind currents or by co-occurring insects (Shah and Pell, 2003).

Very interestingly we have observed a live insect infected followed by fungal growth on the dorsal surface. The same type of observation that conidia of *Entomohthera thripidium* on the fly *S. castrans* were discharged while the host insect was still alive (Steinhaus, 1964; Shah and Pell, 2003).

With entomophthorelean species, cadavers are attached to foliage by fungal rhizoids, which emerge through the ventral surface of mouthparts of the cadavers (Shah and Pell, 2003). In our present investigation we have also observed the cadavers attached to the upper foliage of the cotton leaf, and fungal growth covered the entire *D. cingulatus*. Specialized attachment structures ensure that the fungus remains in the environment of now host for further transmission (Shah and Pell, 2003).

From the experiment we concluded that *M. anisopliae* CPRC18 could be used to manage the sucking pests of cotton, *D. cingulatus* in future. Further elaborated study in this area is needed to find out the nature of attachment and the mode of infection by *M. anisopliae* and field evaluation is also essential. All these are going on in our laboratory.

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In vitro mass production of *Pasteuria penetrans* (Mankau) Sayre and Starr for the management of *Meloidogyne incognita* (Kofoid and White) Chitwood

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ABSTRACT

Plant parasitic nematodes are parasitized and preyed by a variety of soil organisms which includes predatory nematodes, fungi, bacteria, viruses, protozoans, turbellarians, tardigrades, mites and other microarthropods. One such spore forming and an obligate parasite is *Pasteuria penetrans*. The major limitation in the use of *Pasteuria* spp. is their inability to grow in the absence of host nematodes. *In vivo* systems although proven are unlikely to be adopted for large scale application in most farming systems. Attempts were made to culture the organism *in vitro*. Among the symbiotic bacteria used for mass production co-culturing with *Enterobacter* proved to be successful in culturing this obligate organism in a specially developed medium for mass multiplication.

Key words: *Pasteuria penetrans*, *Meloidogyne incognita*, *in vitro*, mass production.

INTRODUCTION

Pasteuria (Metchinkoff, 1888) is a Gram positive endospore forming bacterium. It is one of the most promising biocontrol agents for many nematodes that cause extensive damage to field crops, vegetables, turf grass and ornamentals. The bacterium propagates within the pseudocoelom of the infected nematode host and thus resulting in loss of fecundity (Bird, 1986). The nematodes serve as an amplification medium for the infective spores. Each infected root knot nematode female contains an average of 2-2.5 million spores which are eventually released into the soil. The ability to form spores is a significant advantage in formulating this organism.

MATERIALS AND METHODS

Isolation of *Enterobacter cloacae*

One hundred spores encumbered J₂ were inoculated in tomato plants grown for a week in tumbler pots. The inoculated plants were maintained for 15 days in the glass house. The plants were uprooted and root system was harvested and incubated in cytolase solution (25 %) for 48 h (Chen *et al.*, 1996). The roots were placed in 100 mesh and jet of water was directed towards the root to dislodge the milky white females. The females were carefully collected and rinsed in double distilled water for 10 times. The infected females were squeezed between the cover slips and observed under the microscope to confirm that the bacterium was in vegetative stage. The body content was streaked on nutrient agar plates. Single colonies which appeared after 24 h were separately streaked on slants. Primary identification was made by Gram staining, which stained negative. The strains were confirmed as *Enterobacter* through enterotube test (Martin *et al.*, 1971). The bacterium was sub cultured on NA slants. Symbiotic bacteria from *Steinernema glaseri* and *Heterorhabditis indica* were isolated by the procedure given by Heungens *et al.* (2002).

Preparation of co culture Medium

A special co culture liquid medium was prepared with simple modifications of medium reported by White *et al.* (2006). The composition of co culture medium is Glucose (50 g), Egg yolk (10 ml), Yeast extract (1g), CuSO₄.5H₂O (1g), MnSO₄.4H₂O (1g), ZnSO₄ (2 g), H₃Bo₃ (0.25g), FeSO₄ (0.5g), KNO₃ (1g), Micronutrient solution (10ml), Multivitamin tablet (500 mg) and Double distilled water (1000 ml) with pH 5.5.

The isolate of *Enterobacter cloacae* (M1) symbiotic bacteria of entomopathogenic nematodes *viz.*, *Xenorhabdus poinarii*. (*S. glaseri*) (M2) and *Photorhabdus luminescens*. (*H. indica*) (M3) were inoculated in the co culture medium. After 48 h, the medium was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and filtered through bacterial filter and the medium is made free of any bacterial cells. To the culture filtrates the vegetative stages of *P. penetrans* collected from infected females were added @ 5 crushed females per flask. Observations were made at 25, 35, and 45 DAI by drawing 5ml of aliquot. The experiment was replicated five times.

RESULTS AND DISCUSSION

Inoculation of vegetative stages of *P. penetrans* in the culture flask turned the medium turbid. *P. penetrans* in vegetative stage began to multiply in all the media. Mycelia like structures were found during the early stages. Vegetative stages were observed 25 DAI in all the media. After 35 DAI, vegetative stages were present in M1 and M2 and no vegetative structures were present in M3. In M2 medium, some replications did not show any vegetative stage. Spores were present in M1 after 45 DAI. Some replications showed both the presence of spores and vegetative stages. Spores settled at the bottom of the flask like a precipitate. The spore count of M1 medium was 1×10⁴ spores per flask (Table 1). The culture filtrates

Table 1. Multiplication of *P. penetrans* in different media

Media	DAI	Vegetative stages	Spores	Spore load per 50 ml medium
	25	+	-	-
	35	+	-	-
	45	-, +	+	1×10 ⁴
	25	+	-	-
	35	-, +	-	-
	45	-	+	1000
	25	+	-	-
	35	-	-	-
	45	-	+	100

+ present, - absent

M1 - Culture filtrate of *Enterobacter cloacae*

M2 - Culture filtrate of *X. poinarii*

M3 - Culture filtrate of *P. luminescens*

of the *E. cloacae* supported a moderate growth of *P. penetrans*. In M2, lysis of vegetative structures was noticed and only 1000 spores were produced per flask. Similar reactions were recorded in M3 medium also. The M2 and M3 media supported poor multiplication of *P. penetrans*. Various media had been tested for their ability to support the growth of isolates of *P. penetrans* but they can maintain only the vegetative cells and no spore production was observed (Reise *et al.*, 1988). Bishop and Ellar (1991) screened several microbial media but resulted in failure. After a long time multiplication of this organism is now partially successful. *In vitro* mass production of *P. penetrans* is simple when compared to other methods. The culture filtrates of the *M. incognita* associated bacterium supports the growth of the *P. penetrans*. The biochemical composition of culture filtrate is yet to be defined. The isolate of *E. cloacae* which was isolated in the present study supported a moderate growth of *P. penetrans*. Still efficient isolate is to be isolated. The symbiotic bacterium obtained from entomopathogenic nematodes *viz.*, *S. glaseri* and *H. indica* supported moderate and poor growth of *P. penetrans* respectively. The antibiotics produced by these bacteria should be the limiting factor for multiplication of *P. penetrans*. *P. luminescens* culture filtrate digested the vegetative stages of *P. penetrans*. The late vegetative stages resistant to digestion formed spores. The culture filtrates of *E. cloacae* contains a "helper factor" which helps to maintain and produce spores of *P. penetrans* as reported in WO01/11017A2 (Gerber and White, 2001).

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Studies on the need of Phytosanitary Measures for the Management of the Coffee Berry Borer in Pulney Hills

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ABSTRACT

The study conducted at the Regional Coffee Research Station, Thandigudi in four villages during the year 2004-05 to identify the source of inoculum and pattern of emergence of the coffee berry borer adult from gleanings (fallen fruits) and left-over arabica coffee berries revealed that irrespective of the locations surveyed, the population of coffee berry borer in the left over berries appeared to be the main source of inoculum for carryover of the pest to the next season's crop. The mean number of borer adults that emerged from gleanings was high (21.72) due to rain. Hence, it is important to remove the left-over berries and gleanings to keep the population level low in the next season's crop.

Key words : Coffee berry borer, *Hypothenemus hampei*, emergence pattern, population

INTRODUCTION

The coffee berry borer, *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae), is the most serious pest in many of the major coffee producing countries, causing great yield losses (Le-Pelley, 1968). Coffee berry borer was first noticed infesting coffee in the field in 1901 in Gabon, a Central African country (Sreedharan *et al.*, 2001). It was recorded for the first time in coffee estates in Gudalur liaison zone, Nilgiris district, Tamil Nadu in South India (Kumar *et al.*, 1990). Sreedharan *et al.* (1994) reported that the coffee berry borer entered the neighboring Wayanad district of Kerala from Gudalur in the mid 1990s. In 1991 it was detected in Kutta region of Kodagu district of Karnataka; now it is noticed in all the major coffee growing tracts of Karnataka, Kerala and Tamil Nadu (Anonymous, 2000).

In India, the qualitative loss estimated as blacks / bits and browns is 2.69 for lit at 10% infestation, 22.07 at 50% infestation and 54.9 at 100% infestation (Anonymous, 2001). The percentage infestation due to borer may even reach 100 per cent (Baker, 1999). Since the berry borer thrives on the fruits, the availability of suitable fruits throughout the year makes management of this pest difficult. But in countries like India with a set rainfall pattern it is possible to observe periods during the year when suitable fruits are not available for the berry borer to multiply. During this period the borer survives on fruits left over on the plants after the harvest or on fruits that have fallen to the ground (gleanings). These form the main source of inoculum for carry over of the infestation from one season to the other. Hence, the present study was conducted to understand the population pattern of the berry borer in left over fruits and the emergence of adults from the gleanings.

MATERIALS AND METHODS

Experiments were conducted with gleanings / left over berries on arabica coffee estates in Adalur, Solaikadu, Nallurkadu and Pillaveli villages of Pulney hills in the Dindigul District of Tamil Nadu during 2004-05. The details of materials and methods are furnished hereunder.

Population of Coffee Berry Borer

The left-over berries were collected from 17 locations @ 100 left over berries from five sites at each location in the lower Pulney hills area during May and June of 2004 and 2005 after the main harvest. The fruits were sliced open and the total number of beetles recorded. The mean number of beetles per berry was computed.

Pattern of Coffee Berry Borer Emergence

This study was conducted to understand the triggering mechanism for the emergence of adult berry borer. Infested gleanings were collected from the field after the main harvest. The gleanings were subjected to the treatments *viz.*, (1) water spray, (2) water soaking for 2 minutes., (3) exposure to natural rain, (4) exposure to higher temperature (25 °C), (5) exposure to high relative humidity (90%) and (6) untreated check. Fifty gleanings were used in each treatment and the process was replicated five times. The treated gleanings were kept in plastic containers covered with brass wire mesh on top to allow aeration. The emerging adults were counted periodically up to 5 days.

RESULTS AND DISCUSSION

Population of Coffee Berry Borer

The mean population of coffee berry borer recorded in left-over berries collected from different locations is presented in Table 1. The mean borer population per left-over berry was high in Adalur 48.53 ± 41.39 followed by

Table 1. Coffee berry borer population in left-over berries

Location	Mean number of beetles / gleaning*										
	May 2004		June 2004			May 2005		June 2005			Over all
	Mean \pm SD	Range	Mean \pm SD	Range	Mean(2004) ⁺	Mean \pm SD	Range	Mean \pm SD	Range	Mean (2005) ⁺	Mean ⁺
Adalur	48.53 \pm 41.39	5-144	33.75 \pm 24.37	3-92	41.14(6.41) ^a	39.09 \pm 33.71	4-135	31.36 \pm 23.08	2-76	35.23(5.93) ^a	38.16(6.17) ^a
Kanalkadu	34.13 \pm 32.70	4-131	29.05 \pm 23.47	2-86	31.59(5.62) ^{bc}	30.67 \pm 26.33	4-126	26.12 \pm 20.32	2-81	28.40(5.32) ^d	29.99(5.47) ^d
K.C.Patty	27.96 \pm 30.95	3-121	29.48 \pm 20.97	4-81	28.72(5.35) ^{bc}	29.2 \pm 26.45	2-115	20.71 \pm 19.48	3-69	24.96(4.99) ^{gh}	26.70(5.16) ^{ef}
Kamanur	33.83 \pm 28.59	2-120	29.78 \pm 20.25	3-77	31.81(5.64) ^{bc}	28.40 \pm 25.45	3-99	19.92 \pm 16.70	2-62	24.16(4.91) ^{hi}	27.98(5.28) ^{ef}
Mangalamkombu	26.18 \pm 22.61	3-89	28.18 \pm 19.73	1-71	27.18(5.21) ^k	22.39 \pm 19.07	2-83	20.64 \pm 17.21	1-79	21.52(4.63) ⁱ	24.83(4.98) ^{hi}
Manjalparappu	31.24 \pm 26.03	2-91	26.12 \pm 19.73	1-82	28.68(5.35) ^{ji}	23.60 \pm 21.90	1-78	21.39 \pm 19.76	1-72	25.50(5.04) ^{ef}	25.09(5.00) ^b
Manalur	34.58 \pm 30.02	5-123	25.50 \pm 17.05	2-87	30.04(5.48) ^{ghi}	29.64 \pm 25.13	3-98	23.71 \pm 19.51	2-77	26.68(5.16) ^f	28.35(5.32) ^{ef}
Nallurkadu	42.80 \pm 33.89	7-131	35.00 \pm 22.55	3-99	38.90(6.23) ^b	33.06 \pm 28.82	4-127	32.11 \pm 21.36	2-91	32.59(5.70) ^b	35.74(5.97) ^b
Nerimalai	35.19 \pm 30.15	4-113	30.76 \pm 21.03	2-89	32.98(5.74) ^{def}	25.07 \pm 27.58	3-112	24.12 \pm 19.76	1-86	24.60(4.95) ⁱ	28.78(5.36) ^{ef}
Periyamalai	33.50 \pm 27.51	3-117	24.63 \pm 21.63	2-88	29.07(5.39) ^{hij}	29.84 \pm 28.92	2-105	23.66 \pm 21.32	1-79	26.75(5.17) ^f	27.90(5.28) ^f
Perumparai	29.20 \pm 25.97	2-94	22.13 \pm 18.71	1-74	25.67(5.06) ^{kl}	25.03 \pm 22.68	3-87	20.71 \pm 16.17	2-71	22.81(4.77) ^j	24.26(4.92) ⁱ
Pillaveli	38.49 \pm 34.16	4-137	34.03 \pm 16.60	2-99	36.26(5.02) ^c	32.13 \pm 26.74	3-107	26.11 \pm 19.21	1-83	29.12(5.39) ^e	31.94(5.65) ^e
Pachalur	31.26 \pm 29.17	3-123	26.98 \pm 23.10	2-91	29.12(5.39) ^{hij}	31.16 \pm 25.59	2-115	23.11 \pm 19.91	1-88	27.14(5.20) ^g	28.12(5.30) ^{ef}
Pallathukalvai	37.36 \pm 32.08	5-121	30.33 \pm 21.71	3-97	33.85(5.81) ^d	27.38 \pm 21.76	3-117	25.31 \pm 21.72	1-92	26.35(5.13) ^f	30.09(5.48) ^d
Solaikadu	45.50 \pm 43.63	4-131	32.15 \pm 24.16	2-98	38.83(6.23) ^b	35.66 \pm 28.71	3-131	31.36 \pm 25.38	2-95	33.51(5.78) ^b	36.16(6.01) ^b
Thandigudi	33.43 \pm 24.05	1-91	23.50 \pm 17.10	1-71	28.47(5.33) ^{jk}	20.53 \pm 18.88	1-88	19.46 \pm 16.72	0-76	20.00(4.47) ^k	24.23(4.92) ⁱ
Thadiyankudisai	27.81 \pm 25.38	2-98	22.12 \pm 19.73	1-66	24.97(4.99) ^l	24.43 \pm 22.37	1-87	20.11 \pm 18.66	1-71	22.27(4.71) ^j	23.62(4.86) ⁱ
Mean \pm S.D	34.76 \pm 30.48		28.44 \pm 20.69			28.48 \pm 25.25		24.11 \pm 19.78			

* Mean of five estates sampling per location

+ Figures in parentheses are square root transformed values

In a column, means followed by a common letter(s) are not significantly different by DMRT (P=0.05)

Solaikadu 45.50 \pm 43.63, Nallurkadu 42.80 \pm 33.89 and Pillaveli 38.49 \pm 34.16 while it was low in Mangalamkombu 26.18 \pm 22.61 and Thadiyankudisai 27.81 \pm 25.38 during May 2004 (Table 1). During June 2004, the borer population was high in Nallurkadu 35.00 \pm 22.55 followed by Pillaveli 34.03 \pm 16.60, Adalur 33.75 \pm 24.37 and Solaikadu 32.15 \pm 24.16 as against in Thadiyankudisai 22.12 \pm 19.73, Perumparai 22.13 \pm 18.71 and Thandigudi (June 2004) 23.50 \pm 17.10 where it was slightly low.

The mean borer population per left-over fruit recorded during May 2005 and June 2005, was high in Adalur (39.09 \pm 33.71 and 31.36 \pm 23.08) followed by Solaikadu (35.66 \pm 28.71 and 31.36 \pm 25.38), Nallurkadu (33.06 \pm 28.82 and 32.11 \pm 21.36), and Pillaveli (32.13 \pm 26.74 and 26.11 \pm 19.21). It was less in Thandigudi (20.53 \pm 18.88 and 19.46 \pm 16.72), Mangalamkombu (22.39 \pm 19.07 and 20.64 \pm 17.21) and Manjalparappu (23.60 \pm 21.90 and 21.39 \pm 19.76). The over all mean population of borer per left-over fruit recorded in Adalur, Solaikadu, Nallurkadu and Pillaveli was as high as 38.16 \pm 30.63, 36.16 \pm 30.47, 35.74 \pm 26.65 and 31.94 \pm 24.17 respectively whereas the borer population recorded

in Thadiyankudisai (23.62 \pm 21.54), Thandigudi (24.23 \pm 19.19), Perumparai (24.26 \pm 20.88) and Mangalamkombu (24.83 \pm 21.00) was low. Thus, irrespective of the locations surveyed, the population of coffee berry borer in left over berries was considerable and could form the main source of inoculum for carryover of the population to the next season. In general, the infestation of coffee berry borer gradually declines from January onwards as most of the ripened berries are harvested during this period. The borers then move to left over berries or dry berries or gleanings for shelter and further breeding and multiplication.

Pattern of Coffee Berry Borer Emergence

The data on the emergence of adult berry borer from gleanings exposed to different treatments are presented in Table 2. The mean number of borer adults that emerged per gleaning was high (21.72) in natural rain followed by water spray (12.93) and exposure to higher temperature (25°C) (12.52), respectively. The next in order of borer emergence were in water soaking treatment (11.67) and

Table 2. Effect of moisture and temperature on coffee berry borer beetle emergence from gleanings

Treatment No	Treatments	Mean number of beetles emerged * (Days after exposed)					
		1	2	3	4	5	Mean
T1	Impact of water spraying	24.13(4.12) ^d	26.12s (5.10) ^b	8.09 (2.84) ^c	4.16 (2.03) ^c	2.17(1.47) ^b	12.93(3.59) ^b
T2	Impact of water soaking (2 minutes)	26.31(5.12) ^c	22.41(4.73) ^c	5.32 (2.30) ^d	3.12 (1.76) ^{cd}	1.20 (1.09) ^c	11.67(3.41) ^c
T3	Impact of natural rain	46.16(6.83) ^a	32.11(5.66) ^a	22.09 (4.70) ^a	6.14 (2.47) ^b	2.12 (1.45) ^b	21.72(4.66) ^a
T4	Impact of surface temperature (25°C)	28.13(5.30) ^b	19.07(4.36) ^d	12.12 (3.48) ^b	2.10 (1.47) ^{cd}	1.22 (1.10) ^c	12.52(3.53) ^b
T5	Impact of Relative humidity (90%)	10.19(3.19) ^e	12.12 (3.48) ^e	6.30 (2.50) ^d	8.21(2.86) ^a	7.16 (2.67) ^a	8.79 (2.96) ^d
T6	Untreated check	3.40(1.84) ^f	4.36 (2.08) ^f	2.11(1.45) ^e	1.30 (1.14) ^a	1.20 (1.09) ^c	2.47 (1.57) ^e

* Each value is the mean of five replications

Figures in Parentheses are square root transformed values

In a column, means followed by a common letter(s) are not significantly different by DMRT (P=0.05)

prevalence of high relative humidity (8.79) compared to untreated check (2.47 beetles).

The study indicated that maximum emergence of adult borers was recorded from gleanings exposed to natural shower and minimum emergence from that exposed to high relative humidity of 90%. This is in conformity with the earlier results of Sreedharan *et al.*, (1994) that heavy rain triggered the emergence of the beetles and low humidity (<60% RH, 25°C) provoked rapid evacuation of adults while it was minimum at 90% RH (Baker *et al.*, 1992).

The present study demonstrated the importance of removal of the left-over berries for the management of berry borer population. The left-over fruits on the plant, after main harvest season retained the inoculum for carry over of the berry borer to the next season's crop. Removal of the left over fruits and collection of emerging adults from the gleanings could be the best management tools against the berry borer. As the emergence of adult borers from fallen fruits was maximum after natural rainfall, rainy season is the best period to use any trapping mechanism to trap and kill the borers.

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Qualitative Phytochemical Screening of Some Locally Available Insecticidal Plants

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ABSTRACT

Qualitative phytochemical screening of ten locally available insecticidal plants such as *Adathoda vasica* (Ness) (Acanthaceae), *Cynodon dactylon* (Linn.) Pers. (Poaceae), *Eclipta alba* (Linn.) (Asteraceae), *Morinda pubescens* J.E.Smith (Rubiaceae), *Ocimum tenuiflorum* (Linn.) (Labiatae), *Phyllanthus amarus* (Linn.) (Euphorbiaceae), *Sesbania grandiflora* (Linn.) (Fabaceae), *Solanum surattense* (Linn.) (Solanaceae), *Solanum trilobatum* (Linn.) (Solanaceae), and *Vinca rosea* (Linn.) (Apocynaceae) were investigated. Petroleum ether (40 – 60° C), hexane, chloroform, ethanol and water were used as solvents. Secondary metabolites steroids, alkaloids, phenolic compounds, flavonoids, saponins, tannins, aromatic acids, and xanthoproteins were analysed using standard procedures. Tannins was found from water and hexane extracts of *S. grandiflora* and *E. alba* respectively. Except the chloroform extract of *A. vasica*, petroleum ether, ethanol extracts of *O. tenuiflorum* and petroleum ether and hexane extracts of *V. rosea* had alkaloids.

INTRODUCTION

Phytochemical surveys are now seen as the first step towards the discovery of useful drugs now that the tropical rain forest has been identified as a potential source due to its diverse richness in flora. Screening for biological activity using simple and fast bioassays has now been added to give a better indication of the usefulness of the plants. Comparative phytochemical examinations of 127 species have been studied by Goh *et al.* (1997). However, very little information is available for the phytochemical studies of these plants. The main objectives of the present work was to study the preliminary phytochemistry of the aerial parts of 10 locally available plants such as *Adathoda vasica*, *Eclipta alba*, *Cynodon dactylon*, *Morinda pubescens*, *Ocimum tenuiflorum*, *Phyllanthus ararus*,

Sesbania grandiflora, *Solanum trilobatum*, *Solanum surattense* and *Vinca rosea*. Subramaniam (1993) reported that *Adathoda trilobatum*, *Ocimum* spp. *Solanum* spp. had been considered as insecticidal plants. Hence it is imperative to study the preliminary photochemistry of these plants. The main objective of the present work was to study the preliminary phytochemistry of the aerial parts of 10 locally available plants.

MATERIAL AND METHODS

Aerial parts of 10 selected plants were collected from Thoothukudi district of Tamilnadu, India. They were washed thrice with distilled water and once with tap water and were shade dried for two weeks. 20 g each of the aerial parts powder samples of *Adathoda vasica* (AV),

Table 1. Plants used for the preliminary phytochemical screening

S.No.	Common name	Botanical name	Family	Parts used
1	Adathodai	<i>Adathoda vasica</i> (Ness.)	Acanthaceae	Aerial parts
2	Arukampillu	<i>Cynodon dactylon</i> (Linn.)Pers.	Poaceae	Aerial parts
3	Kayyantakara	<i>Eclipta alba</i> Linn.	Asteraceae	Aerial parts
4	Manjanathi	<i>Morinda pubescens</i> J.E.Smith	Rubiaceae	Aerial parts
5	Tulsi	<i>Ocimum tenuiflorum</i> (Linn.)	Labiatae	Aerial parts
6	Keelanelli	<i>Phyllanthus amarus</i> Linn.	Euphorbiaceae	Aerial parts
7	Agathikeerai	<i>Sesbania grandiflora</i> (Linn.)	Fabaceae	Aerial parts
8	Thoothuvalai	<i>Solanum trilobatum</i> (Linn)	Solanaceae	Aerial parts
9.	Kandan kathiri	<i>Solanum surattense</i> Linn. (= <i>xanthocarpum</i> Schrad & Wendl)	Solanaceae	Aerial parts
10	Nithyakalyani	<i>Vinca rosea</i> Linn.	Apocynaceae	Aerial parts

Table 2. Preliminary phytochemical screening tests

S.No.	Tests	Observation	Inference
1.	Test solution + minimum amount of CHCl_3 + 3 drops of acetic anhydride + 2 drops of conce H_2SO_4 (Lieberman – Burchard test)	Purple colour changing to blue or green	Presence of steroids
2.	Test solution + piece of tin + 3 drops of thionyl chloride	Violet or purple colour	Presence of Triterpenoids
3.	Test solution shaken with 2 N HCl. Aqueous layer formed, decanted and to which are added one or two drops of Mayer's reagent added	White turbidity or precipitate	Presence of alkaloids
4.	Alcoholic solution of test solution + one drop of ferric chloride	Intense colour	Presence of phenolic compounds
5.	Test solution + NaHCO_3	Brisk effervescence	Presence of aromatic acids
6.	Test solution + H_2O and shaken well	Foamy lather	Presence of saponins
7.	Water soluble portion of the extract tested with basic lead acetate solution	White precipitate	Presence of tannins
8.	Test solution + magnesium powder and treated with concentrated HCl and heated. Cool the test tube under the running water	Orange colour	Presence of Flavonoids

Eclipta alba (EA), *Cynodon dactylon* (CD), *Morinda pubescens* (MP), *Ocimum tenuiflorum* (OT), *Phyllanthus ararus* (PA), *Sesbania grandiflora* (SG), *Solanum trilobatum* (ST), *Solanum surattense* (SS) and *Vinca rosea* (VR) separately were successively extracted with petroleum ether ($40^\circ - 60^\circ \text{C}$), hexane, chloroform, ethanol and water in a soxhlet apparatus. The extracts were tested for steroids, triterpenoids, alkaloids, phenolic compounds, flavonoids, saponins, tannins, and aromatic acids. The various phytochemical tests were performed (Brinda *et al.*, 1981) with slight modifications to find out the secondary metabolites are presented in Table 2.

RESULTS AND DISCUSSION

Air-dried aerial parts of *Adathoda vasica* (AV), *Eclipta alba* (EA), *Cynodon dactylon* (CD), *Morinda pubescens* (MP), *Ocimum tenuiflorum* (OT), *Phyllanthus ararus* (PA), *Sesbania grandiflora* (SG), *Solanum trilobatum* (ST), *Solanum surattense* (SS) and *Vinca rosea* (VR) are successively treated with petroleum ether ($40^\circ - 60^\circ \text{C}$), hexane, chloroform, ethanol and water. The results of different extracts have been tested for steroids, triterpenoids, alkaloids, phenolic compounds, saponins, tannins, flavanoids and aromatic acids and are presented in Table 3.

True triterpenoids, steroids, saponins and cardiac glycosides are the classes of terperpenoids. Tannins are considered as an important compound, which act as a

barrier to herbivory (Anathakrishnan, 1992). It was reported only in *E. alba* and *Solanum trilobatum*. Harbone (1984) reported that flavonoids were mainly water insoluble and they could be extracted only with ethanol. However, in the present study, it was recorded that water extract of *Adathoda vasica*, *Cynodon dactylon*, *Morinda pubescens* and *Ocimum tenuiflorum* and petroleum ether extracts of AV showed the presence of flavonoids. Almost all the plants of the plant kingdom posse's flavones and flavonols. Another water insoluble chemicals present in the plant are phenolic compounds (says Harbone, 1984). But the present study shows that both *Ocimum tenuiflorum* and *Vinca rosea* consist of phenolic compounds. Both tannin and flavonoids are collectively called polyphenls. Results showed that none of the 10 plants posses polyphenols. They mainly reduce damage caused by insects through their deterrent and/or antifeedant effects (Echeverri *et al.*, 1991; Palvela, 2006). But tannin and flavonoids were reported in many plants. This study helps the pest control practitioners to select the plants without detailed studies related to phytochemistry of the locally available plants. All the plants possess at least three or more than three secondary metabolites. So they can be utilized as pesticidal plants. However, the quantity may be determined before selecting them for pest management purpose.

Table 3. Phytochemical screening of the aerial parts of 10 selected plants

Extracts	Steroids	Triterpenoids	Alkaloids	Phenolic	Saponins compounds	Tannins	Flavonoids	Aromatic acids
Petroleum Ether (40 ⁰ – 60 ⁰ C)	AV, CD, EA, MP, OT, PA, SG, SS, ST	-	OT, VR	EA, VR	AV, CD, MP, OT, PA, SG, SS, ST	-	AV	AV, CD, EA, MP, OT,
Hexane	EA, VR	-	VR	CD, OT, SG	AV, CD, EA, MP, OT, PA, SG, SS, ST, VR	EA	-	MP, PA, ST
Chloroform	AV, CD, EA, MP, OT, PA, SG, SS, ST, VR	PA, SS, VR	AV,	SG	AV, CD, MP, OT, PA, SG, SS, ST	-	-	OT, VR
Ethanol	AV, CD, EA, MP, OT, PA, SG, SS, ST, VR	-	OT	AV	OT, PA	-	-	AV, CD, MP, SG, ST
Water	OT, SG, VR	-	-	OT, VR	AV, MP, OT, PA, SG, SS	SG	AV, CD, MP, OT	SS

Adathoda vasica – AV, *Eclipta alba*- EA, *Cynodon dactylon*- CD, *Morinda pubescens* – MP, *Ocimum tenuiflorum* – OT, *Phyllanthus ararus*- PA, *Sesbania grandiflora* – SG, *Solanum trilobatum* – ST, *Solanum surattense* – SS and *Vinca rosea* - VR

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Bioefficacy of Crude and Fractions of *Argemone mexicana* against Tobacco Caterpillar, *Spodoptera Litura* Fab. (Noctuidae: Lepidoptera)

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ABSTRACT

The insecticidal activity of crude extracts and fractions of *Argemone mexicana* L. (Papaveraceae) was investigated against *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). The different treatments differed significantly in their efficacy. Pupation was nil in chloroform extract and acetone extract, while water extract treated larvae resulted in least pupal weight and maximum malformed adults. The adult life span was least in acetone solvent followed by hexane and petroleum ether extracts. Most of the treatments resulted in nil fecundity. Among the chloroform fractions, the first fraction arrested the pupation. In addition, disturbed moulting, larval-pupal intermediates and malformed moth emergence/dead pupae were also observed.

Keywords: *Argemone mexicana*, pupation, larval-pupal intermediates, fecundity

INTRODUCTION

The leafworm, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera), a serious but sporadic insect pest causes economic losses of crops from 25.8-100% (Dhir *et al.*, 1992) based on crop stage and its infestation level in the field. It has a large host range of more than 120 host plants including crops, vegetables, weeds and ornamental plants (Ramana *et al.*, 1988). It feeds gregariously on leaves leaving midrib veins only resulting in great yield loss. In India, 40 species of cultivated crops, wild plants and 11 flowering plants (Ali *et al.*, 1999) are affected by this pest. Several outbreaks of this pest on cotton, tobacco and chillies have been reported in Tamil Nadu especially in Coimbatore and Madurai districts (Rao *et al.*, 1983). Further, Rao *et al.* (1983) reported that yield losses due to this pest was in the tune of Rs. 281.98 lakhs in tobacco and Rs.275.5 lakhs in chillies in Andhra Pradesh State alone.

It has developed resistance against a variety of insecticides belonging to almost all the insecticide groups used against it (Anonymous, 1999; 2000, Armes *et al.*, 1997; Kranthi *et al.*, 2002) even against new chemical insecticides like lufenuron (Sudhakaran, 2002). Adverse effects due to synthetic pesticides on pests and their subsequent impact on ecological imbalance (Zadoks and Waibel, 1999) demands ecofriendly alternatives (Parmar, 1993). Botanical is one such alternative and an important component in Integrated Pest Management (IPM) due to its advantages such as availability, least toxicity to beneficials, quick degradation and multiple functions (Isman, 2006). They act as antifeedant, repellent, deterrent,

chemosterilants and growth regulator due to the presence of nearly 30,000 secondary metabolites (Bowers and Nishida, 1980; Schoonhoven, 1993; Isman, 2006).

Argemone mexicana (Family: Papaveraceae) is an erect prickly annual plant with yellow flower and latex. It is a native of tropical America and now widely naturalized in tropics. The plant is available along riverbanks and in Tamil Nadu; it is predominantly present at Yercaud (1400 m) (Matthew, 1983). The plant contains many alkaloids (Sangwan and Malik, 1998) and is used mostly for the treatment of HIV (YuhChwen *et al.*, 2003). A critical literature survey reveals that *Argemone* has not been studied in-depth for its pesticidal character, except against cabbage head caterpillar, *Crocidolomia binotalis* (Facknath and Kawol, 1993) and mosquito, *Aedes aegypti* (Sakthivadivel and Thilagavathy, 2003). Hence, the present study aimed to explore the biopesticidal activity of this plant to combat the devastating pest *S. litura* with the following objectives: to test *A. mexicana* crude extracts against the larval (4th instar) and its adult stages of *H. armigera* and to test *A. mexicana* chloroform fractions against the larval (4th instar) stage of *H. armigera*.

MATERIALS AND METHODS

Collection and Rearing of *Spodoptera litura*

S. litura larvae were collected from infested castor plants from Kannivadi in Dindigul District., Tamil Nadu, India. The larvae collected from castor were maintained in the laboratory at 22 ± 2°C and 70 – 75 % relative humidity (RH). The larvae were reared both on castor and semi-synthetic diet in individual containers to prevent contamination (Santharam, 1985).

Table 1. Bioefficacy of *Argemone mexicana* crude extracts on first generation *S. litura*

Treatment		Characters		
		Larval development		Moth emergence (%)
		Pupation %	Pupal weight (mg)	Malformed/Dead
Hexane	extract	76.6	249	81.9
	solvent	93.3	276	60.0
Petroleum ether	extract	36.6	256	82.2
	solvent	90.0	260	55.5
Chloroform	extract	0.0	—	—
	solvent	66.6	265	70.8
Acetone	extract	0.0	—	—
	solvent	16.6	206	83.3
Water extract		96.6	203	83.3
Untreated		100.0	321	6.6
CD (P=0.05)		17.02	34.86	24.08

Plant Material and Its Extraction

Leaves of *Argemone mexicana* were collected from different parts of Thirukazhukundram and Kannivadi, Tamil Nadu. Collected leaves of *A. mexicana* were shade dried and powdered. One kg of powdered leaves was extracted successively using both non-polar and polar solvents viz., petroleum ether, hexane, chloroform and acetone. The powdered leaf material was soaked for 24h at $30 \pm 2^\circ\text{C}$ in 2.5 litre of solvent, filtered and to the residue the same solvent was added. The extraction was repeated thrice to obtain maximum extractables. All the filtrates were pooled and evaporated under vacuum in a rotary evaporator (Harborne, 1998) at 190 rpm/min (the temperature varies between extracts viz., $40-60^\circ\text{C}$ for petroleum ether, $60-62^\circ\text{C}$ for chloroform and acetone, $66-70^\circ\text{C}$ for hexane).

Bioassay of Host Plants

Growth Inhibition of Larvae

Ten per cent solution of hexane, petroleum ether, chloroform, acetone and water extracts of *A. mexicana* were made in the respective solvents and mixed in the larval diet and fed to the fourth instar larvae only of *S. litura* using 1) normal diet + extract, 2) normal diet + solvent and 3) normal diet (control). Pupation (%), pupal weight (mg) and malformed moth emergence/dead pupae (%) and intermediate forms if any were recorded. Triplicates were maintained for each treatment and the data were analyzed statistically using Agres package version 4.

Adult Longevity, Fecundity and Egg Hatchability of *S. litura*

The adults of the previous (larvae 1st generation) study from the respective treatments, if any, were tested further. Ten per cent solution of hexane, petroleum ether, chloroform, acetone and water extracts of *A. mexicana* were made in the sugar solution with the respective solvents, which was fed to the adult moths, and the longevity, fecundity and hatchability were checked. Solvent control (10%) and 10% sugar solution (normal control) were also maintained. Five pairs of treated adults were released into the mud pot and maintained. Longevity of the moths, eggs laid and hatchability were recorded. Triplicates were maintained for each treatment and the data were analyzed statistically using Agres package version 4.

Fractionation of Leaf Extract

Efficacy of primary fractions of *A. mexicana* on *S. litura* 40 g of chloroform crude extract was dissolved in the respective solvent and fractionated on a silica gel column, using hexane/methanol at 9.8:0.2, 9:1, 8:2, 7:3 and 6:4 ratios. Fraction 1 - (hexane: methanol 98: 2); Fraction 2 - (hexane: methanol 90: 10); Fraction 3 - (hexane: methanol 80: 20) Fraction 4 - (hexane: methanol 70: 30); Fraction 5 (hexane: methanol 60: 40). Five fractions named as Fr 1 (dark yellow with slow fractionation), Fr 2 (light yellow with slow fractionation), Fr 3 (reddish brown with moderate fractionation), Fr 4 (brown with high fractionation) and Fr 5 (green with high fractionation) was eluted.

Only the fourth instar larvae of *S. litura* were bioassayed using 1) normal diet + fraction, 2) normal diet + solvent

Table 2 Effect of chloroform fractions (primary) of *A. mexicana* on the growth of *S. litura* larvae

Treatments	Characters*		
	Larval development		Moth emergence %
	Pupation %	Pupal weight (mg)	Malformed /Dead
Fraction 1	0	—	—
Fraction 2	13.3	254	83.3
Fraction 3	25.0	253	91.6
Fraction 4	25.0	284	72.2
Fraction 5	21.6	248	88.8
Solvent control	11.6	129	83.3
Untreated	96.6	304	3.33
CD (P = 0.05)	9.309	12.556	30.030

*Each value mean of triplicate

and 3) normal diet (control). Pupation (%), pupal weight (mg) and malformed moth emergence/dead pupae (%) and intermediate forms if any were recorded. Triplicates were maintained for each treatment and the data were analyzed statistically using Agres package version 4.

RESULTS AND DISCUSSION

Efficacy of *A. mexicana* Crude Extracts on *S. litura* Larva

S. litura larvae treated with chloroform and acetone extracts showed no pupation, which was superior over the others. This was followed by acetone solvent and petroleum ether extract as against 100 % pupation in the untreated larvae (Table 1). Deterred feeding and significant larval mortality was reported in *S. litura* treated with methanolic extracts of *Melia dubia* (Opender *et al.*, 2000) and *Adathoda vasica* (Sadek, 2003). In addition to no pupation, phagodepression and difficulty in moulting resulted in pre-pupal malformations (Plate 1). This coincided with significant changes in pupal and pre-pupal stages in *S. litura* fed with different doses of hexane

extracts of neem seed kernel (Kaur *et al.*, 2001) and *Tribulus terrestris* (Gunasekaran and Chelliah, 1985). Such potent toxicity leading to high larval mortality exhibited by the fractions of *A. mexicana* could be attributed to the group of toxic bimolecular possessing insecticidal properties particularly, glycosides and alkaloids present in species of the family Papaveraceae (YuChwen *et al.*, 2003). The pupal weight was least in water extract treated ones (203 mg) followed by acetone solvent (206 mg). The hexane extract treated larvae resulted in pupal weight with 249 mg, followed by petroleum ether extract (256 mg) as against 321 mg in untreated control. Similar effects were reported by the extracts of *Ocimum basilicum* against *Helicoverpa armigera* (Pandey *et al.*, 1983), *Melia azedarach* against cabbage diamond-back moth (Dilawari *et al.*, 1994) and *Annona squamosa* against *Helicoverpa* (Ganeshan *et al.*, 1995). Least healthy moth emergence (16.6 %) was recorded in water extract treatment followed by 17.7 % and 18.1 % in petroleum ether and hexane extracts treatment respectively. The control recorded 93.3 % healthy moth emergence. Similarly, furanocoumarin from the dried fruits of *Tetradium daniellii*, exhibited less healthy moth emergence (Tripathi, 2002; Stevenson *et al.*, 2003).

Efficacy of Crude Extracts against First-Generation Adults

The adult longevity was less (0.3 days) in adults emerged from acetone solvent treatment, followed by 1.1 days in hexane and petroleum ether extracts. Chloroform solvent resulted in 2.1 days while 2.5 days survival was observed in hexane solvent and water extract as against 6.0 days in untreated (Fig 1). Petroleum ether extract and its solvent, acetone solvent and water extract treatments recorded no eggs. The fecundity was minimum (17.3 eggs) in hexane extract as against the highest (238) in untreated. Similarly, hexane extract of neem seed kernel induced 86% sterility

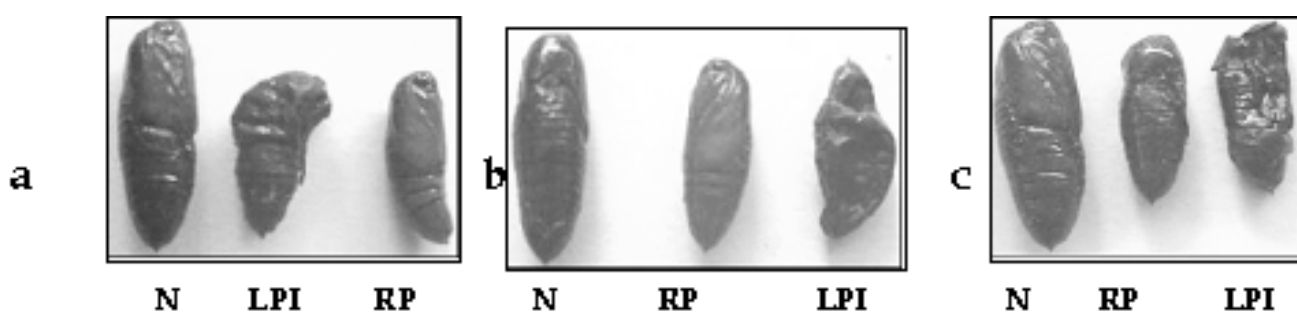


Plate 1. Impact of water (a), hexane (b) and petroleum ether extracts (c) of *A. mexicana* on *S. litura* (N- normal pupa; RP-reduced/affected pupa; LPI-larval-pupal intermediate)

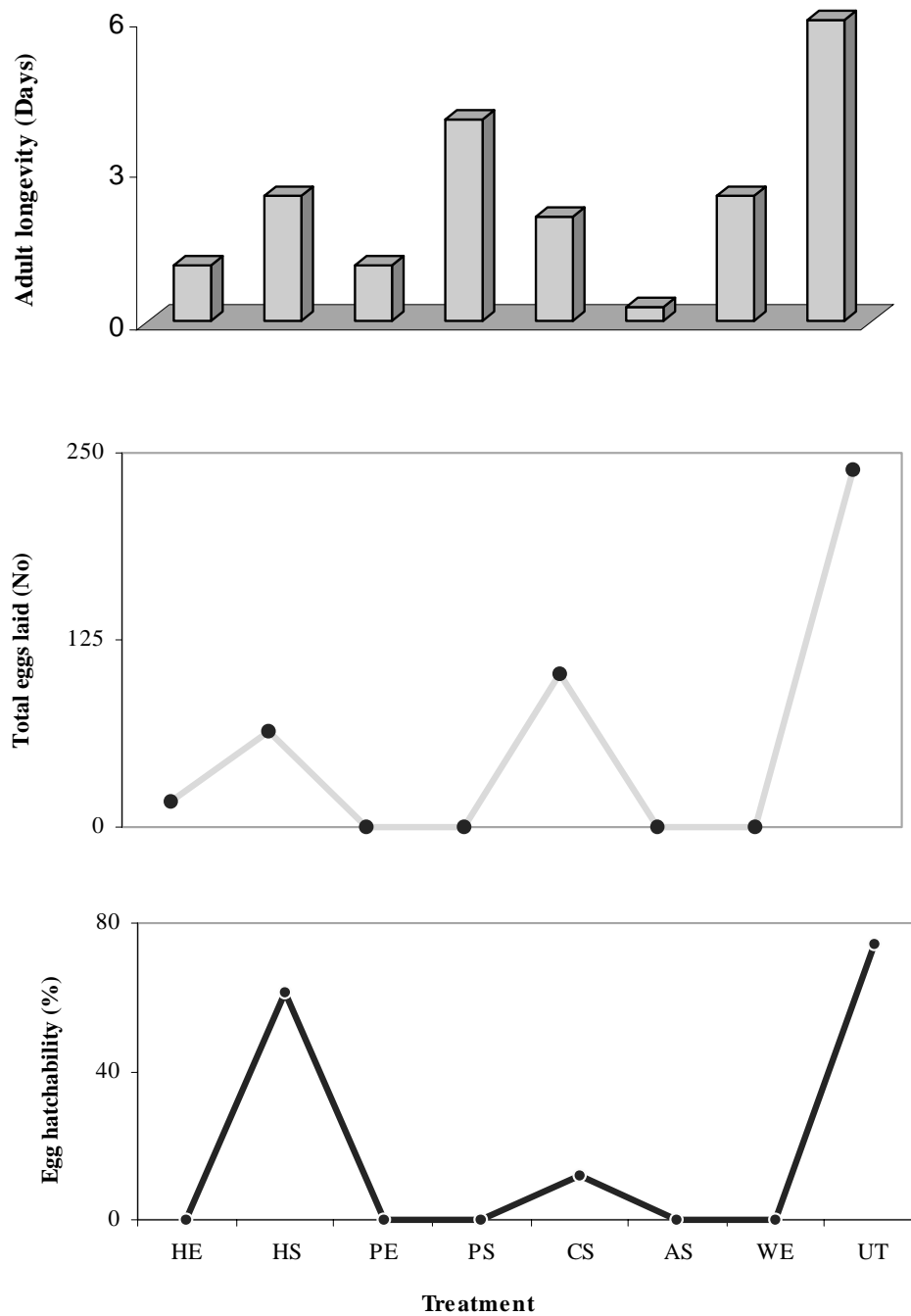
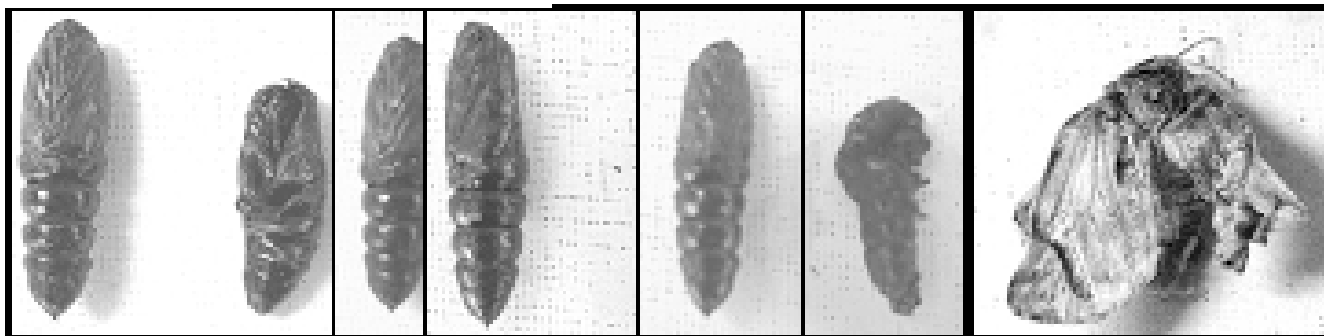


Fig. 1. Efficacy of *A. mexicana* Hexane Extract (HE), Hexane (HS), Petroleum ether Extract (PE), petroleum ether (PS), Chloroform (CS), Acetone (AS) and Water Extract (WE) on longevity (in days) (a), fecundity (b) and hatchability (c) of *S. litura*



i **ii** **iii** **iv** **v** **vi** **vii**

Plate 2. Juvenomimetic effect of *A. mexicana* primary fractions (chloroform) on *S. litura* larvae– normal pupa; ii – solvent treated; iii – 5th fraction – reduced pupal weight; iv – 3rd fraction with reduced pupal weight; v – 1st fraction; vi – 5th fraction larval-pupal intermediates; vii – 3rd fraction – malformed adult

and further suppressed the reproductive performance (Kaur *et al.*, 2001). Oviposition of the cabbage pest, *Mamestra brassicae* was reduced to half the number of eggs per plant by the neem treatment (Shimizu, 1988). The number of eggs that hatched was not affected by the neem treatment, but development of the larva was strongly inhibited and all larvae in the neem treatment died within two weeks without reaching 2nd instar (Selijasen and Meadow, 2006). Except hexane (61%) and chloroform solvent (12%) treatments the rest of the treatments, resulted in nil hatchability. The impairment of gonotrophic cycle of adults might have prevented the eggs from hatching. Similar trend was also observed in *Earias vitella* (Fab.) (Noctuidae) treated with the leaves of *Azadirachta indica*, *Ocimum basilicum*, *Eucalyptus rostrata*, *Lantana camara* and *Allium sativum* which significantly reduced the oviposition and hatchability compared to the control (Shukla and Pathak, 1997).

Efficacy of Chloroform Fractions against *S. litura* Larvae

Maximum growth inhibition was observed in larvae treated with fraction 1 and none of the larvae were able to pupate. This was followed by solvent control (11.6 %) and fraction 2 (13.3 %). The control showed 96.6 % pupation. This was in confirmation with Ganeshan *et al.* (1995) wherein exposure of *H. armigera* larvae to Neem and *Annona* resulted in 100% larval mortalities irrespective of the treatments. Methanol fraction of *M. dubia* inhibited larval growth of neonate *H. armigera* larvae in a dose dependant manner, when added to artificial diet in the range of 100 – 500 ppm of the extract. The extract inhibited larval growth by 50 % at 147 ppm (Koul *et al.*, 2002).

It was also observed that during development, larvae of *S. litura* lost their body weight rapidly when treated with

the fractions and transformed into small sized and shrivelled pupa (Plate 2 - vi). Prolongation in larval developmental periods leading to reduction in pupal weight and malformed larval pupal intermediaries (Plate 2b – iv - vi) are reported to be the physiological effects of the neem (Red Fern *et al.*, 1982; Schmutterer *et al.*, 1983). Similarly, all the fractions of *D. angustifolia* resulted in a drastic reduction in pupal weight and subsequent record of malformed adults (Malarvannan, 2004).

Further, a drastic reduction in pupal weight was recorded in all the treatments. It ranged from 129-284 mg (normal control-304 mg), with 248 mg in fraction 5 (Plate 2 - iii), which subsequently resulted as malformed moths (Table 2; Plate 2 - vii). This may be attributed to the increased energy expenditure in order to detoxify the extracts within the insect body (Schoonhoven and Meerman, 1978; Dowd *et al.*, 1983; Al- Sharook *et al.*, 1991). Similarly, the postembryonic development and subsequent loss in pupal weight was observed in *S. litura* larvae fed with crude extracts of neem+mahua+jatropha (Ganeshan *et al.*, 1995). Similar effects in *Annona* (Kawazu *et al.*, 1990; Rupprecht *et al.*, 1990; Londershausen *et al.*, 1991; Ohsawa *et al.*, 1991) neem (Schmutterer, 1990) and jatropha and mahua (Grainage and Saleem Ahmad, 1988) have been documented earlier. Maximum malformed moth emergence was recorded in fraction 3 (91.6%) (Plate 2e), followed by fraction 5 (88.8%) as against the untreated ones (3.33%) (Table 2). The results obtained from laboratory studies on feeding of *S. litura* with botanicals are in confirmation with the antifeedant effects of neem seed kernel suspension (Joshi *et al.*, 1984), karanja (Deshmukh and Borle, 1975). Sombatsiri and Tigvattannont (1983) reported that survival rate of the larvae to the adult stage of *S.*

litura was 8.6% when treated with 0.1% neem kernel extract.

The experimental results proved that the biopesticides, particularly plant extracts play a major role in combating the pest. Its wide application as a botanical pesticide could be taken up after exploring its toxicity and field trials.

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Effect of Neem oil Extractive (NOE) on Repellency, Mortality, Fecundity, Development and Biochemical Analysis of *Pericallia ricini* (Lepidoptera:Arctidae)

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ABSTRACT

Investigations carried out on the Biological effects of neem oil extractive were assessed against *Pericallia ricini*, revealed that NOE affects both feeding and growth rates of *Pericallia ricini*. The biochemical studies showed that larvae carbohydrate and protein content get reduced in the treated larvae and this reduction is found to be dosage dependent. NOE also influences the number of eggs laid. The hatchability was totally suppressed. The extractive produced malformations in adult and pupae of *P.ricini*.

Key Words: - Castor, *Pericallia ricini*, neem oil extractive, azadirachtin.

INTRODUCTION

Interest in the use of biopesticides with selectivity against phytophagous insects has increased in recent years, particularly in cropping systems that rely on natural enemies as a major component of integrated pest management (Rausell *et al.*, 2000). Use of these natural compounds in the place of conventional insecticides can reduce environmental pollution, preserve non-target organisms, and avert insecticide, induced pest resurgence. The neem tree, *Azadirachta indica* produces the biodegradable and insecticidal liminoid, azadirachtin (Isman, 1999). The compound can be efficiently extracted from neem seeds where its concentration is greatest (Butter Worth and Morgan 1968). The insecticidal activity of azadirachtin has been demonstrated against numerous insect pests and its various modes of activity include, disruption of feeding, reproduction, or development (Walter, 1999). As azadirachtin is selective towards phytophagous insect with minimal toxicity to beneficial insects increases its potential value to pest management (Lowery and Isman, 1995). With all these in view, the present investigation was undertaken to study the effects of a Neem Oil Extractive (NOE) on *Percallia ricini*, a pest of castor.

MATERIALS AND METHODS

Biopesticide Source

Neem oil extractive used in the present investigation was obtained from the Directorate of Khadi and Village industries Commission, Pune, Maharashtra. It is a by product of neem oil and it has 10 percent fraction of crude oil. The product was screened at four concentrations viz., 0.2, 0.4, 0.6 and 0.8 percent to evaluate a range of biological effects on *P. ricini*.

Insect Source and Rearing

P. ricini is a phytophagous insect. The larvae are serious pests on many economically important crops, larvae were collected from the castor (*Ricinus Communis*) and a general culture was maintained to obtain third and fourth instar larvae.

Newly moulted third and fourth instar larvae were introduced in separate containers and were fed with the leaves soaked in different concentrations of the extractive. Distilled water mixed with 2 ml of alcohol forms the control solution. Both the treated and control larvae were maintained under identical conditions till pupation. The duration of larval period was recorded. Feeding budget of both fourth and fifth instar larvae were also calculated following Waldbauer (1968) and Petruszewicz and Macfadyen, (1970). The efficiencies were calculated relating the quantities of food consumed, assimilated and converted.

Other feeding parameters calculated were : CR = Consumption rate (mg / gm / day), AR = Assimilation rate (mg / gm / day), PR = Production rate (mg / gm / day), MR = Metabolic rate (mg / gm / day), AD = Approximate digestibility (%) ECD = Gross Conversion efficiency (%), and ECI = Net Conversion efficiency (%).

Total (carbohydrate), protein and lipid content of the final instar larvae which fed with the leaves soaked in the different concentrations of the NOE were estimated following (Seifter *et al.*, (1950), total protein by method of Lowery *et al.*, (1951) and Bragdon (1951) Standard methods. taking 5 mg of dry samples, from each treatment. Carbohydrate was estimated following the method described by Seifter *et al.*, (1950), total protein by method of Lowery *et al.*, (1951) and lipid by the method of Bragdon (1951).

Table 1. Larval duration (In days) of various instars treated with various concentrations of NOE

Larval instar	NOE Concentration (%)				
	Control	0.2	0.4	0.6	0.8
III	3.5 ± 1.290	3.5 ± 2.03	3.5 ± 4.92	3.5 ± 0.871	3.5 ± 1.46
IV	8.4 ± 2.07	8.4 ± 7.08	8.4 ± 4.71	8.4 ± 7.92	8.4 ± 8.0
V	15.4 ± 2.30	15.4 ± 4.76	16.0 ± 6.07	16.0 ± 7.11	17.5 ± 8.4

Results obtained in the present investigation were subjected to statistical analysis. Statistical tools used in this study were S.D, correlation co-efficient, Regression analysis etc.

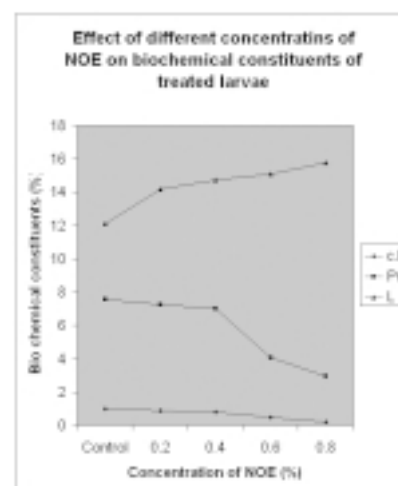
RESULT AND DISCUSSION

The larval duration of various instars treated with various concentration of NOE was given in Table 1. The larval duration is prolonged by two days (15.4 ± 2.30 to 17.5 ± 8.4) when the larvae were fed with 0.8 % NOE treated leaves.

Feeding budget of IV and V instar larvae treated with various concentration of NOE were given in table 2 and table 3 respectively. The relationship between various feeding parameters with the concentrations of the extractive. furnished in table IV. It is clearly seen from the table that the feeding activity is much influenced by this extractive. As the dosages are raised consumption, assimilation, production and their respective rates all found to decline. Consumption decreases from 3673.27 mg in the control to 1464.76 mg in the 0.8 % concentration. Similarly production decreases to about 75 % when the larvae were treated with 0.8 % NOE, assimilation rate is 119.99 mg / gm / day in the 0.2 % concentration which declines to 39.14 mg / gm / day in the final concentration. The assimilation efficiency is also found to be reduced. The declining trends observed in the feeding parameters are in accordance with the works of Ladd *et al.*, (1978). Wartlen (1979) reported that NOE acts as feeding inhibitor for many insect pests.

Figure 1. shows the effect of different concentrations of NOE on bio-chemical constituents of treated larvae. The total carbohydrate and protein contents found to decrease when the dosage increases, and the decline is found to be statistically significant. However, a slight increase in the lipid content was seen. Beck (1950) has reported that the insufficient amount of carbohydrate resulted in sub-optimal growth of *Pyrausta nubilalis*. Similarly lack of protein caused retardation of many physiological processes in insects, and adult insects require protein to promote ovulation and egg development (House, 1963). The treated larvae were allowed to metamorphose into adult and the egg laid by these moths were calculated. At higher concentration the egg laid was totally suppressed. None of the eggs laid by treated insects hatched out.

The extract was also effective in producing malformed adults. At higher doses the adults were unable to extricate from pupal skin. The malformed adults were shorter in length with crumpled wings. The fifth instar larvae was also crumpled and the hairs were found to be lost when treated with 0.8 % concentration of the extractive. The reduction in fecundity in the treated insect may be due to the derangement in the protein metabolism in the insects. In the present study, NOE might have affected the hormonal metabolism resulting in receipt of wrong code by cells inducing to perform wrong functions. This disturbed hormonal metabolism induced by the extractive may be attributed to the malformations seen in the adult. The observed reduction in growth, low fecundity and suppression in hatchability will definitely have a drastic effect on the population density of *Pericallia ricini*.



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Table 2. Feeding Budget Of IV Instar Larvae Treated With Various Concentration Of NOE

NOE (%)	C (mg /g/d)	A(mg/ g/d)	P (mg/ g/d)	M (mg/g/d)	CR (mg/g/d)	AR (%)	PR (%)	MR	AD	ECI	ECD
Control	741.98 ± 0.4744	278.97 ± 45.13	15.49 ± 0.05	266.57 ± 0.50	472.82 ± 0.302	177.7 ± 0.03	9.33 ± 0.23	171.87 ± 7.28	56.79 ± 3.03	1.959 ± 5.81	5.36 ± 0.09
0.2	729.97 ± 0.4998	266.98 ± 0.5015	14.30 ± 0.51	252.67 ± 0.06	467.66 ± 0.316	170.98 ± 0.03	9.16 ± 0.03	160.6 ± ± 0.03	36.66 ± ± 4.50	2.08 ± 5.47	5.55 ± 0.08
0.4	669.45 ± 0.4151	169.45 ± 0.057	14.304 ± 0.05	156.93 ± 0.50	369.23 ± 0.276	101.9 ± 0.03	7.93 ± 0.023	92.3 ± 0.03	37.58 ± 4.82	2.13 ± 6.37	7.58 ± 7.58
0.6	289.65 ± 0.5015	164.53 ± 0.515	12.516 ± 0.051	149.15 ± 0.58	179.46 ± 0.31	93.4 ± 0.029	7.87 ± 0.03	86.5 ± 0.02	25.30 ± 5.83	4.31 ± 0.01	8.44 ± 0.05
0.8	201.23 ± 0.515	108.23 ± 0.051	12.376 ± 0.05	93.92 ± 0.05	127.89 ± 0.35	68.7 ± 0.02	7.75 ± 0.039	59.69 ± 23.2	53.76 ± 0.01	6.15 ± 0.01	11.45 ± 0.04

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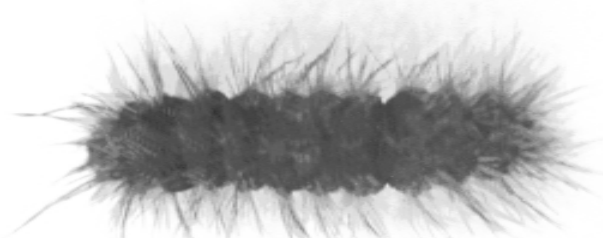
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1. 0.8% NOE treated third instar larva. Size of the Larva is highly reduced



2. Supernumerary larva darker in colour with abnormally developed hairs. obtained on 0.8% NDE treatment.

Table 3. Feeding Budget Of V Instar Larvae Treated With Various Concentration Of NOE

NOE%	C(mg)	A(mg)	F(mg)	M(mg)	CR(mg g/d)	AR(mg /g/d)	PR(mg/ /g/d)	MR(mg /g/d)	AD(%)	ECE(%)	ECD(%)
Control	2930.29 ± 0.5014	956.29 ± 0.50	94.37 ± 0.50	634.87 ± 0.51	2629.19± ± 0.45	857.9 ± 0.04	84.67 ± 1.05	585.43 ± 0.04	32.9 ± 0.1	36.39 ± 3.88	0.976 ± 0.10
0.2	2120.78 ± 0.5012	698.78 ± 0.49	63.91 ± 0.51	537.43 ± 0.55	1982.35 ± 0.82	644.3 ± 0.05	58.93 ± 0.46	557.1 ± 0.05	31.77 ± 2.5	64.91 ± 2.16	1.09 ± 0.03
0.4	1813.28 ± 0.520	576.28 ± 0.52	38.84 ± 0.59	298.92 ± 0.47	1955.62 ± 0.46	597.4± ± 0.07	40.26± ± 0.53	312.5 ± 0.07	16.90 ± ±3.4	67.40 ± 1.04	2.141 ± 0.02
0.6	1484.18 ± 0.523	398.18 ± 0.52	14.49 ± 0.61	201.91 ± 0.59	1880.02 ± 0.53	401.0± ± 0.05	21.76 ± 0.77	301.12 ± 0.04	26.8 ± 0.05	91.46 ± 0.82	3.01 ± 0.02
0.8	1280.40 ± 0.530	216.40 ± 0.53	14.06 ± 0.57	181.72 ± 0.52	1495.24 ± 0.52	335.0 ± 0.08	14.59 ± 1.54	101.70 ± 0.10	32.63 ± 1.22	98.71 ± 0.47	3.22 ± 1.01

TABLE 4. Relationship Between Various Feeding Parameters With The Concentrations Of The Extractive

Parameter	Correlation Coefficient (r)
Consumption	-0.9872**
Assimilation	-0.9986**
Production	-0.8728**
Consumption rate	-0.9598**
Assimilation rate	-1.0003
Production rate	-0.9782**
Assimilation efficiency	-0.8460*
Cross Conversion efficiency	-0.9360**
Net Conversion efficiency	-0.9066**

**= Indicate Significant at 10% level

* = Indicate Significant at 5% level



4. Adult moth developed from NDE treated third instar larva.



3. Deformed pupa with abnormally developed thorax compared with that of control pupa. (0.6% NDE treatment)

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Efficacy of Some Plant Products against Spotted Leaf Beetle (*Hadda* beetle), *Henosepilachna vigintioctopunctata* (F.) in Brinjal.

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ABSTRACT

Ten plant products were evaluated against *Henosepilachna vigintioctopunctata*. They were: *Azadirachta indica* (Neem) leaf extract (@ 5.0 %), *Calotropis gigantea* leaf extract @ 5.0 %, *Lantana camera* leaf extract @ 5.0 %, Neem cake extract @ 5.0 % neem oil @ 2.0 %, Nimbecidine® @ 2 ml /lit %, *Pongamia glabra* (Pungam) leaf extract @ 5.0 %, *Prosopis juliflora* L. leaf extract @ 5.0 %, *Vitex negundo* (Notchi) L. leaf extract (@ 5.0 %), and *Allium sativum* (Garlic) extract (@ 5.0 %. The standard check, carbaryl (Sevin 50 WP) (@ 0.1%) and an untreated check were included. The plant products were able to bring about higher reduction in population of *H. vigintioctopunctata* from 87.86 to 71.97 % on the third day after spray. However, the efficacy was reduced with the increase in days after spray. Higher reduction in population of *H. vigintioctopunctata* was observed in neem oil and was on a par with *C. gigantea*, Nimbecidine and *L. camera*; *P. glabra* neem cake extract and *V. negundo* stood next. However, the plant products were less effective than the standard check carbaryl but better than the untreated check.

INTRODUCTION

Under sustainable farming, brinjal provides regular daily income to meet the day-to-day expenditure like wages for the labour, service charges for the machinery etc. Among the list of insects troublesome to brinjal, the key ones are, fruit borer, *Leucinodes orbonalis* and spotted beetle popularly known as hadda beetle, *Henosepilachna vigintiocto punctata* (Feb). According to Kirtani (1979), ecofriendly less costly measures such as, cropping system approach, botanicals are more advantageous over insecticides, as they fit well in IPM.

METHODOLOGY

In the present investigation, fish oil rosin soap (FORS) and ten botanicals were evaluated – at Agricultural College and Research Institute (TNAU), Killikulam - for their efficacy against the hadda beetle *H. vigintioctopunctata* in brinjal. The standard check, carbaryl (Sevin 50 WP) (@ 0.1%) and an untreated check were included. The Brinjal variety KKM 1 was used in this experiment. 12 treatments were maintained with three replications. They were: T₁- *Azadirachta indica* A. Juss. leaf extract (5.0%), T₂- *Calotropis gigantean*. R.Br. leaf extract (5.0%), T₃- *Lantana camera* L. leaf extract (5.0%), T₄-Neem cake extract(5.0%), T₅-Neem oil(2.0%), T₆-Nimbecidine (2 ml l⁻¹), T₇- *Pongamia glabra* L.leaf extract(5.0%), T₈-*Prosopis juliflora* L.leaf extract (5.0%), T₉-*Vitex negundo* L.leaf extract(5.0%), T₁₀- Garlic (*Allium sativum* L.) extract(5.0%),

T₁₁-Carbaryl (Sevin 50 WP)(0.1%) and T₁₂-Untreated check. The treatments were applied as foliar spray.

Preparation of extracts

Leaves from various plants were collected and shade dried. When well dried, they were ground with a mixie. Then calculated quantity of well powdered leaf material was soaked in one-third of water and kept overnight. Stirring was done frequently. Then the material was filtered through a clean muslin cloth and the clear filtrate was mixed with the remaining two-third portion of water. Neem cake extract was also prepared in the same way. The observations on *H. vignitioctopunctata* were made on three, seven and 14 days after each spray. The number of grubs and adults of *H. vigintioctopunctata* were recorded from three leaves, one each from top, middle and bottom part of ten randomly selected plants; mean was worked out and expressed as number/three leaves. The fruit was harvested at seven days interval commencing from 60 DAT and continued up to 125 DAT. The yield in grams was recorded.

STATISTICAL ANALYSIS

The data gathered were transformed into angular or square-root values for statistical scrutiny, wherever necessary (Gomez and Gomez, 1984). The experiments were subjected to statistical scrutiny following the method of Panse and Sukhatme (1989) and Gomez and Gomez (1984)

Table 1. Efficacy of plant products against *H. vigintioctopunctata* population.

Treatment	Percent reduction in population			Mean
	Days after spray (DAS)			
	3	7	14	
T ₁	78.21 (62.34) ^{Aa}	60.84 (51.32) ^{Aa}	38.30 (38.18) ^{Aa}	59.06 (50.61) ^d
T ₂	86.77(69.97) ^{Aa}	70.22 (57.02) ^{Aa}	50.65 (45.38) ^{Aa}	69.22 (57.46) ^b
T ₃	85.19 (68.63) ^{Aa}	68.88 (56.56) ^{Aa}	48.21 (43.97) ^{Aa}	67.43 (56.39) ^b
T ₄	80.97 (64.89) ^{Aa}	63.78 (53.05) ^{Aa}	42.46 (40.64) ^{Aa}	62.21(52.86) ^c
T ₅	87.86 (70.77) ^{Aa}	70.31(57.07) ^{Aa}	51.52(45.67) ^{Aa}	69.77(57.84) ^b
T ₆	85.52(69.44) ^{Aa}	68.25(55.68) ^{Aa}	48.44(44.09) ^{Aa}	67.59(56.40) ^b
T ₇	81.68(65.19) ^{Aa}	64.06(53.36) ^{Aa}	41.86(40.30) ^{Aa}	62.37(52.95) ^c
T ₈	71.97(58.14) ^{Aa}	53.72(47.15) ^{Aa}	30.02(33.20) ^{Aa}	51.87(46.17) ^e
T ₉	78.48(62.56) ^{Aa}	62.86(52.52) ^{Aa}	40.97(39.78) ^{Aa}	60.39(51.62) ^{cd}
T ₁₀	78.20(63.17) ^{Aa}	60.04(51.16) ^{Aa}	36.65(37.21) ^{Aa}	58.74(50.51) ^d
T ₁₁	100.00(87.14) ^{Aa}	95.98(79.64) ^{Aa}	79.99(62.77) ^{Aa}	91.99(77.31) ^a
Mean	83.17(67.48) ^A	67.18(55.87) ^B	46.28(42.84) ^c	65.51(55.39)

Figures in parentheses are angular transformed values, in a column/row means followed by a common letter are not significantly different at 5% level (LSD).

and the means were compared with Least Significant Difference (L.S.D.).

RESULTS

The results of the investigation with ten plant products against Shoot and fruit borer, *L. orbonalis* are presented hereunder (Table 1 & 2). Variability in the population of *H. vigintioctopunctata* was significant among the treatments, periods of observation and spray rounds. Significant interaction could not be observed between treatment and spray round. The plant products were able to bring about higher reduction in population of *H. vigintioctopunctata* from 87.86 per cent to 71.97 per cent on third day after spray. However, the efficacy was reduced with the increase in days after spray. Overall treatment means indicated that the higher reduction in population of *H. vigintioctopunctata* was observed in neem oil (69.77 per cent) and was on a par with *C. gigantea* (69.22 per cent), Nimbecidine (67.59 per cent) and *L. camera* (67.43 per cent). *P. glabra* (62.37 per cent) neem cake extract (62.21 per cent) and *V. negundo* (60.39 per cent) stood next. However, the plant products were less effective than the standard check, carbaryl (91.99 per cent).

Fruit Yield

The plant products were able to increase the fruit yield significantly over untreated check; but the yield increase

was more than 2 t ha⁻¹, only in neem oil treated plots (Table 2). Neem oil recorded a fruit yield of 14.38 t/ha. Nimbecidine was the next best treatment with 13.99 t/ha.

Table 2. Effect of plant products on fruit yield

Treatment	Yield	
	T ha ⁻¹	Increase over UTC (%)
T ₁	13.05 ^g	6.44
T ₂	13.79 ^d	12.48
T ₃	13.54 ^e	10.44
T ₄	13.89 ^{cd}	13.31
T ₅	14.38 ^b	17.29
T ₆	13.99 ^c	14.11
T ₇	13.25 ^f	8.07
T ₈	12.51 ⁱ	2.04
T ₉	12.85 ^h	4.81
T ₁₀	12.95 ^{gh}	5.63
T ₁₁	16.89 ^a	37.77
T ₁₂	12.26 ^j	-
Mean	13.61	-
Significance	0.01	-
CD (P=0.05)	0.19	-

In a column/row means followed by a common letter are not significantly different at 5% level (LSD)

The yield in control plot was 12.26. The standard check carbaryl recorded 16.89 t/ha.

DISCUSSION

Brinjal is an important dietary vegetable crop and is scourged by a wide range of insect pests. In the recent years, the leaf beetle, *Henosepilachna vigintioctopunctata* (F.) is emerging as a serious pest. It damages the leaves as well as fruits; the scarifications made on the fruit surface reduce the market value. Usually the management of insect pests in brinjal has been insecticide-oriented. However, the obvious limitations and hazards associated with the insecticide applications restrict their use in pest control programmes. Evidently, the safer plant products proved useful in developing sound pest management strategies.

The studies on plant products revealed that some of the plant products were moderately effective in bringing down the damage by *H. vigintioctopunctata*, besides increasing the yield, though not as effective as that of the standard check carbaryl. Neem oil, Nimbecidine were consistently moderately effective.

Neem oil, *C. gigantea*, Nimbecidine, *L. camera*, *P. glabra*, neem cake extract and *V. negundo* were effective. Neem oil and Nimbecidine treated plots had higher yields too, at least two tones ha⁻¹ more. Several earlier workers have also demonstrated the effectiveness of neem oil against *H. vigintioctopunctata* (Mishra *et al.*, 1990; Udaiyan and Ramarathinam, 1994; Shanmugaraj, 1995), Nimbecidine (Udaiyan and Ramarathinam, 1994), *C. gigantea* against *H. vigintioctopunctata* (Rao *et al.*, 1990; Chitra *et al.*, 1992) and *L. camera* against *H. vigintioctopunctata* (Mehta *et al.*, 1995). The present investigation has brought out the efficacy of neem oil, Nimbecidine, *Calotropis gigantea*, *Lantana camera*, *Pongamia glabra*, neem cake extract and *Vitex negundo* against the spotted leaf beetle, *H. vigintioctopunctata* in Brinjal.

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Effect of Botanical Insecticides on Coffee Berry Borer, *Hypothenemus hampei* (Ferrari) (Coleoptera : Scolytidae)

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ABSTRACT

In a laboratory study with eight botanicals against the coffee berry borer, *Hypothenemus hampei* (F.) showed that neem oil (3%) was superior (78.67%) to others in bringing about berry borer mortality, followed by TNAU NO 60 EC A 3%, TNAU NO 60 EC (3%) and NSKE 5%. In the field studies, NSKE 5% was superior to others botanicals and was followed by TNAU NO 60 EC A 3%, TNAU NO 60 EC C 3% and neem oil 3%. It was concluded that the variation noticed in effectiveness of the botanicals in the laboratory and field experiments may be due to the rate of photodegradation.

Key words : Botanicals, neem oil, neem kernel extract, coffee berry borer, *Hypothenemus hampei*

INTRODUCTION

Coffee, being an important commercial crop, is of concern that it is affected by a number of pests (Le-Pelley, 1968). Of them the coffee berry borer, *Hypothenemus hampei*, a native of Central Africa, is the most dreaded one in many of the coffee growing countries. In India, it was first noticed in February 1990 in a few plantations in Gudalur in the Nilgiris District of Tamil Nadu (Kumar *et al.*, 1990). It is now prevalent in coffee growing areas of South India. It can cause 30-80 per cent damage to berries resulting in heavy crop loss.

Application of pesticides for the control of berry borer, though widely practised, is discouraged to avoid pesticide residues in coffee bean and the adverse effect on natural enemies of the berry borer and other beneficial fauna. Hence, there is an urgent need to develop an eco-friendly and effective insect pest management system in coffee. The present study was conducted to evaluate eight botanicals against the coffee berry borer.

MATERIAL AND METHODS

Laboratory Evaluation

Eight botanicals *viz.*, neem (*Azadirachta indica* A.Juss) seed kernel extract (NSKE) @ 5%, neem oils (Commercial, TNAU NO 60 EC A & TNAU NO 60 EC C) (3%), acorus (*Acorus calamus* L.) extract (3%), pungam (*Pongamia glabra* Vent.) oil (3%), illuppai (*Madhuca latifolia* Gmel.) oil (3%), notchi (*Vitex negundo* L.) leaf extract (5%), TNAU NO 60 EC A (3%) (*A. indica*) and TNAU NO 60 EC C (3%) (*A. indica*). Chlorpyrifos 20 EC @ 3 ml/lit was the standard check. An untreated check was maintained. Each treatment was replicated thrice.

Effect of Spray on Coffee Berries

One hundred coffee berries of uniform size were collected and were sprayed with the botanicals at stipulated

concentration using a hand-operated atomizer. Water spray on berries was considered as untreated check. The treated berries were kept over moistened filter paper kept in a plastic container and covered with muslin cloth. The entire set up was replicated thrice. Hundred pre-starved (24 hrs.) beetles were released into each container and the number of berries bored was recorded on the 3rd, 5th, 7th and 10th day after release; per cent infestation was worked out.

Effect of Direct Spray on Adult Berry Borer

In another experiment, botanicals were sprayed on the berry borer beetles (50 numbers), using a hand-operated atomizer. The beetles were provided with bean scrapings as food. Mortality was recorded on the 3rd, 5th, 7th and 10th day after treatment and the corrected per cent mortality was calculated.

Field Evaluation

Two field experiments were conducted at the Regional Coffee Research Station (RCRS), Thandigudi during July 2004 and 2005 in randomized block design (RBD) using the arabica variety Sln.7-3 (S.3708). The plots had coffee plants of eighteen years old with coffee berry borer infestation. The nine treatments as in the laboratory studies and an untreated check were imposed. Each treatment was replicated thrice. The treatments were imposed using a hand-operated knapsack sprayer of 16 litres capacity. Berry borer- infested-plants were tagged using coloured cloth for each treatment. Three rounds of application were done; first one on the day when initial infestation was recorded, followed by second and third on 21st and 42nd day after the first spray. Teepol 0.1 per cent was added as a spreader in all the spray treatments. The total number of berries and number of berries showing

Table 1. Effect of botanicals against coffee berry borer in the laboratory

Treatments	Per cent reduction over untreated check * (Days after application)				
	3	5	7	10	Mean
T1	76.04 ^d	74.67 ^d	71.33 ^e	67.51 ^e	72.38 ^e
T2	81.25 ^b	79.33 ^b	78.84 ^c	77.16 ^b	78.67 ^b
T3	47.92 ^e	48.00 ^e	60.53 ^f	41.12 ^f	46.37 ^f
T4	41.67 ^f	42.67 ^f	48.00 ^g	33.81 ^g	38.81 ^g
T5	31.25 ^h	38.00 ^g	38.33 ^h	28.83 ^h	32.21 ^h
T6	39.38 ^g	34.67 ^h	35.56 ⁱ	26.90 ⁱ	31.39 ⁱ
T7	82.29 ^b	78.67 ^b	91.45 ^b	74.37 ^c	76.91 ^c
T8	78.13 ^c	76.00 ^{cd}	73.62 ^d	72.59 ^d	74.57 ^d
T9	98.96 ^a	97.67 ^a	97.42 ^a	96.45 ^a	97.41 ^a

In a column, means followed by a common letter (s) are not significantly different by DMRT (P=0.05)

T1 = Neem seed kernel extract (5%), T2= Neem oil (3%), T3= Acorus extract (3%), T4= Pungam oil (3%), T5= Illuppai oil (3%), T6= Notchi leaf extract (5%), T7 = TNAU NO 60 EC A (3%), T8= TNAU NO 60 EC C (3%), T9= Chlorpyrifos (0.06%)

bore hole were recorded from two secondary branches of each of the five treated plants per replication on the 5th, 10th, 15th and 20th day after each round of treatment. The per cent berry borer infestation was computed. Data collected from various laboratory and field experiments were analysed using standard statistical procedures. The percentage values were subjected to angular or *arc - sine* transformation; square root transformation was followed

for converting the population / numbers. The treatment means were compared using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1985).

RESULTS AND DISCUSSION

The results of the laboratory and field experiments conducted with eight botanicals against coffee berry borer are presented hereunder (Tables 1-4).

Table 3. Field evaluation of selected botanicals against coffee berry borer (Season 2004-05)

Treatments	Per cent reduction over untreated check* (Days after spraying)															
	First spray					Second spray					Third spray					Over all mean
	5	10	15	20	Mean	5	10	15	20	Mean	5	10	15	20	Mean	
T1	30.82 ^b	45.79 ^b	49.73 ^b	43.39 ^b	42.66 ^b	58.36 ^b	66.34 ^{ab}	75.03 ^b	75.03 ^b	69.07 ^b	80.21 ^b	87.31 ^b	92.19 ^b	94.28 ^b	88.57	66.77 ^b
T2	21.69 ^d	29.84 ^e	39.68 ^e	43.29 ^{bc}	34.18 ^c	48.97 ^d	56.09 ^{bc}	65.78 ^c	62.19 ^e	58.59 ^d	69.23 ^d	74.88 ^d	82.83 ^d	88.53 ^c	78.96 ^e	57.24 ^e
T3	16.07 ^e	26.96 ^f	40.07 ^d	30.62 ^f	28.75 ^c	44.95 ^f	53.42 ^{bc}	62.62 ^f	59.12 ^{gh}	55.36 ^e	63.11 ^e	65.89 ^e	65.77 ^e	66.36 ^d	65.28 ^f	49.80 ^f
T4	10.08 ^a	18.13 ⁱ	30.24 ^h	30.32 ^f	22.72 ^d	41.41 ^g	50.31 ^{bc}	55.12 ⁱ	60.63 ^f	52.25 ^g	60.11 ^f	63.46 ^e	66.15 ^e	64.96 ^d	63.68 ^f	46.22 ^f
T5	16.00 ^e	24.89 ^g	34.91 ^g	34.84 ^{de}	28.15 ^d	45.79 ^e	54.04 ^{bc}	57.09 ^g	62.82 ^e	55.28 ^e	61.25 ^e	65.14 ^e	65.77 ^e	65.40 ^d	64.39 ^f	49.27 ^f
T6	16.25 ^e	23.07 ^h	29.80 ⁱ	31.86 ^{ef}	25.65 ^d	45.62 ^e	53.67 ^c	56.78 ^h	58.15 ^h	53.81 ^f	62.06 ^e	64.58 ^e	65.22 ^e	64.37 ^d	64.05 ^f	47.84 ^f
T7	26.97 ^c	39.02 ^c	44.57 ^c	40.56 ^{bc}	38.06 ^b	52.10 ^c	60.84 ^b	70.56 ^c	70.59 ^c	63.95 ^c	75.99 ^c	82.74 ^c	88.41 ^c	90.96 ^c	84.60 ^c	62.20 ^c
T8	26.73 ^c	30.78 ^d	38.47 ^f	37.08 ^{cd}	33.57 ^c	51.12 ^c	56.29 ^{bc}	67.56 ^d	64.18 ^d	60.11 ^c	69.95 ^d	75.85 ^d	85.43 ^{cd}	89.26 ^c	80.23 ^d	59.97 ^d
T9	38.89 ^a	50.21 ^a	71.35 ^a	78.58 ^a	60.83 ^a	86.58 ^a	91.39 ^a	95.06 ^a	96.31 ^a	92.55 ^a	98.10 ^a	100.00 ^a	100.00 ^a	100.00 ^a	99.55 ^a	4.315 ^a

In a column, means followed by a common letter (s) are not significantly different by DMRT (P=0.05)

Table 2. Effect of botanicals on the mortality of coffee berry borer (In laboratory condition)

Treatments	Corrected per cent mortality + (Days after application)				Mean
	3	5	7	10	
T1	7.15 (15.50) ^d	19.25 (26.02) ^e	42.00 (40.39) ^d	55.66 (48.25) ^d	31.01 (32.54) ^d
T2	8.50 (16.85) ^b	25.25 (30.16) ^b	50.00 (45.00) ^b	66.00 (54.33) ^b	37.43 (36.61) ^b
T3	4.10 (11.68) ^h	14.10 (22.05) ^g	20.31 (26.78) ^{ef}	29.30 (32.77) ^e	16.95 (23.32) ^f
T4	3.00 (9.97) ⁱ	12.30 (20.53) ^h	18.00 (25.10) ^{fg}	25.00 (30.00) ^f	14.51 (21.4) ^h
T5	4.50 (21.4) ^g	12.40 (20.61) ^h	17.10 (24.42) ^g	26.00 (30.65) ^f	15.00 (21.98) ^g
T6	5.00 (12.92) ^f	15.31 (23.03) ^f	22.20 (28.11) ^e	31.30 (34.01) ^e	18.45 (24.51) ^e
T7	6.75 (15.05) ^e	20.25 (23.74) ^d	46.00 (42.70) ^c	61.00 (51.35) ^c	33.50 (33.21) ^c
T8	8.00 (16.43) ^c	22.00 (27.97) ^c	45.35 (42.33) ^c	57.45 (49.28) ^d	33.20 (34.00) ^c
T9	24.00 (29.33) ^a	64.75 (53.58) ^a	100.00 (89.71) ^a	100.00 (89.71) ^a	72.18 (65.58) ^a

+ Data corrected by using Abbott's formula

Figures in parentheses are arcsine transformed values

In a column, means followed by a common letter (s) are not significantly different by DMRT (P=0.05)

Laboratory Studies

Effect of Spray on Coffee Berries

Of the eight botanicals tested, neem oil 3% was found to be the most effective one in inducing mortality of the berry borer. The mortality was 77.16 to 81.25 per cent at all the intervals of observation with a mean of 78.67 per cent mortality over control. The other botanicals in the descending order of efficacy were TNAU NO 60 EC A 3%, TNAU NO 60 EC C 3% and NSKE 5% with corresponding mean per cent mortality of 76.91, 74.57 and 72.38 per cent, respectively (Table 1). Earlier Vijayalakshmi (2000) recorded that the extract of garlic and kemisal (neem product) were found effective in bringing about 90-95 per cent mortality when tested under laboratory conditions but not so promising in the field. Saravanan and Chozhan (2003) reported that 0.05% spray of neem-methanol extract was effective in controlling coffee berry borer.

Effect of Direct Spray on Adult Berry Borer

Evaluation with direct sprays revealed that chlorpyrifos 0.06% was the most effective with the highest mortality of 72.18 per cent over untreated check (Table 2), followed by neem oil (37.43%), TNAU NO 60 EC A (33.50%), TNAU NO 60 EC C (33.20%) and NSKE (31.01%). Sreedharan *et al.* (2001) earlier has also reported that the maximum mortality of coffee berry borer adults recorded was 38 per cent with the neem formulation of Achook 2 per cent.

Field Evaluation

The data on the per cent reduction in berry borer infestation due to different treatments with botanicals and standard check during 2004-05 and 2005-06 are presented in the Tables 3 and 4 respectively. During 2004-05 spray

with chlorpyrifos (0.06%) was, as expected, superior in reducing the berry borer infestation by 60.83, 92.55 and 99.55 per cent, after first, second and third sprays, respectively. Among the botanicals tested, NSKE 5% was the best significantly reduced infestation of berry borer with a mean per cent reduction of 42.66, 69.07 and 88.57 after first, second and third sprays, respectively. The overall mean per cent reduction in infestation of berry borer after the first, second and third spray was 84.31 in chlorpyrifos treatment. This was followed by NSKE, TNAU NO 60 EC A, TNAU NO 60 EC C and neem oil (3%) with corresponding per cent reduction of 66.77, 62.20, 59.97 and 57.24 (Table 3). The trend was similar in the second season (2005 – 06) trial too (Table 4). The overall data of two seasons revealed that chlorpyrifos treatment was the best in reducing the berry borer infestation with a reduction of 83.48 per cent over untreated check. Among the botanicals tested, NSKE was the best with 65.15% reduction followed by TNAU NO 60 EC A, TNAU NO 60 EC C and neem oil 3% with per cent reduction of 60.85, 58.33 and 56.56 per cent respectively. The least reduction of infestation was recorded from pungam oil (40.39%) and notchi leaf extract (40.77%); they were on a par with each other. Illupai oil (44.02%) and acorus extract (44.40%) were better than the former ones (Table 4).

In the present investigation, among the eight botanicals tested, NSKE (5%) proved to be the best recording highest per cent (65.15%) reduction in berry borer infestation. Neem oil (3%) was also good as it caused 56.56 per cent reduction, but next to TNAU NO 60 EC A (3%). This may be due to the repellent and antifeedant properties of neem products. However, the variation noticed in the

Table 4. Field evaluation of selected botanicals against coffee berry borer (Season 2005-06)

Treatments	Per cent reduction over untreated check* (Days after spraying)															
	First spray					Second spray					Third spray					Over all mean
	5	10	15	20	Mean	5	10	15	20	Mean	5	10	15	20	Mean	
T1	31.11 ^b	38.95 ^b	52.30 ^b	44.49 ^b	42.32 ^b	53.52 ^b	68.75 ^b	69.69 ^b	67.43 ^b	64.99 ^b	77.61 ^b	87.37 ^b	92.49 ^b	94.60 ^b	88.13 ^b	
T2	24.25 ^d	26.71 ^e	42.76 ^e	34.48 ^e	33.12 ^e	49.22 ^d	53.83 ^e	65.26 ^c	57.15 ^e	56.53 ^e	65.46 ^e	80.34 ^e	86.24 ^e	87.26 ^e	80.03 ^e	56.56 ^e
T3	9.74 ^h	16.22 ^f	32.69 ^f	27.75 ^f	22.79 ^f	38.75 ^e	47.34 ^f	52.26 ^d	48.41 ^f	46.83 ^f	55.92 ^f	62.35 ^f	68.67 ^f	66.93 ^f	63.60 ^f	44.40 ^f
T4	13.17 ^f	9.37 ^h	28.27 ^h	23.75 ^g	19.96 ^h	32.94 ^g	41.70 ^g	48.66 ^e	43.91 ^h	41.99 ^h	49.65 ^g	41.33 ^g	65.05 ^g	62.91 ^h	59.21 ^h	40.39 ^h
T5	16.10 ^e	13.64 ^g	30.29 ^g	27.23 ^f	25.15 ^f	37.05 ^e	46.00 ^f	51.09 ^d	46.78 ^{fg}	45.39 ^g	50.65 ^g	61.73 ^f	67.71 ^f	65.33 ^g	61.51 ^g	44.02 ^g
T6	11.73 ^g	7.78 ^h	25.87 ⁱ	20.98 ^h	18.68 ^h	35.36 ^f	41.05 ^g	48.99 ^e	45.32 ^{gh}	42.82 ^h	49.17 ^g	60.30 ^f	67.28 ^f	65.93 ^g	60.80 ^h	40.77 ^h
T7	29.92 ^b	34.58 ^c	47.98 ^c	39.79 ^c	39.24 ^c	51.65 ^c	58.04 ^c	65.88 ^c	60.55 ^c	59.10 ^c	70.12 ^c	83.65 ^c	90.62 ^c	91.75 ^c	84.20 ^c	60.85 ^c
T8	25.19 ^c	30.96 ^d	45.77 ^d	35.22 ^d	34.29 ^d	49.86 ^d	56.49 ^d	65.55 ^c	59.14 ^d	57.92 ^d	68.59 ^d	82.58 ^d	88.59 ^d	90.76 ^d	82.78 ^d	58.33 ^d
T9	37.58 ^a	60.77 ^a	70.08 ^a	72.44 ^a	60.96 ^a	81.15 ^a	88.69 ^a	94.76 ^a	96.16 ^a	90.35 ^a	96.86 ^a	99.51 ^a	100.0 ^a	100.0 ^a	99.12 ^a	83.48 ^a

* Each value is the mean of three replications

In a column, means followed by a common letter (s) are not significantly different by DMRT (P=0.05)

effectiveness of NSKE 5% and neem oil 3% on coffee berry borer in the field and laboratory studies may be attributed to photodegradation. This is in corroboration with the findings of Lagunes *et al.* (1998) who found that 2% and 5% aqueous extracts of neem seeds registered 73 and 75 per cent reduction of coffee berry borer infestation respectively. As the botanicals are less toxic than the recommended insecticide, they do not bring about a sudden reduction of the population. The present study demonstrates the value of using botanicals in population regulation of the coffee berry borer.

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Botanical treatment for grain protection and their effects on Seed Germination and Seedling Performance of stored maize

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Apart from being known for having insecticidal activity, several plants and their materials also show effects on seed viability and plant growth. The crude acetone extracts from the seeds of *Piper cubeba* L, coriander, *Coriandrum sativum* L. and the shade dried leaves of the aquatic weed, *Eichhornia crassipes* Mart., wood-apple, *Limonia acidissima* L, the Indian Tamarind, *Tamarindus indica* L., the coconut palm, *Cocos nucifera* L., Indian Badam, *Terminalia catappa* L., the Indian Cherry *Syzygium cumini* L. and Ivy Gourd, *Coccinia indica* Wight & Arn. Were evaluated for finding their influence on germination capabilities and seedling growth of treated *Zea mays* L. seeds. The effect of extract on germination, root and shoot growth and inhibition of seed borne fungi *Aspergillus flavus* have been observed in Petri dish bioassays at a concentrations of 0.5, 1.25 and 2.5 mg /g maize seed. In general the extracts had no adverse effects on seed germination and the fungal growth was also quantitatively similar to that of the controls. Present investigation reveals the importance and potential of plant extracts and their allelopathic effects on stored grain.

Key words: Plant products, Seed germination, Seedling growth, Antifungal.

INTRODUCTION

In India maize is stored for longer period for seed purpose and during this period it is vulnerable to pest and disease infestations. The application of naturally occurring plant products, which are ecologically friendly to agricultural practice, is a promising method of stored product pest control in recent years. Several botanical extracts has been proved to prevent or regulate the pest and disease attack (Usha Rani 2007). Essential oils of thyme and oregano are effective fumigants against fungi, which attack stored grains. This property strengthens the probability of their use as alternative chemicals in the storage of grains. Certain plant essential oils and their components showed inhibitory activity to *A. flavus* in Maize (Belmont, and Carvajal, 1998).

Particularly in the event of methyl bromide ban/ going to ban in several countries there is a growing need for the development and use of natural pesticides. One of the major disadvantages of the use of plant based insect control agents on stored food commodities is their possible adverse effect on seed germination. According to Jood *et al.* (1993) the sorghum grains treated with plant products were normal in colour, appearance and texture after 6 months, but their seed viability and seedling growth adversely affected by either insect infestation or plant products. Majority of plant extracts are safe and a careful utilization of these compounds on food commodities is beneficial in several ways. The plant based chemicals when used as either fumigants or contact toxicants often show high toxicity besides being economical and environmentally safe as well as biodegradable. However, studies are needed to explore the adverse effects if any associated with the use of the botanical pesticides, such

as germination capacity, nutritional value and seedling growth of the treated grain are essential before recommending their use.

In the present study experiments were conducted to evaluate the impact of allelopathic potential of certain plant crude extracts on germination, growth and fungi associated with the germination of maize (*Zea mays* L.). The effects of nine plant extracts on maize kernel protection against *Aspergillus flavus* were studied. Tests were conducted to determine optimal levels of dosages for maize protection, residual effects and toxicity of the extracts to maize plants.

MATERIALS AND METHODS:

Plant extract

Laboratory experiments were conducted to evaluate the effects of various botanical insecticides on the seed germination and seedling performance of stored maize. The plant extracts used were, seed extract of tail pepper, *Piper cubeba* L and the mature leaves of the aquatic weed common water hyacinth, *Eichhornia crassipes* Mart., the dog wood apple, *Limonia acidissima* L, the Indian Tamarind, *Tamarindus indica* L., the coconut palm, *Cocos nucifera* L., Indian Badam, *Terminalia catappa* L., the Indian Cherry *Syzygium cumini* and the Kovai Fruit *Coccinia indica* Wight & Arn. and the seed oil of coriander, *Coriandrum sativum* were harvested from naturally growing population in the campus of the Indian Institute of Chemical Technology, Hyderabad, India. The plant materials were shade dried at room temperature $28\pm 4^\circ\text{C}$ for 72 h, grounded in an electrical mixer-grinder, and extracted with soxhlet using acetone as solvent. The resulting crude extract is concentrated in a rotary

Table 1. Allelopathic effects of various plant extracts on shoot and root length of treated *Zea mays* (10 day old) at different concentrations.

Test Species	Mean (%) inhibition ^a					
	Shoot growth			Root growth		
	0.5 ^b	1.25 ^b	5.0 ^b	0.5 ^b	1.25 ^b	5.0 ^b
<i>E. crassipes</i> (root)	5.77	0.98 a	6.73	6.66	1.14	17.79
<i>E. crassipes</i> (leaf)	6.73	1.93 a	3.85	5.64	2.84	1.42
<i>L. acidissima</i>	6.03	1.21 a	3.61	4.63	1.56	10.76
<i>S. cumini</i>	10.49	2.87 ac	-2.87	35.74	39.30	8.88
<i>T. indica</i>	0.98	4.91 c	19.53	3.07	4.05 a	76.77
<i>P. cubeba</i>	31.78	14.18	3.33	21.94	15.17	9.07
<i>C. nucifera</i>	15.40	17.61	23.21	-13.07	-32.08	2.07
<i>T. catappa</i>	7.45	10.75	14.90	-32.32	-41.51	-4.61
<i>C. indica</i>	22.13	3.16 a	2.11	6.15	4.07 a	30.61
<i>C. sativum</i>	22.47	4.62 c	0.05	3.15	10.28	14.91

^a Each datum represents the mean percentage inhibition = [(control-extracts)/control] X 100

Values followed by the same letter within a column are not significantly different (P < 0.05, tukey test). (N=10/replicate, total 10 replicates for each treatment).

^b indicate the extracts treated at mg/g concentration.

- negative inhibition.

evaporator, re-dissolved in a known amount of acetone to obtain a 50% stock solution. The extracts were stored in the refrigerator for subsequent experiments. Further dilutions were made to prepare test solutions (w/v) just before their use in the experiments.

Seeds of maize (DM 5000) were provided by Directorate of Maize Research (Indian Council of Agricultural Research), Hyderabad.

Seed germination and incidence of seed borne fungi bioassays

The viability of treated and control seeds were tested one day, ten days, 1 month and 6 months after application. For this assay, maize seeds were separately treated with different plant extracts, at the rate of 0.5, 1.25 and 5 mg per gm seeds. The control seeds were treated with the solvent. The seeds were air-dried for 2–3 hours. Then 25 seeds from each treatment and control group were placed separately in plastic containers and stored under laboratory conditions for 6 months. There were 4 replicates for each treatment. Simultaneously incidence of the seed borne fungi was tested for each treatment. The germination of treated seeds and incidence of the seed borne fungi were evaluated after one day, ten days, one month and six months for each treatment. Each group of seeds was placed on moist filter paper in Petri dishes. Plates were incubated in a growth chamber at 25°C under 10 h light periods daily. After 7 days, seed germination and incidence of seed borne

fungi were measured and compared to that of controls. The inhibition percentage calculated by using following formulae. Inhibition percentage (%) = [(control-extracts)/control] X 100

Seedling growth:

The effect of prolonged botanical treatment on seedling growth of the maize grain was evaluated at one day, 10 days, one month and six months after treatment.

The treated maize grains were germinated on the moist filter paper for 24 hours as described earlier. 10 uniform seedlings were selected and transferred into the petri dish (150 X 30 mm) lined with two sheaths of moist whatman No# 1 filter paper. The seedlings were grown for seven days in a growth chamber at 25 °C under 10 h light periods daily. After 7 days, shoot and root length of the treated seeds were measured and compared with that of controls. The inhibition was calculated by using the following formulae:

Inhibition percentage (%) = [(control-extracts)/control] X 100.

Experimental design and analysis

Repeated measurements on each variable were obtained from the same experimental units over time. All the data collected were first homogenised using appropriate logarithmic and square root transformations (Gomez and Gomez, 1984) before being subjected to analysis of variance (ANOVA), repeated measures analysis and means separated using Tukey's test.

Table 2. Allelopathic effects of various plant extracts on shoot and root length of treated *Zea mays* (one month old) at different concentrations.

Test Species	Mean (%) inhibition ^a					
	Shoot growth			Root growth		
	0.5 ^b	1.25 ^b	5.0 ^b	0.5 ^b	1.25 ^b	5.0 ^b
<i>E. crassipes</i> (root)	16.00	4.00	14.00	2.94	35.29 c	5.88 b
<i>E. crassipes</i> (leaf)	2.00	3.00 a	11.00	36.76	63.24	55.88
<i>L. acidissima</i>	11.97	11.11	39.32	-8.33	33.33 b	29.17 b
<i>S. cumini</i>	0.00	100.00	100.00	1.89 a	100.00	100.00
<i>T. indica</i>	7.69 a	17.09	18.80	2.00 a	18.00	6.00 a
<i>P. cubeba</i>	22.79 b	27.94	1.47 a	57.14	34.69 c	30.61 b
<i>C. nucifera</i>	4.48	30.60	2.24 b	5.88	9.80	37.25
<i>T. catappa</i>	23.00 b	5.00 b	3.00	25.00	33.33 b	10.42
<i>C. indica</i>	36.90	2.38 a	-4.76	-2.44	21.95 a	7.32 a
<i>C. sativum</i>	7.69 a	5.77 b	1.92 ab	5.00	22.50 a	0.00

^a Each datum represents the mean percentage inhibition = [(control-extracts)/control] X 100

Values followed by the same letter with in a column are not significantly different (P < 0.05, tukey test). (N=10/ replicate, total 10 replicates for each treatment).

^b indicate the extracts treated at mg/g concentration.

- negative inhibition.

RESULTS

Seed germination and Incidence of seed borne fungi

Almost all the plant extracts utilized in the experiments were effective in promoting the seed germination and none of the extracts hindered the germination capacity of the treated maize seeds. There was significant difference between the percentage seed germinated in treated and controls. In one day stored seeds, very few botanicals like *E. crassipes*, *L. acidissima*, *S. cumini*, *C. sativum* and *C. nucifera* showed the inhibition at higher concentrations. The negative effects of the treated botanicals on seed germination is highly insignificant in this investigation. Irrespective of concentration, all the treated botanicals either promoted or did not effect the seed germination for 10 days, 1 month and 6 months after treatment in stored maize.

In parallel to seed germination, the incidence of seed borne fungi was also studied. One day old seeds, treated with *E. crassipes* root and leaf extracts showed significant suppression of the incidence of seed borne fungi at all concentrations. *S. cumini* and *C. sativum* caused a total inhibition of fungal development on maize kernels. A residual effect of these two extracts were detected up to 6 months. No phytotoxic effect on germination and growth was found with *C. sativum* and *S. cumini* after one month of treatment. *T. indica* and *P. cubeba* at optimal protective dosage of 0.5 mg/g (v/v) was fungistatic on *A. flavus* with a residual effect that lasted for 6 months.

L. acidissima showed inhibition of incidence of seed borne fungi at lower doses only without effecting the seed germination. The effects produced due to the treatment were similar and comparable to that of the controls and

suggest that the extracts did not have any adverse action against the average seed germination.

Seedling growth- Shoot growth

In 10-day-old treated seeds, crude extracts of botanicals caused a mild inhibition of shoot growth. The degree of inhibition was a function of extract concentration. The percentage of shoot growth inhibition ranges from 0.96 to 30% in maize seedlings. *P. cubeba* showed significant inhibition even at lower concentrations and the least inhibition was found in *E. crassipes* root treatment at 1.25 mg/g (Table 1). The lower extract concentrations of *S. cumini*, *C. indica* and *C. sativum* (0.5 mg / g) caused inhibition of the shoot growth, while at higher concentrations their inhibition was insignificant (20 %) as shown in the table 1. The *T. catappa* and *C. nucifera* extracts exhibited dose dependent shoot growth inhibition and their inhibition increased with the increase of concentration.

In one month treated seeds, the inhibitory potential of the extracts, however, was found to vary with the specific species (Table 2). There was no effect on shoot growth at lower concentration of *S. cumini* extract; whereas, at higher concentrations (1.25 and 5 mg / g) extract completely inhibited the growth (Table 2).

The lengths of the maize shoot were inhibited significantly by all the applied concentrations of *T. indica* extracts and were in a concentration – dependent manner. *E. crassipes* root, *T. catappa* and *C. indica* showed greatest inhibition at lower concentrations and their inhibition effect decreased with increase in concentration (Table 2). *C. nucifera* and *P. cubeba* showed high shoot growth

Table 3. Allelopathic effects of various plant extracts on shoot and root length of treated *Zea mays* (six months old) at different concentrations.

Test Species	Mean (%) inhibition ^a					
	Shoot growth			Root growth		
	0.5 ^b	1.25 ^b	5.0 ^b	0.5 ^b	1.25 ^b	5.0 ^b
<i>E. crassipes</i> (root)	16.00	4.00	14.00	2.94	35.29 c	5.88 b
<i>E. crassipes</i> (root)	0.5 ^b	1.25 ^b	5.0 ^b	0.5 ^b	1.25 ^b	5.0 ^b
<i>E. crassipes</i> (leaf)	3.73	1.50 ab	0.68	6.48 b	2.35 a	5.77 a
<i>L. acidissima</i>	-61.10	-22.34	-32.27	6.01 b	6.90	-33.61
<i>S. cumini</i>	1.85 cd	-3.09 bc	18.00 c	-33.25	-28.48	-2.51
<i>S. cumini</i>	0.37 a	21.22	38.55	-2.53 a	0.89	17.56 c
<i>T. indica</i>	0.79 ab	3.79 c	4.37 a	-1.62 a	-21.61	-39.28
<i>P. cubeba</i>	1.48 bc	1.39 a	4.75 a	0.67	4.05	14.63 b
<i>C. nucifera</i>	1.95 cd	11.00 d	11.90 b	4.14	13.95 b	16.03 bc
<i>T. catappa</i>	9.76	11.77 a	17.76 c	2.09	14.39 b	11.63
<i>C. indica</i>	2.33 d	0.94 a	16.11	-15.41	8.75	6.59 a
<i>C. sativum</i>	0.46 a	10.47 d	12.19	6.67 b	2.31 a	6.88 a

^a Each datum represents the mean percentage inhibition = [(control-extracts)/control] X 100

Values followed by the same letter with in a column are not significantly different (P< 0.05, tukey test). (N=10/ replicate, total 10 replicates for each treatment).

^b indicate the extracts treated at mg/g concentration.

- negative inhibition.

inhibition at 1.25 mg / g while least inhibition observed in 0.5 and 5 mg / g concentrations (Table 2). *L. acidissima* extract was comparatively less toxic, with only the highest concentration of 5 mg / g significantly suppressing the shoot growth (Table 2)



Fig.1: Effect of botanical extracts on maize seedling growth of 10 days old treated *Zea mays*: A, Control maize seedling; B, Root and shoot inhibition due to *C. indica*; C, Root growth promoted due to *C. nucifera*; D, Seedling growth retardation due to *P. cubeba*.,

The botanicals had negligible effect on the shoot growth of 6 months old treated seeds and the inhibition ranged from 0.68 to 38.55 %. The greatest inhibition was found with the extract of *S. cumini* (Fig 2 d) at 5 mg / g and the least was caused by *E. crassipes* root at 1.25 mg/g. The leaf extracts of *E. crassipes* promoted the shoot growth at all the concentrations tested (Fig 2 b), while all other plant extracts inhibited it in a concentration dependent manner.

Root growth

When *C. nucifera* and *T. catappa* treated seeds after 10 days when allowed to germinate they promoted the root growth ranging from 13 to 41 % at the lower concentrations tested (0.5 and 1.25 mg / g) (Fig 1 c). The length of the maize root was inhibited significantly by all the applied concentrations of *E. crassipes* root, *L. acidissima*, *C. indica*, (Fig 1 b) *C. sativum* and *T. indica* extracts and these extracts increased the root growth inhibition in a concentration –dependent manner. In contrast, *S. cumini* and *P. cubeba* (Fig 1 d) extracts decreased the root growth inhibition in a concentration–dependent manner. Greatest root growth inhibition was observed in *T. indica* (76.77%) followed by *C. indica* (30.61%) and the least inhibition was found in *E. crassipes* root (1.14%) as shown in the table 1.

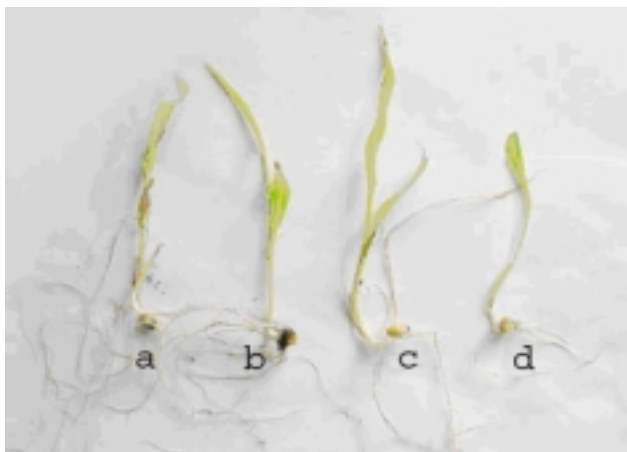


Fig. 2: Effect of botanical extracts on maize seedling growth of 1 month old treated *Zea mays*: a, control maize seedling; b, root growth promoted due to *L. acidissima*; c, root and shoot promoted due to *C. indica* leaf; d, growth retardation due to *E. crassipes* leaf extract.

In one month treated grain, botanical extracts showed significantly high toxicity and root growth inhibition ranging from 2% to 100% (Table 2). There was no effect on root growth at lower concentration of *S. cumini* extract, whereas, at higher concentrations (1.25 and 5 mg / g) the extract had completely inhibited the root growth. Similar results were obtained with *E. crassipes* leaf extract, which showed greater inhibition at higher concentrations (Table 2). However leaf extract of *E. crassipes*, *P. cubeba* and *T. catappa* caused inhibitions even at lower concentrations (Table 2). A distinct response was observed in botanical extracts of *E. crassipes* leaf (Fig 2 d), *T. indica*, *L. acidissima* (Fig 2 b), *T. catappa*, *C. indica* (Fig 2 c) and *C. sativum* plants. At 1.25-mg/ g, these extracts inhibited the root growth, while at 0.5 and 5 mg/ g, the inhibition was insignificant.

In six months treated seeds, the effect of botanicals on the root growth was negligible. A distinct response was observed with *E. crassipes* leaf extract treatment. At higher concentration, the extract had promoted the shoot growth, while at lower concentrations, the extract inhibited the root growth (Table 3). In contrast, *S. cumini* and *C. indica* promoted the shoot growth at lower concentration and inhibited at the higher concentrations tested (Table 3). The extracts of *P. cubeba*, *C. nucifera* and *T. catappa* increased the root growth inhibition in a concentration-dependent manner. *L. acidissima* (Fig 3 c) and *C. indica* promoted significant root growth at all concentrations.



Fig. 3: Effect of botanical extracts on maize seedling growth of 6 months old treated *Zea mays*: a, Control maize seedling; Root and shoot growth promoted due to treated with b, *E. crassipes* leaf; c, *L. acidissima*; d, seedling inhibition due the treatment of *S. cumini* leaf extract

DISCUSSION

From the results of these trials, it was found that the use of treated botanical extracts as natural pesticides in maize storage have negligible adverse effects on seed germination. The botanical treatments caused only marginal changes in seed viability, which should have virtually no impact on the local market value of treated grain.

Results indicate that the seed viability of treated seeds was influenced by the type of plant extract treatment and storage duration. *E. crassipes*, *L. acidissima*, *S. cumini* and *C. nucifera* inhibited the germination at higher concentrations in the grain after 24 hours post treatment. This finding is supported by Sundarraj *et al.* 1996, who found that the degree of inhibition increased at higher concentrations.

The storage of maize seeds up to a prolonged period such as 6 months after treating with various botanicals does not have any adverse effect on the seed viability, which is an important aspect of use of botanicals for storage pests. This concurs with, Pandey *et al.* (1986) and Kasa and Tadese (1995) who reported that the use of crude powders of 17 botanical plant species on sorghum had no effect on seed germination.

The experimental results on seed borne fungus indicate that certain extracts are promising in inhibiting the seed borne fungal growth and the inhibition depends on both concentration and duration of the storage.

In one day old treated seeds, *E. crassipes* root and leaf extracts showed significant suppression of the incidence of seed borne fungi at all concentrations. *S. cumini* and *C. sativum* caused a total inhibition of fungal development on maize kernels. A residual effect of these two extracts were detected up to 6 months. Nidiry (1999) also obtained a similar result with tomato seed extract, which reduced the mycelial growth of *Colletotrichum gloeosporioides*. The degree of inhibition increased with the extract concentration. No adverse phytotoxic effect on treated seed germination and seedling growth was detected with *C. sativum* and *S. cumini* even after one month of treatment. At optimal protective dosage of 0.5 mg/g (v/v), *T. indica* and *P. cubeba* were fungistatic on *A. flavus* with a residual effect that lasted for 6 months and *L. acidissima* showed inhibition of seed born fungal growth at lower doses only without effecting the seed germination and it appears that these plant materials contain chemicals having fungicidal properties.

The crude extracts were more effective in reducing the fungal growth. This is an indication that dilution of the extracts reduced toxic effects of the leaf extracts on the seed-borne fungi. The result agrees with the findings of Zaman *et al* (1997), who also found that the efficacy of *S. cumini*, *T. indica* and *P. cubeba* extracts on seed borne fungi of mustard, declined with increase in dilution and are effective up to 6 months. It has also been revealed in this investigation that the botanical treatments, storage duration and their interaction effects influenced the seedling growth.

It appears that these botanical extracts contain chemicals that are effective only on root growth enhancement but failed to promote the shoot growth. This kind of observations were also made by Leather and Einhellig (1985).

In 1-month-old treated seeds, *S. cumini* showed total inhibition of radicle and hypocotyl growth of seedling, which was negatively correlated to the seed germination. The results of this study add support to previous studies by Chung and Miller (1995), who found that mixture of certain plant extracts significantly reduced hypocotyl length at all concentrations. The plant extracts like *E. crassipes* leaf, *P. cubeba*, *T. catappa* and *C. indica* showed greater seedling growth inhibition in comparison to 10 days old seeds. No linear relationships were detected between the concentration of extract applied and the percentage of seedling growth inhibition. The effects lasted for only one month.

The magnitude of depression of inhibition decreased with storage duration of treated seeds. In 6 months treated seeds, except *S. cumini*, all other treated botanicals showed negligible seedling growth inhibition. In fact the

leaf extracts of *E. crassipes*, *L. acidissima*, *C. indica* and *T. indica* treated and stored for 1 month inhibited the seedling growth moderately, while 6 month old treated grain enhanced to a small extent.

From the results of these trials, it can be concluded that the use of certain botanical extracts as natural pesticides in maize storage significantly reduce the seed borne fungal growth with no adverse effects on seed germination and seedling growth of treated maize seeds up to 6 months. Further tests are under progress to investigate the effects of botanical treatments up to the corn production. Studies on the levels of residues remaining on treated food and their potential adverse effects when consumed should be evaluated before the use of these treatments are institutionally promoted as part of a sustainable insect pest management system for farm level storage.

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Larvicidal activities of some Euro-Asiatic plants against *Culex quinquefasciatus* Say (Diptera: Culicidae)

Roman Pavela

ABSTRACT

Methanol extracts of the aerial parts from 31 Euro-Asiatic plant fourth instar larvae species were tested for larvicidal activity against the mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae) fourth instar larvae, under laboratory conditions. The mortality from six concentrations (5, 10, 25, 50, 100 and 200 ppm) was determined and LD₅₀ was calculated. All plant extracts showed larvicidal activity in 24 h exposure tests. The methanolic extracts of plants *Otanthus maritimus* displayed the highest larvicidal activities with LD₅₀ 7 ppm, followed by *Ammi visnaga*, *Acer pseudoplatanus*, *Acer platanoides* and *Satureja hortensis* with LO₅₀ 9, 23, 28 and 28 ppm, respectively.

Keywords: *Culex quinquefasciatus*, *Otanthus maritimus*, *Ammi visnaga*, *Acer pseudoplatanus*, *Acer platanoides* and *Satureja hortensis*.

INTRODUCTION

Mosquitoes not only cause nuisance by their bites but also transmit deadly diseases like malaria, filariasis, yellow fever, dengue and Japanese encephalitis, contribute significantly to poverty and social debility in tropical countries (Jang *et al.*, 2002). *Culex quinquefasciatus* Say (Diptera: Culicidae) is a pantropical pest and urban vector of *Wuchereria bancrofti*, *Plasmodium* (avian malaria), myxomatosis, and other diseases in some parts of the world (Holder, 1999). It has been shown to be able to carry Murray Valley encephalitis (MVE) virus in laboratory studies and MVE virus has been isolated from it in northern Western Australia. *Culex quinquefasciatus* has yielded an isolate of Ross River (RR) virus during an outbreak in New Caledonia, but from a number of laboratory studies in Australia it appears to be a poor and unlikely vector of MVE, Kunjin, RR and other arboviruses. It is a poor vector of dog heartworm, and of human filariasis in more northern tropical regions (Russell, 1993).

Synthetic insecticides are today in the fore of the mosquito controlling agents. Compared to other controlling measures in the past few decades, synthetic insecticides have been used and have produced a feed back of environmental ill effect, non-target organisms being affected and most of mosquitoes species have becoming physiologically resistant to these synthetic insecticides (VCRC, 1989; Severini *et al.*, 1993). On the other hand, some mosquito species have developed high levels of resistance to microbial control agents (Rao *et al.*, 1995). These factors have created a search for ecofriendly, biodegradable and target – specific insecticides against the mosquitoes.

Plant products have been used traditionally by human communities in many parts of the world against the vector and pest species of insects (Jacobson, 1958; Pavela, 2007). Over the past 35 years, the search for plants with novel insecticidal constituents has been intensive. Among the plant families studied Asteraceae, Lamiaceae, Meliaceae,

Piperaceae, Rutaceae crude extracts or their compounds have showed toxicity (Pavela, 2006), antifeedants (Sadek, 2003; Pavela, 2004a), insect growth regulators (Pavela, 2004b; Pavela, 2005), oviposition deterrence (Dimock and Renwick, 1991; Zhao *et al.*, 1998), suppression of calling behaviour (Khan and Saxana, 1986) and reduction of fecundity and fertility (Pavela *et al.*, 2005). Such a wide variety of effects provides potential alternatives for the use of synthetic chemical insecticides. In the present investigation an attempt was made to evaluate the larvicidal efficacy of some plant extracts against *Culex quinquefasciatus*.

MATERIAL AND METHODS

Plant extracts

Fresh plant material of each of the selected species (see Table 1) was collected in 2006. Voucher specimens of all the plant species studied were deposited in herbaria of our institute. The plant material was shade-dried and powdered. The dry powder was extracted with excess of methylalcohol (500 ml of MeOH for 100g of plant powder) for 24 h. The crude extracts were filtrated and evaporated under reduced pressure in a rotary evaporator.

Test organism

The test organism *Culex quinquefasciatus* Say was reared in the laboratory conditions. (28 ± 2°C, 70 ± 5% RH, and a photo regime of 16:8 (L:D) h.) on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week – old chick for blood feeding.

Larvicidal activity

Mosquito larvicidal assays were carried out according to WHO standard procedures (1996), with slight modifications. The extracts were diluted in dimethyl sulphoxide (DMSO) to prepare a serial dilution of test dosage. Early fourth instar larvae of *C. quinquefasciata* were selected and transferred in 25 ml of distilled water.

Table 1. Plants selected for the screening of larvicidal activity against *Culex quinquefasciata*

Species	Family assayed	Plant part	Yield (%) references	Voucher	Origin
<i>Acer campestre</i> L.	Aceraceae	Leaves	8.7	0760	Prague, Czech Republic
<i>Acer cissifolium</i> (Siebold & Zuccarini) Koch	Aceraceae	Leaves	8.3	0762	Prague, Czech Republic
<i>Acer negundo</i> L.	Aceraceae	Leaves	9.5	0763	Prague, Czech Republic
<i>Acer platanoides</i> L.	Aceraceae	Leaves	9.2	0761	Prague, Czech Republic
<i>Acer pseudoplatanus</i> L.	Aceraceae	Leaves	7.6	0759	Prague, Czech Republic
<i>Achillea millefolium</i> L.	Asteraceae	Stem	8.9	0740	Prague, Czech Republic
<i>Ajuga reptans</i> L.	Lamiaceae	Stem	10.2	0722	Prague, Czech Republic
<i>Ammi visnaga</i> (L.) LAM.	Apiaceae	Seeds	19.3	0732	Prague, Czech Republic
<i>Circium arvense</i> (Savi) Ten.	Asteraceae	Stem	5.8	0750	Prague, Czech Republic
<i>Fraxinus exelsior</i> L.	Oleaceae	Leaves	5.3	0765	Prague, Czech Republic
<i>Glebionis coronarium</i> (L.) Tzvelev. Russia	Asteraceae	Flower	9.2	0758	Krasnodarskiy region,
<i>Humulus japonicus</i> Sieb. & Zucc.	Cannabidaceae	Leaves	7.9	0725	Prague, Czech Republic
<i>Hysopus officinalis</i> L.	Lamiaceae	Stem	8.7	0748	Prague, Czech Republic
<i>Laburnum anagyroides</i> Medik.	Leguminosae	Leaves	15.0	0734	Prague, Czech Republic
<i>Lavandula officinalis</i> L.	Lamiaceae	Flower	3.1	0227	Prague, Czech Republic
<i>Limonium. Bonduelii</i> . O. Kuntze	Plumbaginaceae	Stem	2.8	0746	Prague, Czech Republic
<i>Matricaria maritima</i> L.	Asteraceae	Flower	6.8	0766	Prague, Czech Republic
<i>Matthiola tricuspidata</i> (L.) R. Brown Russia	Brassicaceae	Stem	7.2	0754	Krasnodarskiy region,
<i>Melisa officinalis</i> L.	Lamiaceae	Stem	5.7	0742	Prague, Czech Republic
<i>Ocimum basilicum</i> L.	Lamiaceae	Stem	7.6	0753	Prague, Czech Republic
<i>Origanum vulgare</i> L.	Lamiaceae	Stem	7.4	0729	Prague, Czech Republic
<i>Otanthus maritimus</i> (L.) Hoffmanns & Link Russia	Asteraceae	Stem	5.6	0755	Krasnodarskiy region,
<i>Salvia farinacea</i> Benth.	Lamiaceae	Stem	5.4	0713	Prague, Czech Republic
<i>Salvia viridis</i> L.	Lamiaceae	Flower	4.7	0731	Prague, Czech Republic
<i>Salvia officinalis</i> L.	Lamiaceae	Stem	11.3	0721	Prague, Czech Republic
<i>Satureja hortensis</i> L.	Lamiaceae	Stem	7.7	0717	Prague, Czech Republic
<i>Satureja nervosa</i> Desf .	Lamiaceae	Stem	4.2	0756	Crete, Greece
<i>Sonchus arvensis</i> L.	Asteraceae	Stem	5.8	0744	Prague, Czech Republic
<i>Tanacetum vulgare</i> L.	Asteraceae	Flower	6.1	0736	Krasnodarskiy region, Russia
<i>Teuricum capitatum</i> L.	Lamiaceae	Stem	5.8	0757	Crete, Greece
<i>Thymus vulgaris</i> L.	Lamiaceae	Stem	5.0	0718	Prague, Czech Republic

For experimental treatment, 1 ml of serial dilutions was added to 224 ml of distilled water in a 500-ml glass bowl and shaken lightly to ensure a homogenous test solution. The selected larvae were transferred in distilled water into a bowl of prepared test solution with final surface area 125 cm² (25 larvae/beaker). Four replicates were of maintained for all the five dosages (5, 10, 25, 50, 100 and 200 ppm) separately. The assays were placed in a growth chamber (L16:D9, 26 °C). The mortality was determined after 24 h of exposure, during which time no food was offered to the larvae. The control mortality was corrected

by Abbott's formula (Abbott, 1925) and LD₅₀, LD₉₀, regression equation and the 95% confidence limit was calculated by using probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

The results of larvicidal activity of plant extracts are presented in Table 2. All plant extracts showed larvicidal activity in 24 h exposure tests. The methanolic extracts of plants *Otanthus maritimus* displayed the highest larvicidal activities with LD₅₀ 7 ppm, followed by *Ammi visnaga*, *Acer pseudoplatanus*, *Acer platanoides* and *Satureja hortensis* with LD₅₀ 9, 23, 28 and 28 ppm, respectively.

Table 2. Insecticidal activity of plant extracts against fourth stage larvae of *Culex quinquefasciata*

Species	Average mortality ^a (%) ±SE	LD ₅₀ (CI95) ^b	LD ₉₀ (CI95)
<i>Acer campestre</i> L.	100.0±0.0	52(48-75)	86(82-98)
<i>Acer cissifolium</i> (Siebold & Zuccarini) Koch	100.0±0.0	70(62-84)	123(110-132)
<i>Acer negundo</i> L.	100.0±0.0	51(45-63)	89(80-93)
<i>Acer platanoides</i> L.	100.0±0.0	28(25-39)	62(54-73)
<i>Acer pseudoplatanus</i> L.	100.0±0.0	23(18-33)	76(60-89)
<i>Achillea millefolium</i> L.	100.0±0.0	120(115-126)	159(142-169)
<i>Ajuga reptans</i> L.	46.0±3.8	>200	>200
<i>Ammi visnaga</i> (L.) LAM.	100.0±0.0	9(8-18)	45(38-52)
<i>Cirsium arvense</i> (Savi) Ten.	44.6±3.8	>200	>200
<i>Fraxinus exelsior</i> L.	21.0±3.2	>200	>200
<i>Glebionis coronarium</i> (L.) Tzvelev.	100.0±0.0	53(46-58)	110(102-123)
<i>Humulus japonicus</i> Sieb. & Zucc.	100.0±0.0	25(21-32)	87(76-110)
<i>Hysopus officinalis</i> L.	92.7±5.9	150(140-163)	>200
<i>Laburnum anagyroides</i> Medik.	98.0±8.1	111(103-126)	198(156-220)
<i>Lavandula officinalis</i> L.	100.0±0.0	59(57-72)	123(118-128)
<i>Limonium. Bonduelii</i> . O. Kuntze	52.2±12.3	>200	>200
<i>Matricaria maritima</i> L.	100.0±0.0	72(60-90)	139(126-142)
<i>Matthiola tricuspidata</i> (L.) R. Brown	9.0±3.3	>200	>200
<i>Melisa officinalis</i> L.	44.5±6.5	>200	>200
<i>Ocimum basilicum</i> L.	100.0±0.0	32(29-45)	69(53-82)
<i>Origanum vulgare</i> L.	88.6±7.2	156(148-172)	>200
<i>Otanthus maritimus</i> (L.) Hoffmanns & Link	100.0±0.0	7.3(6.5-9.3)	15(12-18)
<i>Salvia farinacea</i> Benth.	68.3±3.3	195(183-199)	>200
<i>Salvia viridis</i> L.	100.0±0.0	110(105-132)	183(153-191)
<i>Salvia officinalis</i> L.	84.6±10.2	159(133-168)	>200
<i>Satureja hortensis</i> L.	100.0±0.0	28(22-37)	56(48-72)
<i>Satureja nervosa</i> Desf .	32.5±8.5	>200	>200
<i>Sonchus arvensis</i> L.	100.0±0.0	68(55-78)	118(110-132)
<i>Tanacetum vulgare</i> L.	92.1±4.4	178(158-188)	198(186-201)
<i>Teuricum capitatum</i> L.	48.8±12.9	>200	>200
<i>Thymus vulgaris</i> L.	100.0±0.0	48.2(39-82)	98(79-102)
control	0.0±0.0		

^a The average mortality (± standard error) established in concentration 200 ppm as a maximal tested concentration.

^b The lethal concentrations with the corresponding 95% confidence intervals are shown in are shown in parenthesis.

Today, the environmental safety of an insecticide is considered to be of paramount importance. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world (Pavela, 2007). The screening of locally available plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health.

The crude extracts of the five plant has been found to possess larvicidal activity against the mosquito *Culex quinquefasciatus*. The biological activity of this plant extracts might be due to the various compound, including phenolics, terpenoids, and alkaloids exist in plants these compounds may jointly or independently contribute to produce larvicidal and adult emergence inhibition activity against *Culex quinquefasciatus*.

The larvicidal efficacy of *O. maritimus*, *A. visnaga*, *A. pseudoplatanus*, *A. platanoides* and *S. hortensis* are

comparable to well established insecticidal plant species. Omena *et al.* (2007) studied larvicidal activities of 51 Brazilian medicinal plants against *Aedes aegypti*. Eleven of 84 extracts (obtained from different plant parts) showed significant LD₅₀ < 100 ppm and seven species showed significant LD₅₀ < 10 ppm (from genus *Annona* sp., *Derris* sp., *Erythrina* sp. and *Pterodon* sp.). Pizzarro *et al.* (1999) studied the activity of the saponine fraction of *Agave sisalana* and estimated the LD₅₀ and LD₉₀ against 3rd instar larvae of *Culex quinquefasciatus*, that were 183 and 408 ppm, respectively. These concentrations were much higher than those reported in this study, but these authors suggested its use for control of this mosquito. The leaf extracts of *O. maritimus*, *A. visnaga*, *A. pseudoplatanus*, *A. platanoides* and *S. hortensis* is superior to various neem extracts, which are reported to be effective with LD₅₀ values ranging from 55-65 ppm against mosquito larvae (Ascher and Meisner, 1989). The median lethal concentrations (LD₅₀) of various parts of *Melia azederach* ranging from 30-40 ppm against larva of *Culex pipiens* (Al-Sharook *et al.*, 1991). The effect of the various neem extracts and various parts of *Melia azederach* was slightly lower than that reported for the *C. asiatica* leaf extract. The adult emergence inhibition activity of *C. asiatica* is also comparable to different species of plant extract in different families (Muthukrishnan *et al.*, 1999; Pushpalatha and Muthukrishnan, 1999). In our study, five extracts possess remarkable larvicidal activity against mosquito *Culex quinquefasciatus*. Further investigations are needed to elucidate this activity against a wide range of all stages mosquito species and also the active ingredients of the extract responsible for larvicidal and adult emergence inhibition activity in *Culex quinquefasciatus* should be identified and utilized, if possible, in preparing a commercial product / formulation to be used as a mosquitocidal.

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Distribution of predatory arthropod communities in selected sandal provenances of south India

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ABSTRACT

Detailed study was undertaken to explore the diversity of predatory arthropods in six-sandal provenances viz., Bangalore, Thangali and Mandagadde in Karnataka, Javadis and Chitteri in Tamil Nadu and Marayoor in Kerala. The study revealed the presence of 74 species of predatory insects and 24 species of spiders and their distribution in different sandal provenances were discussed in this paper.

Key words: sandal, predators, arthropods, spiders

INTRODUCTION

Santalum album Linn., commonly known as sandalwood occupies a pre-eminent place among the forest crops which are of great economic value. Its heartwood oil, commercially known as "East Indian Sandalwood oil" is well known scented oil in the world. It alone has significantly contributed to revenue around Rs.160 million by exporting around 27 tons/year (Ananthapadmanabha, 2000). Current sandalwood and oil prices are indicated at Rs.12 lakhs/tons and Rs.22000/Kilogram respectively (Ananthapadmanabha 2002). The sandalwood prices have increased from Rs.365/ ton in 1990 to Rs.6.5 lakhs/ton in 1999-2000. The current price of Indian Sandalwood is over Rs.3500 per kilogram and that of oil is about Rs. 70,000 per kilogram, whereas the price at the international market is about 15 to 20% higher than the domestic market. Increase in price is due to large gap between demand and supply.

Of the various limiting factors, the insect pests are amongst the most important in successful establishment of sandal. During the past decade, there has been increased interest in the employment of natural enemies for the regulation of forest insect pests. Furthermore, for biocontrol of insects, there has been "a shift in emphasis from the introduction of exotic parasites and predators to the recognition of the importance of naturally occurring biological control agents and this approach is gradually becoming one of the major topics in applied entomology" (Brader, 1980). The knowledge gained from study of natural enemies may be of immense practical value in insect pest management (Kidd and Jervis, 1996). The review of insects associated with sandal by Sundararaj *et al.* (2006) includes 155 species of probable predators representing 13 families under 5 orders. Sundararaj *et al.* (2007) in their review listed 61 species of parasitoids representing 14 families under 2 orders on insects infesting sandal. In the present study extensive surveys were

undertaken to document the distribution of predatory arthropods in selected provenances of sandal and the findings are presented in this communication.

MATERIAL AND METHODS

The detailed study on the distribution of predatory arthropods in sandal dominant ecosystems was conducted for two years from 2004 to 2006. For these purpose six provenances of sandal from south India viz., Bangalore, Thangali and Mandagadde in Karnataka, Marayoor in Kerala, and Javaddis and Chitteri in Tamil Nadu were selected. The details of the study sites were furnished in the Table-1. The survey was conducted two times in a year representing summer and winter season. Blocks of the size 50 x 50 ft in five replications were marked in all the selected sandal provenances for sampling. From each block five trees were selected at random and observed for the predatory insects active on the selected areas. The spiders were sampled by hand picking, sweep net and pit-fall traps and the collected specimens were preserved in 70 per cent alcohol. The representative insect and spider specimens and were identified with the help of taxonomic experts.

RESULTS AND DISCUSSION

The survey indicated the presence of 74 species of predatory insects in all the selected provenances of sandal (Table-2). It includes 22 species each of Odonata under 5 families and Coleoptera under 4 families, 15 species of Mantodea under 2 families, 7 species of Hemiptera under 3 families, 5 species of Neuroptera under 4 families and one species each of Diptera, Hymenoptera and Lepidoptera. Among the families of Coleoptera, the family Coccinellidae was dominant with 17 species. The dominance of Coccinellidae confirms the earlier report of Mani and Krishnamoorthy (1993). On the basis of number of identified species of Odonata, Libellulidae was the most

Table 1. Details of sandal provenances selected for the study

Potential provenance	Forest Division & State	Latitude & longitude	Altitude (m)	Mean annual (mm)	Temp–Max / Min (°C)	Soil Type	pH	TSS EC Mohs / Cm
Bangalore	Bangalore Karnataka	12°58'N 77°38'E	1000	850	36.8/12.2	Red loam	6.3-6.5 Acidic	251.1µ mohs
Thangali	Chickmagalur	13°40'N 76°00'E	766	1500	44.0/10.5	Red loam & alluvium	7.5-7.8 Alkaline	2.3µ mohs
Mandagadde	Shimoga, Karnataka	13°9'N 75°40'E	650	2000	38.1/13.0	Red loam & alluvium	5.5-5.8 Acidic	317.0µ mohs
Chitteri	Harur, Tamil Nadu	12°0'N 78°6'E	1050	1000	35.2/8.2	Red sandy loam	6.0–6.3 Acidic	327.3µ mohs
Javadis (Kavalur)	Tirupattur, Tamil Nadu	12°3'N 78°7'E	930	1200	38.0/12.4	Red loam	6.6-6.7 Acidic	432.5µ mohs
Marayoor	Munnar, Kerala	10°1'N 77°1'E	1000	1450	36.0 / 10.0	Black clay	6.2-6.7 Acidic	362.0µ mohs

dominant family with 15 species, followed by Coenagrionidae by 4 species and Aeshnidae, Euphaeidae and Gomphidae each by 1 species. Many earlier workers reported the dominance of family Libellulidae in the Indian sub continent (Prasad, 2002; Kumar, 2002; Vashishth *et al.* 2002; Kandibane *et al.* 2005; Emiliyamma, 2005 and Emiliyamma *et al.*, 2005). Under the order Mantodea 15 species belonging to 4 families were recorded with dominance of 11 species of the family Mantidae. The dominance of Mantidae is in conformity with the results of Thulsi Rao *et al.* (2005), who reported 12 species out of 26 species from Andhra Pradesh under this family. The order Hemiptera is represented by 7 species under four families with Reduviidae as dominant family with 4 species while the order Neuroptera is represented by 5 species under four families with dominance of the family Chrysopidae with 3 species. One predatory insect each represented Diptera, Lepidoptera and Hymenoptera. Among the provenances Bangalore recorded maximum number of predatory insects being 67 followed by 52 in Marayoor, 45 in Chitteri, 43 in Thangali, 38 in Mandagadde and 34 in Javaddis.

A total of 24 species of spiders belonging to 11 families viz., Araneidae, Clubionidae, Eresidae, Miturgidae, Philodromidae, Pholcidae, Oxyopidae, Salticidae, Scytodiidae, Theridiidae, and Thomisidae were recorded in different provenances of sandal (Table-3). Among them the family Salticidae was dominant with 7 species followed by Thomisidae by 5 species, Araneidae, Philodromidae and Oxyopidae each by 2 species and Clubionidae, Eresidae, Miturgidae, Pholcidae, Scytodiidae and Theridiidae by one species each. Among the sandal provenances, Bangalore recorded the maximum of 14

species of spiders followed by Thangali, which recorded 9 species. The other provenances viz, Chitteri and Javadis (8 species), Mandagadde (7 species) and Marayoor (5 species) recorded lower number of species. These differences could be attributed by several factors such as human interference, climate of the study area, deforestation, habitat destruction, fragmentation etc., (Padhye *et al.*, 2006). Spiders are key components of all ecosystems as they are non-specific predators. Simmonds *et al.* (1994) studied the response of spiders to ecological disturbances and they reported maximum dominance of spiders in semi-evergreen forests. Spider species are well adapted to survive in forest ecosystem and their number increased due to presence of sufficient prey, non existent of competitors, lesser predators and non interference by humans (Sugumaran *et al.*, 2005). Their potential for suppressing the pest abundance in natural ecosystem has been reported by many earlier workers (Ito *et al.*, 1962; Barrion, 1980). The review of insects associated with sandal by (Sundararaj *et al.* 2006) includes 155 species of probable predators representing 13 families under 5 orders. The present study proved that the sandal provenances were rich with predatory arthropods and the non-outbreak of insect pests in natural sandal-dominated ecosystem might be due to the presence of these predators that they play a very valuable role by devouring harmful insect pests and of keeping the insect pest populations under control.

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Table 2. Predatory insects collected from different provenances of sandal

S. No.	Species name	Sandal provenances					
		1	2	3	4	5	6
ORDER: COLEOPTERA							
(1). Family: Carabidae							
1	<i>Abacetus</i> sp.	+	+	+	+	+	+
2	<i>Anthia sexguttata</i> Fabr.	+	+	+	+	+	+
(2). Family: Cicindelidae							
3	<i>Cicindela collicia</i> Acciavatti & Pearson	+	+	+	+	+	+
(3). Family: Coccinellidae							
4	<i>Anegleis cardoni</i> (Ws.)	+	+	+	+	+	+
5	<i>A. perrotti</i> (Mulsant)	+	+	+	+	+	+
6	<i>Brumus suturalis</i> Fabricius	+	-	+	-	-	+
7	<i>Cheilomenes sexmaculata</i> (Fabr.)	+	+	+	+	+	+
8	<i>Chilocorus nigrita</i> (Fabr.)	+	+	+	+	+	+
9	<i>Coccinella septumpunctata</i> Linn.	+	+	+	+	+	+
10	<i>Cryptolaemus montruizeri</i> Mls.	+	+	+	+	+	+
11	<i>Cybocephalus indicus</i> Tian & Ramani	+	+	+	+	+	+
12	<i>Harmonia octomaculata</i> (Fabr.)	+	-	-	-	-	-
13	<i>Illeis cincta</i> (Fabr.)	+	-	-	+	-	-
14	<i>Jauravia albidula</i> Motschulsky	-	+	-	+	-	+
15	<i>Nephus regularis</i> Sic.	+	-	-	-	-	-
16	<i>Pharoscymnus flexibilis</i> (Muls.)	-	+	-	+	-	-
17	<i>Pseudaspidemerus circumflexa</i> Motsch	+	-	-	-	-	-
18	<i>Pullus coccidivora</i> Ayyar	+	+	-	-	-	+
19	<i>Pullus gratiosus</i> Wse.	+	-	-	+	-	+
20	<i>Scymnus</i> sp.	+	+	+	+	+	+
(4). Family: Nitidulidae							
21	<i>Cybocephalus indicus humeralis</i> (Fab.)	+	-	-	-	-	-
22	<i>Haptoncus? humeralis</i> (Fab.)	+	-	-	-	-	-
ORDER: DIPTERA							
(1). Family: Syrphidae							
23	<i>Ishindon scutellaris</i> (Fab.)	+	+	+	+	+	+
ORDER: HEMIPTERA							
(1). Family: Lygaeidae							
24	<i>Geocoris tricolor</i> Fab.	+	-	+	-	-	-
(2). Family: Pentatomidae							
25	<i>Canthecona furcellata</i> (Wolff.)	+	+	+	+	+	+
26	<i>Erthesina fullo</i> Thunb.	+	-	+	+	+	-
(3). Family: Reduviidae							
27	<i>Acanthaspis quinquespinosa</i> Fab.	+	+	+	+	+	+
28	<i>Brassivola hystrix</i> Dist.	+	+	+	+	+	+
29	<i>Epidaus</i> sp.	+	+	+	+	+	+
30	<i>Isyndus herso</i> (Fabr.)	+	+	+	+	+	+
ORDER: HYMENOPTERA							
(1). Family: Formicidae							
31	<i>Oecophylla smaragdina</i> Fabr.	+	+	+	+	+	+

ORDER: LEPIDOPTERA							
(1). Family: Lycaenidae							
32	<i>Spalgis epius</i> (Westw.)	+	+	+	+	+	+
ORDER: MANTODEA							
(1). Family: Amorphoscelidae							
33	<i>Amorphoscelis</i> sp.	+	-	-	-	-	+
(2). Family: Empusidae							
34	<i>Gongylus gongyloides</i> Linn.	+	+	-	-	-	-
(3). Family: Hymenopodidae							
35	<i>Creobroter</i> sp.	+	-	-	-	-	-
36	<i>Ephestiasula</i> near <i>intermedia</i> Werner.	+	+	-	-	-	-
(4). Family: Mantidae							
37	<i>Amantis</i> sp.	+	-	-	-	-	+
38	<i>Amantis biroi</i> Giglio-Tos	+	+	-	+	-	-
39	<i>Dysaules</i> sp.	+	-	-	-	-	-
40	<i>Dysaules longicollis</i> Stal	+	+	-	-	-	-
41	<i>Elmantis</i> sp.	+	-	+	-	+	-
42	<i>Euantissa pulchra</i> (Fabr.)	+	-	-	-	-	-
43	<i>Hierodula</i> sp.	+	-	-	+	-	+
44	<i>Humbertiella</i> sp.	-	+	-	-	-	+
45	<i>Humbertiella indica</i> Saus.	+	-	-	-	-	-
46	<i>Mantis religiosa</i> Linn.	+	+	+	+	+	+
47	<i>Parathespis humbertiana</i> Sassure	+	-	+	+	+	+
ORDER: NEUROPTERA							
(1). Family: Chrysopidae							
48	<i>Chrysopa</i> sp.	+	+	-	+	+	+
49	<i>Chrysoperla cornea</i>	+	+	+	+	+	+
50	<i>Mallada boninensis</i> (Okamoto)	+	+	+	+	+	+
(2). Family: Hemerobiidae							
51	<i>Micromus australis</i> Hagen	+	-	-	-	-	+
(3). Family: Mantispidae							
52	<i>Mantispa indica</i> Westw.	+	+	-	-	-	-
ORDER: ODONATA							
(1). Family: Coenagrionidae							
53	<i>Ceriagrion cerinorubellum</i> (Brauer)	+	-	-	+	-	+
54	<i>Ceriagrion coromandelianum</i> (Fabricius)	+	+	+	+	+	+
55	<i>Pseudagrion r. rubriceps</i> Selys	+	+	+	+	+	+
56	<i>Ischnura a. aurora</i> (Brauer)	+	+	+	+	+	+
(2). Family: Euphaeidae							
57	<i>Anisopleura comes</i> Hagen	-	-	+	+	-	-
(3). Family: Gomphidae							
58	<i>Ictinogomphus rapax</i> (Rambur)	+	-	-	+	-	+
(4). Family: Aeshnidae							
59	<i>Anax immaculifrons</i> Rambur	+	-	-	-	-	-
(5). Family: Libellulidae							
60	<i>Orthetrum pruinosum neglectum</i> (Rambur)	+	+	+	+	+	+
61	<i>O. s. sabina</i> (Drury)	+	+	-	-	-	+
62	<i>O. t. triangulare</i> (Selys)	-	-	+	+	-	+

63	<i>Acisoma p. panorpoides</i> Rambur	+	+	-	+	-	+
64	<i>Brachythemis contaminata</i> (Fabricius)	+	-	+	-	-	+
65	<i>Crocothemis s. servilia</i> (Drury)	+	+	+	+	-	+
66	<i>Diplacodes trivalis</i> (Rambur)	+	+	-	+	+	+
67	<i>Neurothemis t. tullia</i> (Drury)	-	-	+	+	-	+
68	<i>Trithemis aurora</i> (Burmeister)	+	-	-	+	+	+
69	<i>T. festiva</i> (Rambur)	+	-	-	+	-	+
70	<i>T. pallidinervis</i> (Kirby)	-	+	-	-	-	+
71	<i>Palpopleura s. sexmaculata</i> (Fabricius)	+	+	-	-	-	+
72	<i>Tramea virginia</i> (Rambur)	+	-	-	+	-	+
73	<i>Pantala flavescens</i> (Fabricius)	+	+	+	+	+	+
74	<i>Tholymis tillarga</i> (Fabricius)	+	+	+	-	+	+
	Total	67	43	38	45	34	52

Table 3. Predatory spiders collected from different provenances of sandal

Sl.No	Species name	Family	Sandal provenance					
			1	2	3	4	5	6
1	<i>Araneus nympha</i> (Simon)	Araneidae	+	+	+	+	+	+
2	<i>Asemonea</i> sp.	Salticidae	-	-	-	-	-	+
3	<i>Carrhottus vidhuus</i> C.L. Koch	Salticidae	-	-	-	+	-	
4	<i>Cheiracanthium melanostomum</i> (Thorell)	Miturgidae	+	-	+	-	-	-
5	<i>Clubiona drassodes</i> O.P.-Cambridge	Clubionidae	-	-	-	-	+	-
6	<i>Crossopriza lyoni</i> (Blackwall)	Pholcidae	-	+	+	-	-	-
7	<i>Hyllus semicupreus</i> (Simon)	Salticidae	+	+	-	-	-	-
8	<i>Myrmarachne</i> sp.	Salticidae	+	-	-	+	-	-
9	<i>Neoscona vigitans</i> (Blackwall)	Araneidae	+	-	-	-	-	+
10	<i>Oxyopes</i> sp.	Oxyopidae	+	+	+	+	+	+
11	<i>Oxyopes birmanicus</i> Thorell	Oxyopidae	+	+	+	+	+	+
12	<i>Plexippus</i> sp.	Salticidae	-	+	-	-	-	-
13	<i>Rhene</i> sp.	Salticidae	+	-	-	+	-	-
14	<i>Runcinia</i> sp.	Thomisidae	+	-	—	-	-	-
15	<i>Runcinia affinis</i> Simon	Thomisidae	+	-	+	+	-	-
16	<i>Scytodes thoracica</i> (Latreille)	Scytodiidae	-	+	-	-	+	-
17	<i>Stegodyphus sarasinorum</i> Karsch	Eresidae	-	-	-	+	-	-
18	<i>Strigoplus netravati</i> Tikader	Thomisidae	+	-	-	-	-	-
19	<i>Telamonia dimidiata</i> (Simon)	Salticidae	-	+	+		+	-
20	<i>Thanatus</i> sp.	Philodromidae	-	+	-	-	+	-
21	<i>Theridion</i> sp .	Theridiidae	+	-	-	-	-	-
22	<i>Thomisus</i> sp.	Thomisidae	+	-	-	-	-	-
23	<i>Thomisus pugilis</i> Stoliczka	Thomisidae	-	-	-	-	+	-
24	<i>Tibellus</i> sp.	Philodromidae	+	-	-	-	-	-
	Total		14	9	7	8	8	5

1. Bangalore, 2. Thangali, 3. Mandagadde, 4. Chitteri, 5. Javadis, 6. Marayoor

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Host Preference of *Trathala flavoorbitalis* on Brinjal shoot and Fruit Borer and Rice Leaf Folder

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ABSTRACT

The study was conducted both in the greenhouse and field from August 2003 to August 2004. *Trathala flavoorbitalis* is an efficient parasitoid of both Brinjal shoot and fruit borer (BSFB) and Rice leaf folder (RLF) but its parasitism efficiency on BSFB larvae is higher than that of RLF larvae. The highest parasitism rate by *T. flavoorbitalis* on BSFB larvae in August is 36% & July is 47% and on RLF larvae in November is 26% & Mid March-April is 28%. The parasitism rate of *T. flavoorbitalis* was always significantly higher in BSFB larvae than that of RLF. The parasitism of BSFB larvae was 1.52 to 2.14 folds higher than that of RLF. In the confined condition of cage or microplot, when *T. flavoorbitalis* was given a choice to parasitize BSFB and RLF larvae, they preferred more BSFB larvae.

INTRODUCTION

The major constraint of brinjal production is the infestation by a plethora of insect pests. It can be attacked by as many as 53 species of insect pests (Nayar *et al.*, 1995). Among them, Brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis* Guenee (Lepidoptera:Pyralidae) is the most destructive pest of brinjal in Bangladesh (Alam, 1969; Chattopadhyay, 1987). Rice leaf folder (RLF), *Cnaphalocrocis medinalis* Guenee were previously considered minor or sporadic pest (Capco, 1957; Lim, 1962), but in recent years their importance has increased (Kalode 1974; Soejardjan and Iman, 1980). *Trathala flavoorbitalis* (Cameron) has been reported as an important parasitoid of BSFB in Srilanka (Beesan and chatterjee, 1935; Chu and Hsiu, 1937), India (Naresh *et al.*, 1986, Malik *et al.*, 1989) and Bangladesh (Alam and Sana, 1964). Recent studies done by Alam *et al.* (2005) in Bangladesh showed that this parasitoid has significant influence in reducing damage to brinjal crop by parasitizing BSFB. A noteworthy success in classical biological control of the leaf folder was reported in Fiji with the introduction of a larval parasitoid *T. flavoorbitalis* (Cameron) from Hawaii (Hinckley, 1963). Wada and Shimazu (1978) observed in Japan that parasitization of *C. medinalis* due to *T. flavoorbitalis* was 30%. It may also be apprehended that the RLF may be one of the alternate hosts of *T. flavoorbitalis* in Bangladesh. The parasitoid can utilize both the host during the crucial period of insecticides applications. The intensity of pesticide application is seven times higher in brinjal fields than that of rice field (PAB, 1999). Probably due to that reason, *T. flavoorbitalis* is abundant in rice fields than heavily sprayed brinjal fields. They move to brinjal fields to attack BSFB when the insecticide use is restricted (Alam *et al.*, 2005). Yet it is not identified which one is the most preferred host of *T. flavoorbitalis*, BSFB or RLF.

Now-a-days, the role of parasitoids in an integrated pest management system is well recognized. Integrated pest management practice likes removal of BSFB infested shoots and fruits and RLF infested rice leaves, biological control, mass trapping with sex pheromone and minimal or no use of toxic pesticides to encourage proliferation of different parasitoids particularly of *T. flavoorbitalis*. These approaches can facilitate to develop a low cost sustainable management practice to reduce pesticide use and yield losses caused by BSFB and RLF for farmers of south Asia (Alam *et al.*, 2003). To considering the importance of parasitoid, *T. flavoorbitalis* in suppressing BSFB and RLF and to explore the environment friendly management practices for the production of brinjal and rice, this study was undertaken with the following objectives: to determine the relative preference and parasitism efficiency of *T. flavoorbitalis* for BSFB and RLF.

MATERIALS AND METHODS

The study was carried out at the experimental field and greenhouse of the Entomology Division, Bangladesh, from August. Agricultural Research Institute (BARI), Gazipur, Bangladesh from August, 2003 to August, 2004. The land was opened by a tractor drawn disc plough and harrowing was done on the following day for proper pulverization. For ensuring good tilt, power tiller was used. Tractor drawn labeler was used to level the land. In this study, cow dung and other chemical fertilizers were applied as recommended by Rashid (1999) for commercial brinjal cultivation.

Maintenance of Brinjal and Rice

Brinjal seeds (variety: Chega, Jessore local) were collected from Horticulture Division, Bangladesh Agricultural Research Institute (BARI) Joydebpur, Gazipur. A seedbed measuring 5m x 1m was prepared and seeds were sown. Standard seedling raising practice was followed (Rashid,

Table 1. Comparison of *T. flavoorbitalis* parasitism efficiency on its host BSFB and RLF, in cage condition under greenhouse.

Treatments	No. of BSFB/RLF released	No. of <i>T. flavoorbitalis</i> released	No. of BSFB/RLF parasitised	Percent parasitism
Observation 1				
BSFB + parasitoid	24	8	21.33±0.67 ^a	88.89±2.78 ^a
RLF + parasitoid	24	8	14.00±1.56 ^b	58.33±4.82 ^b
Observation 2				
BSFB + parasitoid	24	8	22.00±1.57 ^a	91.67±4.82 ^a
RLF + parasitoid	24	8	13.33±1.77 ^b	55.56±6.36 ^b
Observation 3				
BSFB + parasitoid	24	8	20.67±1.77 ^a	86.11±7.36 ^a
RLF + parasitoid	24	8	10.67±2.67 ^b	38.87±5.56 ^b

Means followed by the same letter (observation wise) are not significantly different ($P>0.05$, t-test)

1999). The seed beds were lightly irrigated, mulched regularly for ensuring germination as well as proper growth and development of the seedlings. Thirty-six days-old (3/4 leaf stage) healthy brinjal seedlings were transplanted three times in the experimental plots (10mX10m). Brinjal plants were transplanted for the first time in last week of May 2003, second time in last week of October 2003 and lastly in the last week of March 2004. So, there was continuous brinjal cultivation in the field throughout the year.

Rice seedbed (60 m²) was cultivated by plough and power tiller was used latter to ensure good tilt. Ploughing was done for puddling with required water. Then the whole plot was submerged with irrigation water and preserved for 7 -10 days. Rice seedbed measuring 1.25m x 0.5 m was prepared with green manure, 7 g urea/m² and gypsum. Forty five day-old healthy rice seedlings were transplanted in the experimental field plots (15mX12m) during first week of July (T. Aman) and first week of February (Boro).

Efficiencies and preference of *T. flavoorbitalis* on BSFB and RLF

Studies on i. Parasitism efficiencies/preference of *T. flavoorbitalis* on BSFB in the field, ii. Parasitism efficiencies/preference of *T. flavoorbitalis* on RLF in the field, iii. Parasitism efficiencies/preference of *T. flavoorbitalis* on BSFB and RLF in caged conditions and iv. Parasitism efficiencies/preference of *T. flavoorbitalis*

on BSFB and RLF in Microplot conditions were undertaken. These aspects of parasitism efficiencies and host preference of *T. flavoorbitalis* on BSFB and RLF were conducted in the laboratory, green house and in the field of BARI. The details of the studies are described as follows:

Parasitism efficiencies of *T. flavoorbitalis*

Forty infested brinjal shoots, thirty infested fruits and forty infested (folder) rice leaves were collected per plot (replication) at fortnightly interval from the brinjal and rice fields from August 2003 to August 2004. Infested brinjal shoots, fruits and rice leaves were collected with the help of secateur. Collected specimens were carried to the laboratory in perforated polythene bags and placed on plastic tray (35cmX 22 cm) having sterilized sand (4 cm depth). The excised portion of infested brinjal shoots and fruits and infested folded rice leaves were wrapped with wet cotton sheet to keep them fresh for few days. The tray was then placed in a wooden rearing cage (42 x 30 x 27 cm) having windows netting with 32 mesh net for proper ventilation. The rearing cages were kept on wooden table inside laboratory maintaining 27±2°C. The infested shoots, fruits and leaves were kept in the cages until the larvae came out from the shoots and fruits and pupation starts. Close observations were made till the adult RLF and *T. flavoorbitalis* emerged from the infested leaves. To determine the parasitism efficiency of *T. flavoorbitalis* in

Table 2. Comparison of *T. flavoorbitalis* parasitism efficiency on its hosts BSFB and RLF in micro plot of greenhouse.

Treatments	No. of BSFB/RLF released	No. of <i>T. flavoorbitalis</i> released	No. of BSFB/RLF parasitized	Percent parasitism
Observation 1				
BSFB + parasitoid	30	12	84.33±2.03 ^a	81.11±6.77 ^a
RLF + parasitoid	30	12	11.33±1.45 ^b	37.77±4.84 ^b
Observation 2				
BSFB + parasitoid	30	12	23.33±1.77 ^a	77.78±5.89 ^a
RLF + parasitoid	30	12	12.33±1.02 ^b	41.11±4.01 ^b
Observation 3				
BSFB + parasitoid	30	12	24.33±1.77 ^a	81.11±5.88 ^a
RLF + parasitoid	30	12	13.33±2.67 ^b	44.44±2.94 ^b

Means followed by the same letter (observation wise) are not significantly different ($P>0.05$, t-test)

the field population of RLF larvae infesting rice were arranged side by side and rice plots were kept without any application of pesticides. So, there was ample opportunity of conservation of the *T. flavoorbitalis*.

Calculation of Parasitism (%)

The number of adult BSFB/RLF and parasitoids *T. flavoorbitalis* emergences were counted and recorded everyday. The percent parasitisms were then calculated using the following formula: Percent parasitization = Number of parasitoid adults emerged/ Number of BSFB/RLF adults + parasitoid adults emerged X 100. Mean parasitism percentages of BSFB in shoots, fruits and RLF in leaves were calculated. Comparison between the means of shoots and fruits were also analyzed by student's t-test and plotted in the graphs.

Parasitism and Preference of *T. flavoorbitalis*

Cage and Microplot studies were conducted in temperature-controlled glasshouse (maintained at 28-30°C) of Entomology Division, BARI during September-October 2003 and April-May 2004, respectively to observe host preference of *T. flavoorbitalis* for the BSFB larvae and RLF larvae. The cage (2ft X 1.5ft X 1.5ft) was made by wood and mesh nylon net. Three sides and door were

rounded by mesh nylon net. A total of 24 RLF and 24 BSFB neonate larvae @ 8 larvae/pot were released along with 8 female 2-3 days old parasitoids, *T. flavoorbitalis* per cage (2ft X 1.5ft X 1.5ft). Another cage (2ft X 1.5ft X 1.5ft), having 24 RLF larvae and 24 BSFB larvae were kept without any parasitoids and considered as the control. There were 4 sets of such cages and one such cage was considered as one replication. On the other hand, in microplot a total of 30 BSFB and 30 RLF neonate larvae was released along with 12 female of 2-3 days old parasitoids of *T. flavoorbitalis* per micro plot after 40 days of transplanting of brinjal and rice. Another micro plot, having 30 BSFB larvae and 30 RLF larvae was kept without any parasitoid release and considered the control. Therefore, there were four sets of micro plots with same type of setting and one plot was considered control and one set was regarded as one replication. After 10 days of release of host and parasitoids larvae in cage and microplot shoots and fruits and rice plants were cut from ground level and thoroughly checked for live larvae. As the parasitoid, *T. flavoorbitalis* can parasitize all instars of the host larvae but the parasitized neonate larvae can not survive (Alam *et al.*, 2003), except those who were escaped from parasitism. The number of escaped alive larvae were collected and recorded from every brinjal shoot and fruits and rice leaves. To eliminate the effect of other mortality

factors acting on the pests and to determine the parasitism rate, percent mortality figures were adjusted, following Abbott's (1925) formula in both studies.

RESULTS AND DISCUSSIONS

Parasitism efficiencies of *T. flavoorbitalis*

There was a seasonal effect on the number of BSFB as well as its parasitoid, *T. flavoorbitalis*. The number of both the host and parasitoids were highest during the hot humid period of September-October and lowest in cool dry period of December-February. The average number of *T. flavoorbitalis* adults recovered from the field-collected shoots and fruits was 4.33 amounting to parasitism of 16% BSFB larvae (Figure 1). But the number of parasitoids from the same number of damaged shoots and fruits increased to 40.67, amounting to parasitism of 49% within 12 months from the plots without insecticides application. *T. flavoorbitalis* had an affinity to parasitize more BSFB larvae feeding within the shoot than that in the fruit (Figure 2). Alam *et al.*(2005) reported that from December to February, the BSFB population as well as their parasitoid; *T. flavoorbitalis* was generally lower than rest of the year.

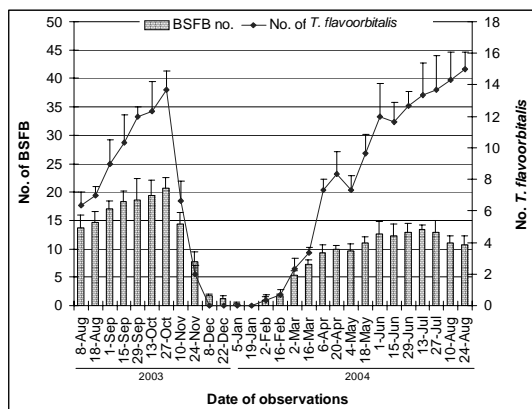


Figure 1. Population fluctuation of BSFB and parasitoid *T. flavoorbitalis* in brinjal shoots in the non-sprayed brinjal plots.

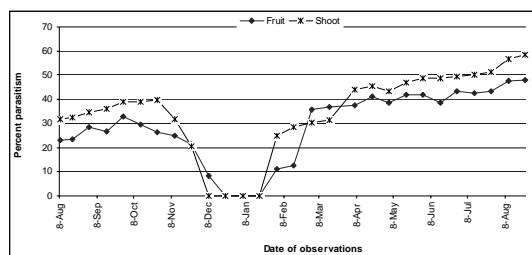


Figure 2. Comparison of parasitism rate by *T. flavoorbitalis* on BSFB in brinjal fruits and shoots in the non-sprayed brinjal fields.

The average year-round parasitism rate of *T. flavoorbitalis* on BSFB larvae attacking both fruits and shoots of brinjal and RLF larvae infesting rice leaves was plotted on the (Figure 3).

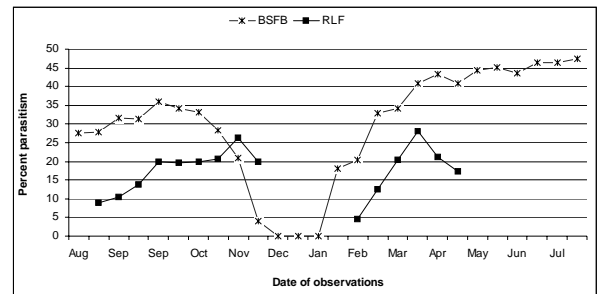


Figure 3. Comparison of parasitism rate by *T. flavoorbitalis* on BSFB larvae in brinjal and on RLF larvae in rice in the non-sprayed brinjal and rice fields.

It was observed from the figure that parasitism rate of *T. flavoorbitalis* on BSFB larvae in August was 26% and in July was 47% and on RLF larvae in November were 26% and Mid March-April it was 28%. So, the parasitism rate of *T. flavoorbitalis* was always less in RLF larvae than that of BSFB. Similarly Sakai *et al.* (1942) reported that in the rice field they found numbers of parasitoids infesting rice leaf folder's eggs, larvae and even pupae. On the other hand in brinjal, the list of natural enemies attacking BSFB is not very long. So, *T. flavoorbitalis* probably is facing less competition in parasitizing BSFB larvae than that of RLF. This may be the reason for more parasitism in BSFB larvae than RLF larvae. It has been reported that high parasitism of *T. flavoorbitalis* occurred on the later instars of the pests, especially in the 3rd and 4th instars (Sandanayake and Edirisinghe, 1992). They further observed that when given a choice for oviposition, the parasitoid prefers 3rd to 5th instars larvae to 1st and 2nd instars. Under natural conditions, however, the parasitoid may attack the early instars when older instars are scarce or absent. In case of RLF the later larval instars hide themselves inside the folded leaves and the BSFB larvae kept them inside the feeding tunnels made by them. But in BSFB an exit hole prevails through which the live excreta of the pest is generally coming out. That is an external sign of the presence of the live larvae of BSFB and which attract the parasitoid, *T. flavoorbitalis*. So, to locate the BSFB larvae is probably easier for the parasitoids than the rice leaf folder. Due to these reasons the parasitism by *T. flavoorbitalis* on BSFB larvae might be higher than that of RLF larvae.

Preference of *T. flavoorbitalis* under cage RLF

In the caged condition percent parasitism of *T. flavoorbitalis* was significantly higher on BSFB larvae

than that of RLF larvae in all the three observations (Table 1). In the 1st observation the parasitism on BSFB larvae was 88.89% while in RLF larvae it was 58.33%. In the 2nd and 3rd observations, 91.67% and 86.11% BSFB larvae and 55.56% and 38.87% RLF larvae were parasitized, respectively.

Preference of *T. flavoorbitalis* microplot RLF house

In microplot studies, *T. flavoorbitalis* parasitized significantly higher number of BSFB larvae than that of RLF larvae in all the observations (Table 2). In the 1st observation, 81.11% BSFB larvae and 37.77% RLF larvae were parasitized by *T. flavoorbitalis*, and in 2nd and 3rd observations, 77.78% and 81.11% BSFB larvae and 41.11% and 44.44% RLF larvae were parasitized by the parasitoid. In both the cage and microplot studies, when *T. flavoorbitalis* was given a choice to parasitize BSFB and RLF larvae, they preferred BSFB larvae. In the cage and microplot studies neonate larvae were released and Alam *et al.* (2003) observed that *T. flavoorbitalis* could be attack the early instars of larvae when the older instars were scarce or absent. So, in this case the neonate larvae were attacked by the parasitoids and due to parasitism the neonates could not survive. In both the studies, the neonate larvae were released on the leaves of rice and brinjal. In rice, the RLF larvae started feeding on the leaves and later part started weaving threads to fold the leaves. On the contrary, after releasing the BSFB larvae on the brinjal leaves, they started searching shoots or fruits to bore inside and start feeding. During crawling to search for shoots they were easily identified by the parasitoids and that is the most vulnerable time for BSFB larvae to be parasitized by the parasitoids. In the natural eco-system the similar scenario also happened. BSFB lays eggs on the underside of the brinjal leaves. After hatching the neonate larvae are looking for suitable shoots or fruits and during that time they are exposed to predation or parasitism. In addition, at later instars of BSFB larvae, parasitoids locate the feeding holes made by the borer inside shoots and fruits of brinjal and parasitize them easily by inserting their long ovipositor through the holes. On the other hand, in rice field, the RLF lays their eggs on rice leaves, living and after hatching, they start feeding and start weaving threads to fold the leaves, living inside. There is ample hiding spaces of RLF larvae in the rice eco-system to escape from *T. flavoorbitalis* parasitism compared to that of BSFB in brinjal eco-system. So, it is clear from the above discussion that the parasitism efficiency of *T. flavoorbitalis* is higher on BSFB larvae than that on RLF larvae.

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Evaluation of an IPM Module against the Leafhopper, *Amrasca devastans* (Distant) in Cotton

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ABSTRACT

To develop an IPM module against cotton leafhopper different tactics like, leafhopper resistant cultivar (KC 2) were integrated in different combinations and were evaluated. When the susceptible LRA 5166 was raised from imidacloprid treated seed with cluster bean intercrop and need based application of dimethoate 0.03 % leafhopper population was reduced by 79.01 per cent and seed cotton yield increased by 31.76 %. The pest reduction was 57.08 % with mere introduction of resistant cultivar *viz.*, KC 2; the yield increase was 58.82 %. The yield increase was 125.88 % when the resistant cultivar KC 2 was raised from imidacloprid treated seeds, grown with cluster bean intercrop and applied with dimethoate on need basis.

Key words: Cotton, *Amrasca devastans*, intercrop, seed treatment, imidacloprid

INTRODUCTION

Pesticide load in crop ecosystem has culminated in many undesirable effects such as resistance, resurgence, residues *etc.*, disturbing the agro-ecosystem. Sprays and soil application of pesticides are costly and cumbersome to adopt. In India, 45 percent of the pesticides are applied (Chaudhary and Laroia, 2001) in cotton alone. So it is imperative to find out eco-friendlier components of integrated pest management. Eco-friendly, less costly measures such as cropping system approach, seed treatment, plant products are fitting well in Integrated Pest Management (IPM) as they are more advantageous over insecticides (Kiritani, 1979). The choice of methods in the IPM strategy depends upon the locality, insect species complex and efficiency and economics of pest control measures. There is a great potential for management of different insect pests of cotton based on IPM technology (Simwat, 1994; Gautam, 1998). Hence, a study was made with an objective of developing an IPM Module integrating resistant cultivar, cropping system approach, seed treatment, need based application of insecticides against the cotton leafhopper *Amrasca devastans* (Distant).

METHODOLOGY

Field experiments on the integration of different tactics of pest management *viz.*, resistant cultivar, seed treatment, cropping system approach and need based application of insecticides were taken up during Summer 2003 at Thirupanikarisalkulam farmer's field with twelve treatments. The experiment was replicated thrice in randomized block design. The plot size was 80 m². In the plots with intercropping treatment a row of cluster bean was raised in between every paired row of cotton. The total population of cotton plants was maintained as that of pure crop. The treatments were: T₁-LRA 5166 – no treatment; T₂- LRA 5166 + seed treatment with imidacloprid 17.8 SL (10 ml kg⁻¹); T₃- LRA 5166 + cluster bean; T₄-

LRA 5166 + spraying with dimethoate 30 EC (0.03 percent) at ETL; T₅- LRA 5166 + seed treatment with imidacloprid 17.8 SL (10 ml kg⁻¹) + cluster bean; T₆- LRA 5166 + seed treatment with imidacloprid 17.8 SL (10 ml kg⁻¹) + cluster bean + spraying with dimethoate 30 EC (0.03 percent) at ETL; T₇- KC 2 – no treatment; T₈-KC 2 + seed treatment with imidacloprid 17.8 SL (10 ml kg⁻¹); T₉- KC 2 + clusterbean; T₁₀-KC 2 + spraying with dimethoate 30 EC (0.03 percent) at ETL; T₁₁-KC 2 + seed treatment with imidacloprid 17.8 SL (10 ml kg⁻¹) + cluster bean; and T₁₂- KC 2 + seed treatment with imidacloprid 17.8 SL (10 ml kg⁻¹) + cluster bean + spraying with dimethoate 30 EC (0.03 %) at ETL.

The acid delinted (using concentrated sulphuric acid @ 100 ml kg⁻¹ of seed) seeds were used for the experiments. In case of imidacloprid seed treatment, to treat one kg of seed 0.5 g of *Acacia* gum powder and 20 ml of water were used. Gum was dissolved in water and then mixed with the 10 ml imidacloprid 17.8 SL. The seeds were thoroughly mixed with gum + insecticide mixture, dried under shade and kept for 24 hours before sowing. Untreated acid delinted seeds served as untreated check (UTC).

Statistical Analysis

The data were transformed into angular or square-root values for statistical scrutiny, wherever necessary (Gomez and Gomez, 1984). The experiments were subjected to statistical scrutiny following the method of Panse and Sukhatme (1989) and Gomez and Gomez (1984) and the means were compared with Least Significant Difference (L.S.D.).

RESULTS

The result of the field experiment conducted on thee integration of different tactics of pest management *viz.*, resistant cultivar, seed treatment, cropping system approach and need based application of insecticides is

presented in Table 1. Variability in the leafhopper population among the treatments was distinguishable. Mean leafhopper population ranged from 0.89 to 4.24 and 0.58 to 1.82 for LRA 5166 and KC 2 respectively. KC 2 crop grown from imidacloprid treated seeds, raised along with cluster bean and sprayed with dimethoate based on ETL (0.58/3 leaves) recorded the least incidence of leafhopper. It was followed by LRA 5166 crop grown from imidacloprid treated seeds, raised along with cluster bean and sprayed with dimethoate based on ETL (0.89/3 leaves) followed by KC 2 crop grown from imidacloprid treated seeds and raised with cluster bean intercrop (1.00/3 leaves); however the latter treatment was on a par with KC 2 crop sprayed with dimethoate (1.12/3 leaves) and KC 2 crop raised from imidacloprid treated seeds (1.28/3 leaves).

LRA 5166 grown from imidacloprid treated seeds and raised along with cluster bean (1.48/3 leaves) equaled the former treatments as well as LRA 5166 crop protected with dimethoate at ETL (1.54/3 leaves), KC 2 crop raised along with cluster bean (1.59/3 leaves) and imidacloprid seed treated LRA 5166 (1.90/3 leaves). Untreated KC 2 (1.82/3 leaves) was better than LRA 5166 with cluster bean intercrop (2.22/3 leaves) and untreated LRA 5166, but was on a par with LRA 5166 under need based protection, KC

2 raised with cluster bean and LRA 5166 crop raised from imidacloprid treated seeds. Among the treatments with LRA 5166, LRA 5166 infused with all tactics (560 kg ha⁻¹) and LRA 5166 crop under need-based protection (535 kg ha⁻¹) were able to register significantly higher yield than other treatments and untreated LRA 5166 (425 kg ha⁻¹); whereas with respect to the treatments with KC 2, all the treatments were able to register 5.19 to 42.22 percent higher yield than untreated KC 2 (675 kg ha⁻¹).

DISCUSSION

The most viable option to manage the cotton pests is the integrated pest management. Actual integration involves proper choice of compatible tactics and blending them so that each component potentiates or complements the other. Probably, the earliest example of integration of techniques was the use of a combination of resistant varieties and sanitation practices as prophylactic measures combined with application of calcium arsenate at high population level in case of boll weevils on cotton in USA during first quarter of the twentieth century. In cotton, a number of cultural and mechanical practices were successfully implemented along with judicious use of insecticides for the management of bollworm under an ICAR sponsored

Table 1. Influence of Integrated pest management tactics on leafhopper population and cotton yield

Treatments	Leafhopper		Pad Kapas content		Cotton Seed Yield	
	no. / 3 leaves	Per cent over T ₁	%	Per cent over T ₁	kg /ha	Percent T ₁
T ₁	4.24 (2.09) ^h	---	18.56 (25.52) ^e	--	425	--.
T ₂	1.90 (1.43) ^{ef}	55.19	16.75 (24.56) ^{ef}	9.75	440	3.52
T ₃	2.22 (1.60) ^g	47.64	14.91 (22.71) ^d	19.67	465	10.58
T ₄	1.54 (1.41) ^{ef}	63.68	13.08 (21.20) ^b	29.53	535	25.8
T ₅	1.48 (1.35) ^{de}	65.09	15.13 (22.89) ^d	18.48	475	11.76
T ₆	0.89 (1.15) ^b	79.01	12.25 (20.48) ^{ab}	33.99	560	31.76
T ₇	1.825 (1.46)	57.08	16.82 (24.21) ^f	9..38	675	58..82
T ₈	1.28 (1.27) ^{cd}	69.81	15.58 (23.25) ^{dc}	16.06	710	67.05
T ₉	1.59 (1.40) ^{ef}	62.50	12.41 (20.61) ^{ab}	33.14	690	62.35
T ₁₀	1.12 (1.26) ^{cd}	73.59	11.58 (19.89) ^a	37.61	795	87.06
T ₁₁	1.00 (1.18) ^{bc}	76.42	13.68	26.29	885	108.23
T ₁₂	0.58 (1.01) ^a	86.32	11.55 (19.86) ^a	37.77	960	125.88
Mean	1.64 (1.38)	--	14.36 (22.21)	--	635	
Significance			0.01	---	--	9
CD (p=0.05)			0.93	--	--	

Operation Research Project (ORP) in Tamil Nadu (Sundaramurthy and Chitra, 1992). Success stories on the effect of various components of integrated pest management on cotton bollworms are available from Punjab (Sandhu *et al.*, 1978).

Simwat (1994) and Murugesan *et al.* (2006) enumerated the different management practices *viz.*, resistant cultivar, intercropping and need based application of botanicals or systemic insecticides. However, the present study may probable the first in India to establish the utility of integration of several tactics *viz.*, resistant cultivar, seed treatment, intercropping and need based application of synthetic insecticides. When the susceptible LRA 5166 was raised from imidacloprid treated seed along with cluster bean and need-based application of dimethoate (0.03%) was able to reduce the leafhopper population by 79.01 per cent and increase the seed cotton yield by 31.76 per cent. Mere introduction of resistant cultivar *viz.*, KC 2 resulted in 57.08 percent reduction in the pest population and 58.82 per cent increase in the yield.

The yield increase was 125.88 per cent when KC 2 (resistant cultivar) was raised from imidacloprid treated seeds, grown with cluster bean intercrop and applied with dimethoate on need basis. Singh and Dhaliwal (1994) suggested location specific pest management practices with simple combinations of different methods of control, keeping in view of farmer's acceptability, which is ecologically, economically and sociologically accepted needs to be developed to have sustained crop production. Host plant resistance is a vital tool of IPM. It suppresses the pest population with least disturbance to cotton ecosystem and also reduces the dependence of insecticides. Several studies proved the worthiness of resistant variety to be used as the basement over which other strategies can be pyramided to have effective IPM (Adkisson and Dyck, 1980). The major advantage of using resistant variety is to induce a constant level of pest suppression in each generation. Moreover, the number of pests produced on a resistant variety usually decline over time, making control with insecticides much easier.

Several earlier workers also reported better growth of the plants of imidacloprid treated seeds in cotton (Dandale *et al.*, 2001; Gupta and Roshan Lal, 1998). The effectiveness of imidacloprid seed treatment gains support from earlier studies (Dandale *et al.*, 2001; Karabhantanal *et al.*, 2001; Murugesan *et al.*, 2006) in cotton.

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Evaluation of biosynthesized silver nanoparticles against fungal pathogens of mulberry *Morus indica*

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ABSTRACT

Biologically synthesized silver nanoparticles were subjected for *in vitro* studies against fungal pathogens *Cerotelium fici*, *Cercospora moricola* and *Phyllactinia corylea* of mulberry. Biologically synthesized silver nanoparticles showed promising antifungal activity against assayed fungus.

Key words: Nanotechnology, silver nanoparticles, fungal diseases, mulberry.

INTRODUCTION

Sericulture has been rightly adjudged as one of the tools for rural development by the planners (Datta, 1994). Introduction of new technology of sericulture has made the industry a highly remunerative crop as reflected in increasing acreage being brought under mulberry cultivation of the step-up in the raw silk output being witnessed every year (Anonymous, 2000; Datta, 2000; Arundhati Choudhury *et al.*, 2004).

Mulberry silkworm, *Bombyx mori* L. sustains its nutrition from mulberry, *Morus indica*. Quality of mulberry leaves plays an important role in the success of sericulture industry and this directs its economics. Foliar diseases of mulberry plays a critical role in silkworm rearing. Leaf rust, leaf spot, powdery mildew caused by *Cerotelium fici*, *Cercospora moricola* and *Phyllactinia corylea* are some of the major pathogens of fungal diseases of mulberry. They reduce the leaf yield to the extent of 10-15% in terms of premature defoliation and 20-25% by destruction of leaf lamina. Nutritional values of leaf also get depleted by reducing its total proteins, total sugars, chlorophyll and moisture contents. Therefore, disease management in mulberry is one of the prerequisites for successful silkworm rearing. In the present investigation an attempt was made on the nanotechnology application for the management of fungal borne diseases of mulberry. Therefore, the goals of this study were in investigate the influence of biologically synthesized silver nanoparticles on the control of fungal pathogens of mulberry, *Morus indica*.

MATERIAL AND METHODS

Investigation made on the effect of biologically synthesized silver nanoparticles role on the control of fungal species *Cerotelium fici*, *Cercospora moricola* and *Phyllactinia corylea* of mulberry *M.indica*. Field study was conducted at Karasamangalam, which is 15km away from Thiruvalluvar University campus. Fungal infected

part of the mulberry field was identified for the study. The biologically synthesized silver nanoparticles was sprayed on the mulberry garden @ 5lt/ha. The treatment with a water spray was kept as control.

Synthesis of silver nanoparticles was mediated using *Spirulina platensis* as described by Singaravelu and Ganesh Kumar, (2006). Briefly one gram of *S.platensis* was dissolved in 50ml of 10^{-3} M concentration of aqueous $AgNO_3$ solution. After completion of reaction the nanoparticles solution was characterized using UV-vis spectrophotometer and Transmission electron microscope (Figure 1).

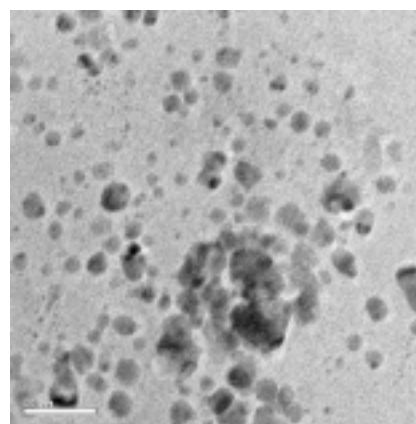


Figure.1 Transmission electron microscopic Photograph showing the *Spirulina platensis* mediated silver nanoparticles.

One gram of the diseased leaves were weighed in a sterile container and homogenized in a pre-sterilized homogeniser. The homogenized samples were streaked separately on mycological agar plates and all the inoculated plates were incubated at room temperature ($28^{\circ}C \pm 2^{\circ}C$) for 2 to 4 days. After incubation period, the fungal colonies were picked up from the agar plate and stored for

Table 1. Effect of biologically synthesized silver nanoparticles on the control of fungal pathogens of mulberry *Morus indica*.

Silver nanoparticles	Fungal pathogens	Zone of inhibition (mm)
20µl	<i>Cerotelium fici</i>	18.12
	<i>Cercospora moricola</i>	17.23
	<i>Phyllactinia corylea</i>	21.15
50µl	<i>Cerotelium fici</i>	23.00
	<i>Cercospora moricola</i>	25.00
	<i>Phyllactinia corylea</i>	27.23
100µl	<i>Cerotelium fici</i>	25.12
	<i>Cercospora moricola</i>	27.00
	<i>Phyllactinia corylea</i>	28.00

identification. The spores slant culture was mixed up with one or two drops of lacto phenol cotton blue which was kept over the clean glass slide. A cover slip was put over the culture and observed under the microscope to identify the fungal organism. Fungicide activity of biosynthesized silver nanoparticles was studied at different concentrations. Fungal species collected from the infected mulberry leaves were subjected for microbial culture. Well Diffusion assay was employed to assess the inhibitory effect of biosynthesized silver nanoparticles at a 20µl, 50µl and 100µl concentrations.

Results

The results on the *in vitro* studies on the efficacy of biosynthesized silver nanoparticles against *Cerotelium fici*, *Cercospora moricola* and *Phyllactinia corylea* are presented in Table I. The diameter of inhibition zones around the subjected three fungal strains clearly indicates the effect of biosynthesized silver nanoparticles. The antifungal activity of biosynthesized silver nanoparticles seems to be same among the three different species, however the inhibitory effect was high in the concentration of 100µl. Table 2 depicts the results of field evaluation of biosynthesized silver nanoparticles on the control of fungal pathogens of mulberry *M.indica*. The subjected fungal infected part of the mulberry garden get free of fungal infection in 10 days after the application of biosynthesized silver nanoparticles. It maintains till 30 days.

Discussion

The use of chemical pesticides may achieve a measure of control of those mulberry diseases but there remains the problem of residual toxicity in the treated plants. The toxicity of nematocide furadan results in reduced

palatability of the leaves to the feeding silkworm larvae, reduction in growth of the larvae and also in silk production (Paul *et al.*, 1995). The problems reported due to the application of pesticide includes reduced palatability, reduction in growth, oviposition behaviour and economical parameters of silkworm *Bombyx mori* L. Foliar diseases reduce the leaf yield and quality, thus affecting the silkworm rearing. Leaf yield will be reduced by the premature defoliation due to diseases and by way of reducing the total consumable area in those leaves, which do not defoliate, but are diseased. Similarly, diseases also reduce the protein and moisture content in the leaves. The commercial characters were greatly reduced when the larvae were fed by the leaf affected with rust, leaf spot and powdery mildew.

Nanoscience is a relatively new branch of science dedicated to the improvement and utilization of devices and structures ranging from 1 to 100nm in size, in which new chemical, physical and biological properties, not seen in bulk materials can be observed (Roco, 1998). Nanomaterials have received considerable attention because of their potential for application in a wide spectrum of areas that include biology and medicine (Albers *et al.*, 2001; Park *et al.*, 2002). Much of this interest stems from the fact that nanomaterials possess interesting optoelectronic properties and is also due to their size compatibility with a variety of biologically active molecules. Nanoparticles could also serve as excellent delivery vehicles for a variety of biomolecules such as proteins, DNA and drugs (Hrushikesh *et al.*, 2006). Reducing the particle size of materials is an efficient and reliable tool for improving their biocompatibility. In fact, nanotechnology helps in overcoming the limitations of size and can change the outlook of the world regarding science (Mirkin and Taton, 2000). Furthermore, nanomaterials can be modified for better efficiency to facilitate their applications in different fields such as bioscience and medicine.

The usefulness of silver as an antimicrobial agent has been known for a long time. It is an effective agent with low toxicity, which is especially important in the topical antibacterial treatment of burn wounds, where transient bacteremia is commonly cited (Mozingo *et al.*, 1997). Combination of different antibiotics with silver nanoparticles exhibit synergistic antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Shahverdi *et al.*, 2007). The mechanism of inhibitory action of silver ions on microorganisms is partially known. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag⁺ treatment (Feng *et al.*, 2000). In addition it was also shown that Ag⁺

Table 2: Impact of fungal pathogens on the fungal disease burden under field condition.

Fungal Pathogens	Duration of observation on the fungal disease burden					
	10th day		20th day		30th day	
	Control	Experimental	Control	Experimental	Control	Experimental
<i>Cerotelium fici</i>	+++	-	+++	-	+++	-
<i>Cercospora moricola</i>	+++	-	+++	-	+++	-
<i>Phyllactinia corylea</i>	+++	-	+++	-	+++	-

binds to functional groups of proteins, resulting in protein denaturation (Spadaro *et al.*,1974). The obvious question is how nanosized silver particles act as biocidal material against *E.coli*. There are reports in the literature that show that electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is crucial for the activity of nanoparticles as bactericidal materials (Stoimenove *et al.*,2002; Hamouda and Baker,2000).

With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. Such problems and needs have led to the resurgence in the use of silver (Ag)-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics (Jones *et al.*,2004)

The antibacterial effects of Ag salts have been noticed since antiquity (Silver and Phug, 1996) and is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds (Catauro *et al.*,2004 and Crabtree *et al.*,2003). In fact, it is well known that Ag ions and Ag-based compounds are highly toxic to microorganisms, showing strong biocidal effects on as many as 12 species of bacteria including *E. coli* (Zhao and Stevens,1998). Aymonier *et al.*,(2002) showed that hybrids of Ag nanoparticles with amphiphilic hyperbranched macromolecules exhibited effective antimicrobial surface coating agents. Results of the present investigation clearly indicate the antifungal activity of biologically synthesized silver nanoparticles. The study also confirms the non-toxic nature of biologically synthesized silver nanoparticles. Since the normal growth of the silkworm, which fed with biosynthesized silver nanoparticles treated leaves not affected by any way.

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