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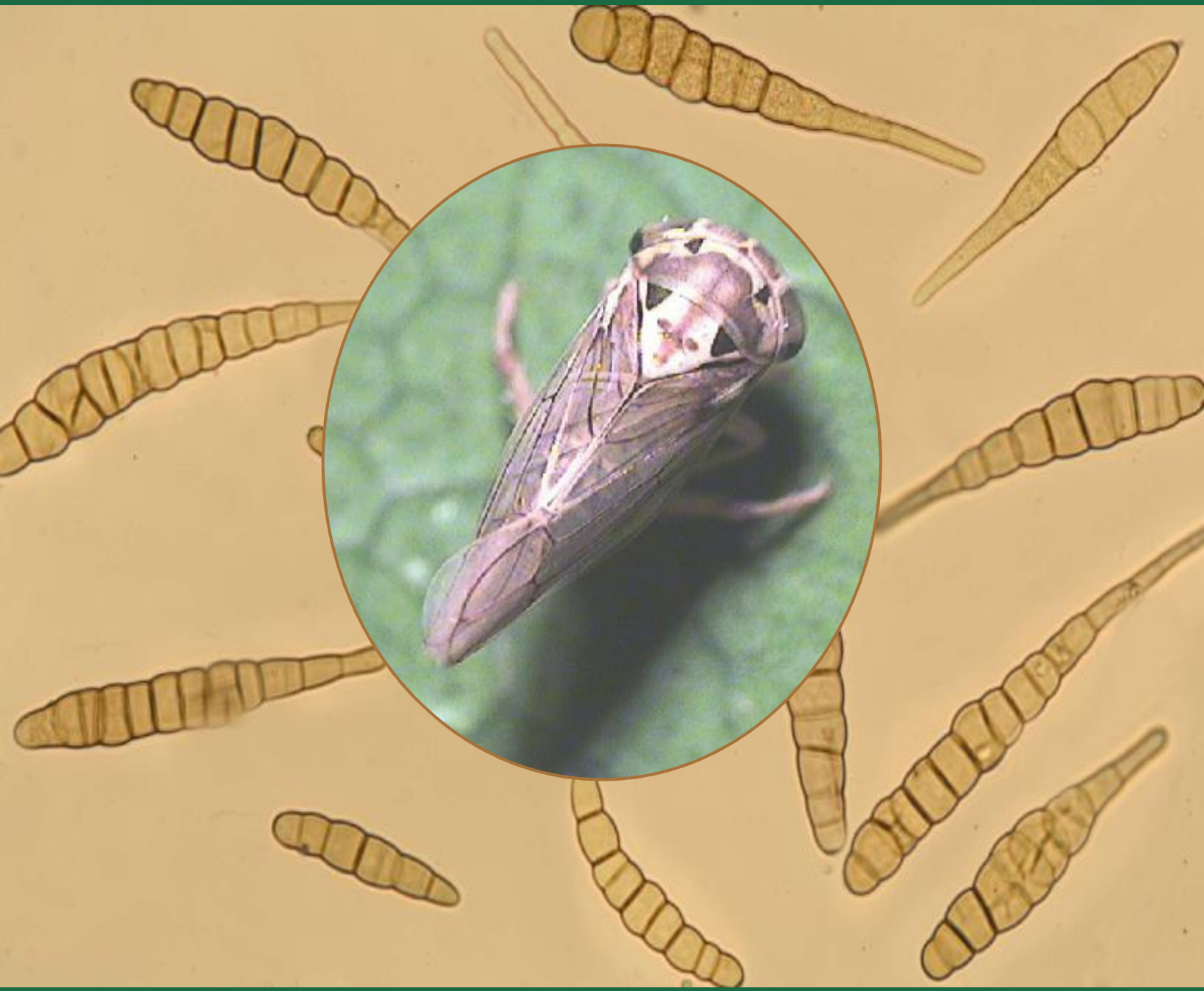

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Pest Management in Horticultural Ecosystems

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All papers and editorial matters are to be addressed to the Chief Editor, Division of Entomology, Indian Institute of Horticultural Research, Bengaluru – 560 089, India. E-mail: **editorpmhe@gmail.com**

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NAAS 2012 Rating : 3.7

COVER PHOTOS : Inset : Mango hopper, *Amritodus atkinsoni* (Courtesy : Ms. S. Devi Thangam, IIHR)
Background : Conidia of *Alternaria brassicae* (Courtesy : Dr. P. Chowdappa & Mr. S.P. Mohan Kumar, IIHR)

Editor's Choice

Beware of invasive pests!

An unwanted fall out of the globalization is the potential risk of exotic pests (insects, pathogens, weeds etc.,) getting entry to the new regions. A list of 100 world's worst invasive alien species compiled by the Invasive Species Specialist Group (ISSG) comprises several insects, diseases and plant species of agricultural importance which have high potential to either interfere with or erode the local biodiversity. From India's point of view, there are about a dozen dreadful species like Mediterranean fruit fly, the Western flower thrips, cotton boll weevil etc., just awaiting an opportunity to enter. This underscores the importance of stringent plant quarantine regulations. Rightly, in the recent past, biosecurity and plant quarantine, have become focus areas of every scientific gathering related to pest management. Considering their importance, the present issue carries a special article on invasive species whose authors hold very high position in the plant protection and quarantine set up in India.

Emphasizing the relevance and the importance of the biosecurity issue, for the benefit of stake holders, major highlights of the proposed Agricultural Biosecurity Bill, 2013 which was introduced in the Lok Sabha on March 11, 2013 by the Union Minister of Agriculture are provided below.

- The Bill aims to establish an integrated national biosecurity system covering plant, animal and marine issues to combat threats of bio-terrorism from pests and weeds to increase the national capacity to protect human health and agricultural production, and also to equip the country to meet obligations under several trade and sanitary agreements in food and agricultural products.
- The Bill repeals the Destructive Insects and Pests Act, 1914 and the Livestock Importation Act, 1898.
- The Bill establishes the Agricultural Biosecurity Authority of India with head office at Faridabad. The Authority shall be headed by a Director General, and comprise experts in plant and animal pests and diseases, and representatives of various ministries and organisations. The functions of the Authority shall include: (i) regulating the import and export of plants, animals and related products; (ii) preventing the introduction of quarantine pests from outside India; and (iii) implementing post-entry quarantine measures
- No person shall import any plant, animal, and plant or animal products in contravention of notifications or guidelines issued by the Authority. Exceptions shall be provided to those imports that are issued permits by the Authority, and imports with sanitary or phytosanitary certificates.
- The Customs Act, 1962 or other laws in force, that prohibit the import of certain customs and goods shall also apply to those pests, plants and animals, which require permits or are prohibited by the Authority.
- No person shall possess, move, grow, raise, culture, breed or produce any plant, animal and related products if he has reason to believe that such a product is or may be carrying a quarantine pest.
- The Authority may notify any pest to be a quarantine pest. It can also notify an area to be a controlled area if it suspects or determines that the area is infested or infected with a quarantine pest.

- The Authority may recommend that the central government declare a biosecurity emergency in an area in case of an outbreak, distribution, or spreading of a pest or organism, which has the potential to cause a significant loss to biosecurity.
- The penalty for contravening the provisions of this Act is an amount that can extend up to Rs two lakh. The punishment for interfering with seized items is imprisonment up to six months and a fine extendable to Rs two lakhs.

(SOURCE: http://www.prsindia.org/billtrack/the-agricultural-biosecurity-bill-2013-2674/)

So next time when your flight baggage contains a flower bouquet or a few fresh fruits from abroad, remember you may as well be bringing in a dreadful quarantine pest! And may also liable to be prosecuted.

P.V. RAMI REDDY
Chief Editor

Congratulations!

AAPMHE's Hall of fame is becoming richer day by day. Dr. Abraham Verghese, the former Chief Editor of PMHE, and the Head, Division of Entomology and Nematology, IIHR, Bengaluru, has assumed the charge as the Director of the National Bureau of Agriculturally Important Insects (NBAII), Bengaluru in April 2013. It is a proud moment to the AAPMHE's fraternity. Dr. Verghese needs no introduction to the readers of PMHE, as he was the Chief Editor for sixteen long years, and instrumental in nurturing and shaping the success of this journal. An entomologist of international repute, Dr. Verghese has been the leading light of fruit entomology research in the country. Having more than hundred and fifty research papers, several commercialized technologies, patents and awards to his credit, Dr. Verghese is an acclaimed scientist, admired teacher and an able administrator. Dr. Verghese is one of those very few entomologists, who have been conferred with the prestigious Fellow of Royal Entomological Society, London. Though an 'insect control expert' by profession, by passion, Dr. Verghese is an ardent naturalist and bird lover. He enjoys analyzing the ecological complexities of insect life. Hope this transition from being a 'crusader' of insect pests to the 'custodian' of insect species will bring many more laurels and under his guidance, Agricultural Entomology in India reaches newer heights. On behalf of all the members and the Executive Council of AAPMHE, I congratulate and wish Dr. Verghese all the best and success in his new assignment.

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REVIEW ARTICLE

Management of the giant African snail, *Achatina fulica* (Bowdich) (Stylommatophora: Achatinidae) in India

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ABSTRACT : The giant African snail, *Achatina fulica* (Bowdich) is one of the most extensively studied snails because of its economic, ecological and medical importance. An array of literature concerning its management is on record, both at global and the Indian context. This review provides an account of all management measures devised against *A. fulica* in different agri-horticultural ecosystems of India, serving as an update of currently known measures and possible impetus for designing future measures. Recommending an integrated approach involving cultural, mechanical, chemical and biological methods seems inevitable to manage the impact of the snail on Indian agriculture.

Keywords: *Achatina fulica*, giant Aftrican snail, IPM, molluscicides, metaldehyde baits

INTRODUCTION

The giant African snail, *Achatina* (*Lissachatina*) *fulica* (Bowdich) (Stylommatophora: Achatinidae), a native of east Africa has invaded many countries in the world and established as a polyphytophagous pest (Raut and Barker, 2002). It is reported to feed on at least 500 different types of plant species (Capinera, 2011) and is extensively studied snail of economic, ecological and medical importance (Mead, 1979; Raut and Ghose, 1984; Srivastava, 1992; Raut and Barker, 2002; Fontinilla *et al.*, 2007).

BIOLOGY

Achatina fulica carries a narrow, conical shell, half as wide as its length. The shell of fully grown adults has 7 to 9 ridges, or whorls. It is normally reddish-brown with faint yellowish vertical markings. The length of the adult shell may exceed 20 cm (8"), but usually averages between 5 and 10 cm and weighs, on average, 32 g. They are obligate-outcrossing hermaphrodites. The eggs, measuring 4.5 mm to 5.5 mm in diameter, hatch at temperatures above 15°C. Hatched snails become mature adults in 6 to 12 months and remain fertile for 400 days. A snail lays up to 100 and 500 eggs during the first and second years, respectively. Although the fertility rate declines after the second year, giant African snails may live up to 5 years, yielding a total of up to 1,000 eggs (Raut and Barker, 2002; Invasive Species Specialist Group, 2012). Raut and Barker, (2002) have enumerated numerous management measures practiced world over in addition to those in India. The present review is updated in addition to strategies mentioned in the former, thus serving as an update of currently known *A. fulica* management measures and intended to serve as a possible source for designing future measures.

GLOBAL PEST STATUS

Achatina fulica has been recorded in every continent except Antarctica and is a major crop pest across the globe (Mead, 1979; Raut and Barker, 2002) (Fig. 1). It is a classic example of an introduced species and has been listed as one of the world's 100 most invasive species by the International Union for Conservation of Nature and Natural resources (IUCN) (Lowe *et al.*, 2000).The giant African snail has gained attention due to its large size, supposed medicinal properties and its potential as human or animal food source (Mead, 1979; Raut and Barker, 2002) and its success as an introduced species is attributed to several factors *viz*., high reproductive capacity, voracious feeding habit, inadequate quarantine arrangements and human aided dispersal (Fontinilla *et al.*, 2007).

In India, it was believed to have been introduced in Chouringhie gardens of Calcutta in 1847 by the British Conchologist William Henry Benson and from there on spread to many states of the country in course of time (Mead, 1961). According to Malacologist Naggs (1997),

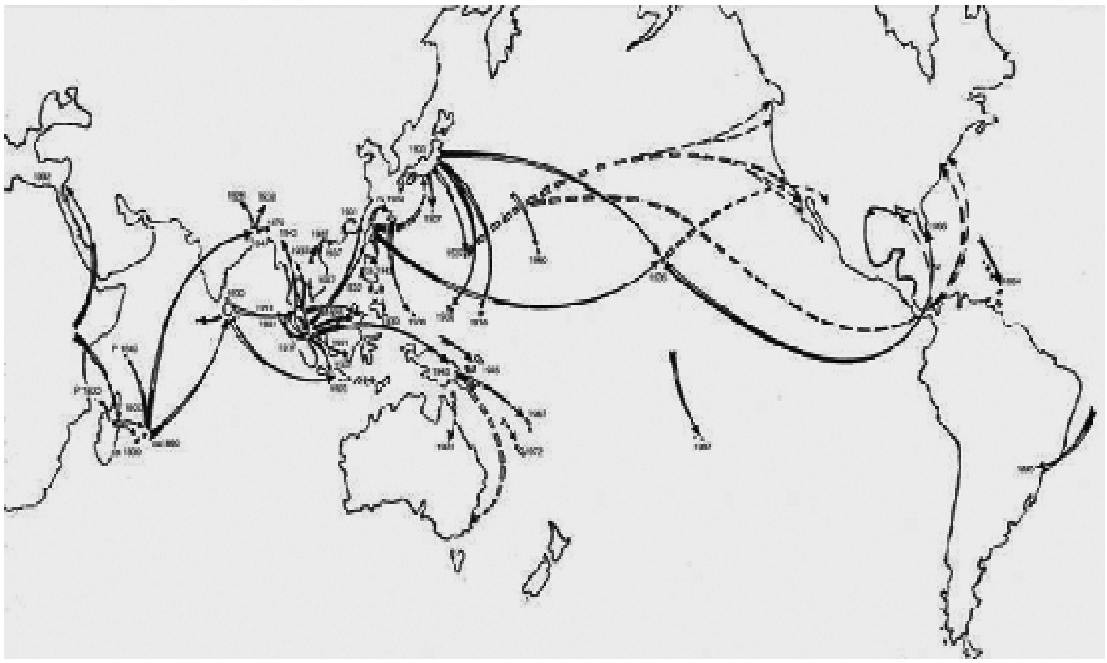

Fig. 1. Global dispersal of *Achatina fulica* (Bowdich) (Raut and Barker, 2002)

although Benson had got the snail to India from Mauritius, he had handed over them to a friend before leaving India and it was the friend who released them in his garden. This perhaps has led to the large scale invasion of the snail in different parts of the country. Recent genetic studies indicate that all *A. fulica* now occurring throughout South Asia, Southeast Asia and the Pacific region are derived from one haplotype (Fontinilla *et al.*, 2007), the source being a single pair of specimens released in Chouringhie in 1847. On Andaman's, it is reported to have been introduced by the Japanese during the Second World War, indicating a second route of introduction of the pest into India (Srivastava, 1992).

DISPERSAL AND PEST STATUS IN INDIA

Surveys carried out in India have revealed that, since its introduction, more than 140 years ago in Calcutta, *A.fulica* has spread and established in different parts of the country (Raut and Ghose, 1984; Srivastava, 1992; Raut and Barker, 2002) (Fig.2), reflecting the snails alarming invasive characteristics *viz*., high reproductive rate (Fig.3) and generalist consumptive patterns. It is known to cause serious damage on different crops in India (Table 1 and Figures 4-6).

MANAGEMENT

The snail almost invariably assumes pest status when introduced to areas of favorable climate clearly pointing to the lack of significant population regulation by pathogens, parasites and predators, at least in the early phases of invasion by the pest. In pursuit of managing the pest, diverse management options including cultural, mechanical, biological and chemical, have continued to play a major role. Some of the main pest control methods practiced in Indian agro-ecosystems is reviewed in this article.

Cultural control

Sharma and Agarwal (1989) have recommended control measures including field sanitation by removing possible hiding places such as bushes and debris from fields to manage the snail menace.

Mechanical or Physical control measures

Adults of *A.fulica* are large enough to be seen easily and handpicking them from resting sites (garbage, weeds and house refuse) in the evening and the morning is crucial for managing the snails (Sharma and Agarwal, 1989; Shah, 1992). Snails are susceptible to different types of traps like food bait traps, hence several trapping devices have been used to monitor and manage the nocturnal snails. Physical barriers to prevent movement of snails reported by different authors in Raut and Barker (2002) include the use of a strip of bare soil around the crop, a fence with a screen of corrugated tin or security wire mesh. Ditches dug around the field and daily collection of snails. Protection of valuable horticultural plants during vulnerable seedling stage by ringing them with a strip of cardboard dipped in a suspension of metaldehyde. However no such physical barriers are

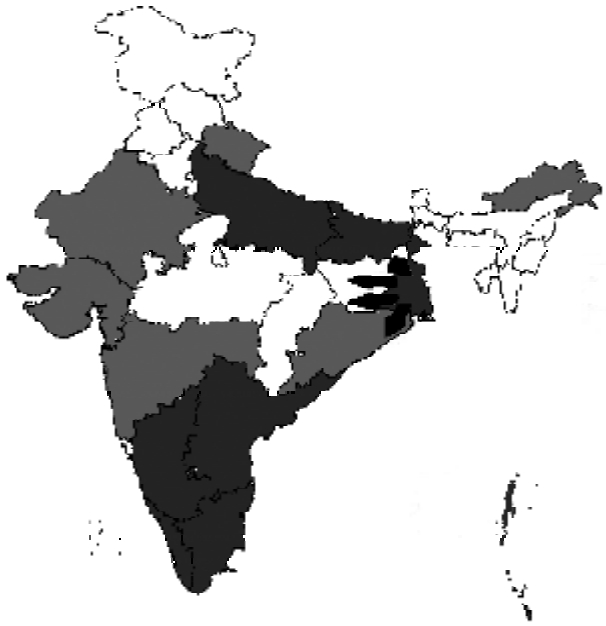

Fig. 2. DIVA-GIS generated map indicating current status and distribution of *A. fulica* in different states of India

Reported as serious pest
Reported, further details on pest status unavailable
Reports unavailable
Point of introduction into India and probable human aided dispersal

reported from India. Ravikumara *et al.,* (2007) recorded highest number of snails attracted to papaya stem waste, followed by vegetable waste, fishmeal waste, areca leaf waste, banana sheath waste, sugarcane bagasse, leaf litter and farmyard manure. Handpicking, involving a considerable labour expense was found to be very effective in commercial nurseries in Bangalore south taluk (Jayashankar *et al.,* 2009, 2010). As fecundity is a best indicator of invasive population of the snail (Jayashankar, 2010), management efforts therefore should include understanding of the reproductive behaviour to predict and perhaps impede, its spread in the introduced area. The snail is regarded sacred and referred to as *Basavana hula* (in Kannada - The holy bullock incarnation) hence, there is a sense of stigma in eradicating it, more over some farmers believe it to be harmless facilitating crop growth (Jayashankar, 2011).

Chemical control measures

Molluscicides like metaldehyde are indispensable for the management of malacofaunal pests in agri-horticultural ecosystems and there has been increasing interest in the use of different chemical formulations to curtail their imminent havoc. However, in most cases they are found to be non-selective and believed to endanger the survival of non-target snails, including the endemic fauna (Prasad *et al.,* 2004).

Organic compounds

Metaldehyde

Utility of different dosage of metaldehyde is reported by different workers in different horticulture ecosystems. Sharma and Agarwal (1989) reported 5 % metaldehyde pellets at 25 kg/ha to manage the snails and Basavaraju *et al.,* (2000) reported 2.5% metaldehyde pellets effective in controlling the snail occurring on betel vine (*Piper betle*) in Karnataka during kharif. Under lab conditions Basavaraju *et al.,* (2001), found metaldehyde pellets as most effective against *A. fulica*, compared to methomyl and carbofuran. The results of field experiments showed that Metaldehyde application (25 kg 2.5% metaldehyde pellets/ha) all along the borders of newly-established betel

Table 1. Incidence of *A.fulica* on different hosts in India

S.No.	Host Plant	Reference
1	*Eranthemum spp. Bougainvillea, Brassica oleracea* var. *botrytis*; *B.* o. var. *capitata, B. campestris* var. *rapa*, *Phaseolus aureus, Vigna radiata*	(Balasubramanian and Kalayanasundaram (1973)
2	*Vitis vinifera, Cajanus cajan, Sesamum indicum, Amaranthus tricolor, Beta vulgaris, Trichosanthes dioica*	Singh and Roy (1977)
3	Ornamental plants	Veeresh *et al.* (1979)
4	*Ipomoea batatas, Cucurbita maxima, Tagetes patula, Carica papaya, Luffa cylindrica, Lochnera rosea*	Singh and Roy (1979)
5	*Morus rubra, M. indica, M. laevigata, M. serrata*	Thangavelu and Singh (1983)
6	*Camelia sinensis*	Kakoty and Das (1988)
7	*Allium oleraceum, Solanum melongena,Lactuca sativa, Zea mays, Sorghum, Saccharum,* B.o. var. *caulorapa*	Sharma and Agarwal (1989)
8	*Morus* sp.	Shree and Kumar (2002)
9	*Piper betle, Capsicum annum, Areca catechu, Musa* sp., *B.capitata Solanum lycopersicum, Moringa sp.*	Javaregowda (2004)
10	*Averrhoa carambola, A. bilimbi, Moringa oleifera*	Prasad *et al.* (2004)
11	*Abelmoschus esculentus, Cucumis sativus, Solanum melanogena, Solanum lycopersicum, Tagetes patula, Catharanthus roseus, Luffa aegyptiaca, Vigna unguiculata, Cucurbita pepo, Amaranthus tricolor, Tricosanthes dioica*	Sheela Thakur (2004)
12	Home gardens and Flower pots	Mavinkurve *et al.* (2004)
13	Coffee, *Musa* sp., *Phaseolus, B. capitata, Cucumis sativus, Solanum lycopersicum, Capsicum annum*	Reddy and Sreedharan (2006)
14	*Vanilla planifolia*	Vanitha *et al.* (2008)
15	*Solanum lycopersicum, Cucumis sativus, Morus sp, Rosa sp. Tagetes sp, Arachis hypogaea, Phaseolus vulgaris, Carica papaya*	Sridhar *et al.* (2012)

vine gardens was effective against snails. Rao and Singh (2002) reported mortality ranging from 49 to 74 snails per plant using SnailKil (a.i., metaldehyde). According to Javaregowda (2006), metaldehyde (2.5%) was most effective and registered highest mortality after one day of application, followed by monocrotophos bait. Ravikumara *et al.* (2007) found highest number of snails attracted to papaya stems waste bait along with metaldehyde (6 kg metaldehyde bait per acre). Metaldehyde based traps placed during or just after the onset of monsoon were effective in controlling snails in vanillary in Tamil Nadu (Vanitha *et al.*, 2008). Metaldehyde 2.5% pellets (25 Kg/ha) along with methomyl 40SP@10g/Kg of fermented food bait (50 Kg wheat bran+5 Kg Jaggery+yeast 30 g/ha) gave 70% control (Shevale and Bedse, 2009).

Organophosphates

Basavaraju *et al.,* (2001) recorded 100% mortality using baits prepared with monocrotophos (600 ml monocrotophos 36 SL + 60 kg rice bran + 6 kg jaggery/ ha), carbofuran, methomyl and metaldehyde pellets under laboratory conditions. However monocrotophos bait recorded the lowest snail mortality compared to

Fig 3. A mating pair of *A. fulica*

Fig. 4. *A. fulica* on papaya plant

Fig 5. *A. fulica* cluster on a stone pillar in gherkin field

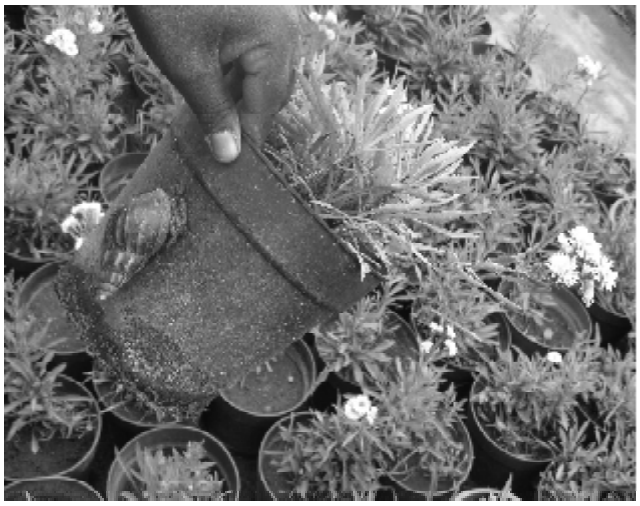

Fig. 6. *A. fulica* adhering to nursery pots

metaldehyde followed by methomyl and carbofuran. Panigrahi and Raut (1993) injected dichlorvos at 1.0 ml into 15-20 g pieces of vegetable or fruit baits (*Solanum lycopersicum, Cucumis sativus, Trichosanthes dioica, Vigna unguiculata* and *Hibiscus esculentus*), against *A. fulica* and showed that the poisoned tomato and *T. dioica* was accepted by the snail and in all cases the pests died after consuming the baits. Saxena and Mahendra (2000) found wet wheat flour (5-10g/snail) bait to be effective compared to gram, barley and conflour and dichlorvos (1.2, 2.5, 3.0 & 3.5 mg/snail) provided more than 90% mortality in *A. fulica* with the mentioned concentrations compared to malathion, trichlorofon and mexacarbate less than 96 hours. The control trial set by Justin *et al.,* (2008) against the snails suggested that spray of garlic extract (2%) or Neemazal (2 ml/L) as effective as soil application of phorate (Phorate 5G; 5 g/vine) granules in controlling the snails on vanilla vines.

Carbamates

Basavaraju *et al.,* (2001) observed 100% mortality under laboratory conditions using carbofuran (600 g carbofuran 3G + 60 kg rice bran + 6 kg jaggery/ha) bait against the snail. However it recorded less mortality compared to metaldehyde followed by methomyl and monocrotophos. Basavaraju *et al.,* (2001) used methomyl (600 ml methomyl 12.5 L + 60 kg rice bran + 6 kg jaggery/ha) bait against the snails and recorded 100% mortality under laboratory conditions. However, methomyl registered less mortality in the field compared to Metaldehyde. Shevale and Bedse (2009) recorded 70% control using methomyl 40SP at the rate of 10g/Kg of fermented food bait (50 Kg wheat bran+5 Kg

Jaggery+1500g yeast)/ha and metaldehyde (2.5%) pellets (25 kg/ha).

Pyrethroids

Rao and Singh (2002) found cypermethrin to be effective against *A. fulica*.

To summarize the understanding on management strategies using baits, a few precautions are mandatory to follow. It is good practice to apply baits after a site is watered or irrigated, as this stimulates snail activity, increasing the likelihood that baits will be eaten. However, none of the baits are completely effective because molluscs sometimes learn to avoid toxicants or may detoxify pesticides, recovering from sub-lethal poisoning. (http://ohioline.osu.edu/ent-fact/pdf/0020.pdf). Also, the ability of the snail to develop resistance is not monitored and remains to be explored.

Inorganic compounds

Kakoty and Das (1988) recorded 100% mortality using copper sulphate to manage the snails infesting tea estates. Sajeev (2011) adopted a control method of initially baiting the snail using cabbage leaves followed by spray with Tobacco decoction, made with Copper sulphate mixture (TDCS mixture). In addition to molluscicides, sodium chloride (common table salt), is proven to be an effective dehydrating agent and may be used as a barrier application on the perimeter of known/ suspected snail-infested areas (Shah, 1992). However, frequent renewal of such barriers during periods of rain or high relative humidity was recommended. Karnatak *et al*. (1998) recorded hundred per cent mortality after 96 hours at 5 per cent spray of sodium chloride.

Botanicals

The most effective concentration of the extract from Kernels of the fruit of *Thevetia peruviana* (Pers.) was 20 per cent; it killed 100 per cent of snails within a day, when the extract was exposed on the soil in experimental trays or when it was applied to potato slices offered as food to the gastropods (Panigrahi and Raut, 1994). Rao and Singh (2002) found *Cedrus deodara* oil to be more toxic among molluscicides of plant origin against *A. fulica* in single treatments while in binary treatments a combination of *Cedrus deodara+Allium sativum* to be more toxic. Softwood cutting fences made of alligator apple (*Annona glabra*) acted as snail repellents to protect the nursery beds (Prasad *et al.*, 2004). The control trial set by Justin *et al.,* (2008) against the snails suggested that spray of garlic extract (2%) or Neemazal (2 ml/L).

Biological control measures and Natural enemies

A. fulica almost invariably assumes pest status when introduced to areas of favourable climate clearly points to the lack of significant population regulation by pathogens, parasites and predators, at least in the early phases of pest invasion (Simberloff and Gibbons, 2004).

Microbes **:** A solution of bacterial pathogen that causes leucoderma-like disease extracted from dead snails sprayed on healthy snails provided significant control. Raut and Panigrahi (1989) reported 8 different types of pathogen caused disease in *A. fulica*. Spraying an aqueous extract of diseased *A. fulica* onto snails and their food plants gave satisfactory control (Srivastava *et al.,* 1985).

Invertebrates **:** The predatory turbellarian flatworm, *Platydemus manokwari*, introduced in late 1981 and early 1982 effectively reduced the snail population infesting cover crops (*Pueraria* sp. and *Centrosema* sp.) in about 20 months in the Philippines (Muniappan *et al.,* 1986). Such introductions has not yielded necessary relief in India wherein effective bio-control agents including *P. manokwari, Bipalium indica*, predatory snails *Euglandina rosea* (Ferussac) and *Gonaxis quadrilateralis* (Preston) have either led to destruction of native snails after introduction or failed to establish due to low reproductive potential when compared to the high reproductive potential that of the giant African snail (Srivastava *et al.,* 1992). Neither did reliance on the local carnivorous snail, *Gulella (indoennea) bicolor*, served any success as it was susceptible to chemicals used against the pest (Srivastava *et al.,* 1985). In Andaman Islands, the former authors also recorded a predatory millipede, *Orthomorha* sp., and hermit crabs, *Coenobita* spp., actively predating the snails and were not affected by molluscicides.

Vertebrates**:** Several vertebrate enemies of *A. fulica* have been reported by various workers in the Indian subcontinent. Avian fauna including *Centropus sinensis, Dendrocitta vagabunda, Tyto alba* and *Bubulcus ibis* and ducks are reported as effective predators of *A. fulica* (Mead, 1961; Raut and Ghose, 1984; Srivastava, 1992; Tehsin and Sharma, 2000; Jayashankar, 2011). The bandicoot rat, *Bandicota indica* was observed to be an effective predator especially for its nocturnal habit and the ability to locate both active and aestivating snails (Raut and Barker, 2002; Jayashankar, 2011). However, to employ a serious pest to control another pest is not advisable (Raut and Barker, 2002). The mongoose,

Herpestes mungo is reported feeding on the giant African snail in Ceylon (Sri Lanka) (Srivastava, 1992). Buddha and Naggs (2007) have reported live individuals of *A. fulica* being eaten by crows, monitor lizards, common Mongoose, Swans and Pigs in Nepal. Das (1995) mentioned that, India star tortoise, *Geochelone elegans* though herbivorous in nature is known to eat animals including *A. fulica,* in captivity.

Miscellaneous initiatives

Feed and fertilizer: According to the information gathered by Buddha and Naggs (2007) from local people in rural parts of Nepal, *A. fulica* is used by fish farmers as a diet for *Clarias batrachus*. Srivastava *et al*. (1984) and Aboua (1990) recommend the use of snail both as feed and fertilizers due the high percentage of phosphate and lysine in the snail flesh. Educational campaign such as "Operation – Eat *Achatina fulica*" has been recommended and practiced in some countries with dense population of *A. fulica* to promote its consumption (Asamoah, 1999) and thereby regulate the snail population. Though there are no reports of snails consumed as part of nutritional diet in India, similar awareness would facilitate the use of snails as feed to poultry, piggery and also to be used in scientific studies.

***Public participation*:** Creating awareness among public and farmers should be an active component of any control method adopted for wide area management. Sajeev (2011) has initiated such an effort in Kerala to manage the giant snail menace. The awareness program is designed to bait the snail using cabbage leaves followed by spraying tobacco decoction - Copper sulphate mixture (TDCS mixture).

CONCLUSION

Despite different management measures described above it has been observed that naturalised populations of *A. fulica* often eventually decline greatly following establishment. Such a decline is often preceded by an exponential increase phase and a stable phase (Mead 1961, 1979, Raut and Barker, 2002). Simberloff and Gibbons (2004) suggest a do-nothing approach to management as a potential rationale for species in which spontaneous collapse has been repeatedly observed. In conclusion an integrated approach is emphasized by various workers to manage the outbreak of the snail menace in different agro-ecosystems of the country. Tackling the menace of *A. fulica* warrants community approach as in case of other pests like rats, for its effective minimization or eradication.

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Germplasm evaluation of mango for preference of the mango hopper, *Idioscopus nitidulus* (Walker) (Hemiptera: Cicadellidae) : The first step in understanding the host plant resistance

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ABSTRACT: Mango hopper, *Idioscopus nitidulus* (Walker) (Hemiptera : Cicadellidae) is a serious pest of mango that breeds on new shoots in September – November and on inflorescence in December – February. The population from the shoots becomes the precursor to the population on mango inflorescence. So, preference of *I. nitidulus* to various accession of mango germplasm during vegetative shoot stage is crucial. A study was carried out at the Indian Institute of Horticultural Research, Bangalore, India (12^0 8'N; 77^0 35'E) during two alternate years 2010 and 2012. Of 392 accessions evaluated, 32 accessions were identified as less preferred which in spite of conducive breeding/feeding niches for hoppers (new shoots) showed zero hopper population in both the years. If found validated when challenged with hoppers, these can be utilized by breeders for developing resistant accession. For the moderate to highly susceptible accessions correlation analysis revealed positive significant correlation between the mean percentage shooting and mean hopper population confirming their susceptibility.

Keywords: Feeding preference, germplasm, *Idioscopus nitidulus*, mango

INTRODUCTION

Mango (*Mangifera indica* L), known as the king of fruits, is an important commercial fruit crop grown in tropical and sub-tropical countries of the world (Abdullah and Shamsulaman, 2008). India is believed to be the home of several mango accessions (Butani, 1979). Production and quality of mango are mainly hampered by the incidence of about 400 insects pests (Tandon and Verghese, 1985, Peña *et al.*, 1998) of which leafhoppers are major pests (Verghese, 2000). Among the mango hoppers, so far 22 species (Dalvi *et al.*, 1992) have been reported, of which *Idioscopus nitidulus* (Walker) (Verghese and Tandon, 1988, Reddy and Dinesh, 2005) is more destructive causing (20-100%) loss (Sohi and Sohi, 1990). This hopper breeds on both shoots and inflorescence unlike *I. clypealis* and *I. nagpurensis*, which breeds only on inflorescence (Verghese and Devi, 2011).

In mango, the hopper activity coincides with maximum emergence of inflorescence and new shoots (Zagade and Chaudhari, 2010). Both adults and nymphs suck and desap the inflorescence and shoots causing them to wither (Nachiappan and Bhaskaran, 1983). As a result of feeding, leafhoppers excrete honey dew on to the flowers and leaves on which sooty mold develops. This is reported to affect the photosynthetic activity of the trees (Tandon *et al.*, 1989, Peng and Chirstian, 2005). The breeding population of *I. nitidulus* on the new shoots of mango from September-November (off-season) forms the residual population that infests the inflorescence subsequently in December/February causing economic damage. Though several natural enemies (Anon., 1993) have been reported on mango hoppers, insecticidal control has been the only viable strategy. Insecticides like imidacloprid, lambda cyalothrin or azadiractin have been recommended (Verghese, 2000). Sprays at flowering is fraught with danger of decimating pollinators which are crucial in effecting good fruit set in mango, as mango is cross pollinated by insects (Verghese and Tandon, 1990). Generally *I. nitidulus* is found almost throughout the year. Initially the population build-up is on the tender shoots (as precursor population) which gradually migrate to the inflorescence. Thus, it was felt to evaluate the preference of the precursor populations of *I. nitidulus*.

Identification of tolerant/resistant cultivars that deter breeding of precursor hopper population on new shoots prior to flowering is more valid than screening during flowering, as preventing flare up of population is possible if precursor population is prevented. If a good source of resistance during new shoots can be found the establishment of a precursor population that can potentially infest the inflorescence subsequently can be prevented. Identification of resistant commercial accession is of relevance as these can be recommended as a component in IPM (Integrated Pest Management) of hoppers. Hence, a preliminary study was carried out to study the feeding preferences which can later be used to identify genotypes for breeding host plant resistance to hoppers, an alternate tool in eco friendly, cost effective viable IPM strategy.

MATERIALS AND METHODS

The present study was conducted in the mango orchards of Indian Institute of Horticultural Research (IIHR), Bangalore, (12^0 8'N; 77^0 35'E), India during 2010 and in the alternate year 2012. The year 2011 was not considered, to exclude the influence of alternate bearing cycle of mango, if any. The mango germplasm collection of IIHR is one of the largest in the southern peninsular India. The collection comprises of several indigenous accessions in which both mono-embryonic and poly-embryonic types are conserved. Root stocks are mainly polyembryonic accessions like Olour, Vellaikulamban, etc. Prior to planting of the germplasm collection, the field has been so selected to minimize heterogeneity, as different germplasm are to be evaluated for their intrinsic variations. The orchard site had a uniform red loamy soil with a pH of 6.5. As all the trees were in one contiguous block there was no differential environmental factors affecting them. The agronomic practices followed for the trees were the same for all accessions. Adjacent to this block were orchards of regularly recorded commercial mango and these supported high infestations of *I. nitidulus*. Thus, this experimental germplasm block was well suited for the present study for evaluating the feeding preferences of *I. nitidulus*. The germplasm block was kept free of insecticides from three months prior to new shoot period, and this ensured the presence of adequate hoppers in the study area.

A total of 392 accessions occupying an area of 25 acres were assessed for the preference of *I. nitidulus*. Each accession had three replications (at 10m x 10m spacing). Three shoots /tree were randomly selected and therefore a total of nine shoots/ accession were chosen. Visual count of *I. nitidulus* nymphs was recorded with the help of a hand lens. Simultaneously, visual gradation on 0-100 scale to assess the percentage of new shoots for each tree was carried out where 0- complete absence of new shoots and 100- abundant new shoot emergence. Accordingly, mean percentage shooting was graded for each accession. Visual grading of hopper population was carried out on a 0-3 scale based on nymphs (0 = complete absence of nymphs; 1 = 1-3 nymphs/shoot, 2= 4-6 nymphs/ shoot and 3= >6 nymphs/shoot) and mean hopper population grade for each accession was calculated and rounded to nearest value. These grades were used to group accessions into three categories *viz*, shoots having zero grade hopper population were grouped as less preferred (Category I), shoots having hopper population grade < 2 were moderately preferred (Category II) and shoots with > 2 hopper population grade were grouped into highly preferred (Category III). Further, correlation analyses were carried out between the mean hopper populations and mean percentage shoots for both the study years separately and pooled, with '*r*' the coefficient of correlation as selection criterion and significance was tested at p= 0.05 (Little and Hills, 1978).

RESULTS AND DISCUSSION

Three categories of accessions were identified based on the hopper population grade *viz*., category I Not preferred accessions that included zero hopper population in spite of new shoots which was consistent across years, category II Moderately preferred accessions that included the graded hopper population < 2 across the years, category III Highly preferred accession that included the hopper population grade > 2. Thus in category I (Not preferred), category II (Moderately preferred) and category III (Highly preferred) there was 32, 52 and 19 accessions with consistent result (Table 1). These accessions serve as potential host plant resistance source for hopper breeding programmes. There was a significant positive correlation between hopper population and mean percentage shooting for moderately preferred accessions (p =0.05) in 2012 and pooled analysis (r = 0.54; 0.32). In highly preferred accessions significant positive correlation was obtained for in both the years *viz.*, 2010, 2012 and pooled analysis (r = 0.61; 0.43; 0.34 respectively) (Table 2).

Mango hopper, *I. nitidulus* breeds and feeds on inflorescence and shoots. After harvest (May to July) trees put forth fresh shoots by the month of September - November under south Indian conditions and become vulnerable to *I. nitidulus* infestation. The adults lay eggs in the midrib of new leaves, causing leaf distortion, while

Table 1. Feeding preference of leafhopper *I. nitidulus* to mango germplasm

S.No.	Accession	2010		2012	
		Mean shooting (%)	Mean Hopper Grade	Mean shooting (%)	Mean Hopper Grade
1	Anfas	6.67	0	5.00	0
2	Hyder Saheb	0.33	0	5.67	0
3	Olour	40.00	0	20.00	0
4	Jamedar	0.00	0	4.66	0
5	Kadari	0.00	0	18.33	0
6	Kasturi Mamidi(RD)	3.33	0	3.33	0
7	Black Andrews	0.00	0	4.00	0
8	Bombay Natasala	0.00	0	5.00	0
9	Panakalu	0.00	0	10.33	0
10	Lemon	1.67	0	15.00	0
11	Jeerige Neermavu	25.00	0	18.66	0
12	Huli Appekai	40.00	0	11.66	0
13	Sundar Langra	0.00	0	25.00	0
14	Bada phattar	28.33	0	30.00	0
15	Halsage	26.67	0	5.00	0
16	Karpoora Jeerige	6.67	0	30.00	0
17	Kadikai	13.33	0	31.66	0
18	Potte	40.00	0	15.00	0
19	Nagin	56.67	0	13.33	0
20	Malgesh	18.33	0	58.33	0
21	Al Fazli	20.00	0	43.33	0
22	Shahjahan	45.00	0	40.00	0
23	Ramphalya	20.00	0	17.33	0
24	Sepia	38.33	0	18.33	0
25	Rebello	0.00	0	28.33	0
26	Harsha	15.00	0	5.00	0
27	Ruswani	31.67	0	13.33	0
28	KU-8	30.00	0	25.00	0
29	Siroli	52.67	0	25.33	0
30	Thambva	29.00	0	8.33	0
31	Mitigiri	7.50	0	17.5	0
32	Naagarappe	20.00	0	20.00	0
33	Panchadara Kalasa	15.00	2	13.33	1
34	Langra	9.00	1	25.00	1

35	Nazuk Badan	19.00	2	25.00	2
36	Kurd	6.67	2	35.33	2
37	Sensation	31.67	1	26.6	2
38	Latif	48.33	2	26.66	1
39	Bhutto Bombay	26.67	1	25.00	1
40	Jehangir	21.67	1	42.33	2
41	Sardar	24.00	1	28.33	2
42	K-O-32	16.67	1	16.67	2
43	Allampur Baneshan	33.67	2	7.67	1
44	Rumani X Neelum	61.67	1	15.00	1
45	Himsagar	1.00	1	0.66	1
46	Himayath Pasand	36.67	2	27.00	2
47	Rangoon Goa	76.67	1	56.66	2
48	Amini	87.50	1	75.00	1
49	Pacharasi	56.67	1	41.66	1
50	Goa Mankurd	26.67	1	33.33	2
51	Bobbili Punasa	20.00	1	23.33	1
52	Nekkare	30.00	2	24.00	2
53	Ratna	30.00	1	43.33	2
54	Maharaja Pasand (L)	47.00	1	21.66	2
55	Janardnana Pasand	43.33	1	55.00	2
56	Royal Special	31.67	2	68.33	2
57	Mahmoozda	48.33	2	33.33	2
58	Starch	45.00	2	8.66	1
59	EC95862	56.67	2	13.33	1
60	Swarna Jehangir	53.33	2	40.00	1
61	Chimut	35.00	2	12.00	1
62	Pahilwan	36.67	1	6.6	1
63	Aryavarthana Rasala	2.67	1	18.33	1
64	Kerala Kalepad	61.67	2	26.66	2
65	Mallika	8.33	1	3.33	1
66	Rumani	46.67	1	30.5	1
67	Dwarf Rumani	23.33	1	45.00	2
68	Creeping	51.67	2	26.66	2
69	Rajapuri	3.33	2	2.66	1
70	Samar Totapuri	33.33	1	5.00	1
71	Manibhatta Appe	16.67	1	18.66	1
72	H- 85 (kal x allam bane)	58.33	2	20.33	1
73	PKM-2	18.33	2	5.66	1

74	Tambva	25.00	1	31.66	1
75	Lord	53.33	1	16.00	1
76	Santhoor Collection	70.00	1	23.66	1
77	Bada Aam	20.00	1	15.00	1
78	Whiteseri	27.67	1	41.66	1
79	Keitt	10.00	1	21.66	1
80	Gaddemar	23.33	1	10.00	1
81	Sai Sugandh	35.67	1	30.66	1
82	Maharaja of Mysore	36.67	1	39.00	1
83	Bandar Bandal	10.00	0	30.33	1
84	Himam Pasand	5.00	1	51.66	1
85	Neeleshan	28.33	2	51.66	3
86	Bombay Green	5.67	0	80.00	3
87	Amrapali	46.67	3	40.00	3
88	Mulgoa	12.00	1	80.00	3
89	Bombay Peda	50.00	2	50.00	3
90	Sundar Shan	32.33	3	45.00	3
91	Gola	63.33	3	36.67	2
92	Royal Special	31.67	2	68.33	3
93	Kalapadi	71.67	3	28.33	2
94	Hybrid 11/14 (Neex Hims)	63.33	3	25.00	2
95	Mahmood Bahar	45.00	3	33.33	2
96	Rehaman Pasand	50.00	3	45.00	3
97	Hamlet	61.67	3	51.66	2
98	Mutwar Pasand	43.33	3	23.66	2
99	Paiyur-1	16.67	3	33.33	3
100	Moreh	46.67	3	26.66	2
101	Vanraj	21.67	3	8.33	1
102	Neeluddin	55.00	3	21.00	2
103	Neelgoa	60.00	3	13.33	3

nymphal feeding leads to shriveling of leaves with sooty mold formation. The hopper nymphs moult to adults and overwinter till flowering (December- January) or continue breeding if new shoots are available till flowering and cause major economic loss in terms of withering of flowers and poor fruit set. Generally, the hopper population gets noticed only during the flowering season. The hopper population that breeds on the vegetative shoots during September - November serves as the precursor to the population that infests during flowering. So, the hopper populations on the vegetative shoots are critical, especially if it is a mono-varietal, monocrop orchard. Therefore in this study, 392 accessions in two seasons in 2010 and 2012 were studied to understand the feeding preference of *I. nitidulus* to various mango accessions. As many mango accessions have a biennial vegetative and reproductive cycle, study was taken up in alternate years.

There were 32 accessions that were not preferred by hoppers consistently for both the years. These accessions were grouped as resistant as there was zero hopper population in spite of availability of new shoots on breeding/feeding niches. Few accessions like Kadari, Lemon, Sundar langra had less percentage of shoots in 2010 but had high shooting in 2012 but still had zero population of hopper confirming their resistance. In some accessions it was vice versa *viz.*, Huli appekai, Halsage

Table 2. Correlation analysis between mean percentage shooting and mean hopper grade (Only for accessions with consistent results):

	'r'			
	2010	**2012**	**Pooled**	**Remarks**
Less preferred for host plant resistance	-	-	-	Resistant, can be used in breeding
Moderately preferred plant resistance	0.19	0.54*	0.31*	Susceptible cannot be used for host
Highly preferred plant resistance	0.61*	0.43*	0.34*	Susceptible cannot be used for host

*Significant at p = 0.05

the percentage fresh shooting was high in 2010 but low in 2012 with zero hopper population confirming resistance. The reasons for non-preferences may be anatomical, morphological, secondary metabolites or a combination of above all. Here we rule out chance or escape as the area had high population of *I. nitidulus.* Fifty two accessions were moderately preferred **(**Table 1) which had < 2 hopper grade indicating these accessions were preferred by the hoppers for feeding and colonizing proving their susceptibility. In category III, there were 19 accessions which were highly preferred (Table 1). In this category there were accessions like Bombay green which recorded zero population in 2010 due to zero shooting but was highly susceptible when the percentage shooting increased. Similarly, in case of Vanraj in 2012 due to low percentage shooting the variety was moderately susceptible but the same variety in 2010 had high percentage of shooting and hence was highly susceptible.

Results of correlation analysis between percentage new shoots and hopper population showed positive significant correlation between the two variables for susceptible accessions. Thus increase in shoots reflected increased hopper colonization. This was true only in categories II and III, both being susceptible. In case of moderately preferred accessions (category II) there was no significance in 2010 and positive significance in 2012, indicating irrespective of the extent of shooting these accessions were preferred. Fluctuation in shooting would have led to significance in one year and non significance in another year. However, overall showed a positive significant correlation between mean percentage shooting and mean hopper grade indicating these were still preferred and had to be treated as susceptible. Further, from the non-significant correlation in 2010 for category II, it may be extrapolated that in resistant accessions, in spite of shoots, the correlation will tend to be non-significant even under low hopper population. However in 2012, the trees showed positive significant correlation implying trend to susceptibility. In case of category III (highly preferred accession), there was a significant positive correlation in both the years. Hence category II and III have to be regarded as susceptible. In the resistant accessions, consistently in both the years, correlation analysis was not possible as population was zero, indicating irrespective of shooting there was no hopper incidence. However, it can be concluded that in resistant accessions, in spite of new shoots and source of infestation around, hoppers were not recorded, confirming their non-preference.

Thus from 392 accessions evaluated, only 103 accessions had consistent results. Only category I in both years showed zero population of *I. nitidulus* and therefore can be short listed as resistant. These 32 accession can be further challenged with field populations using field cages (as *I. nitidulus* is not amenable to mass rear in laboratory) and can be taken forwarded for breeding resistant accessions, if found less preferred by *I. nitidulus*, post challenging.

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Genetic analysis of fruit and shoot borer (*Earias vittella* Fab.) resistance in okra (*Abelmoschus* spp.)

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ABSTRACT : Four okra varieties *viz.,* Arka Anamika, Salkeerthy, Sel 2 and KL 9 of cultivated species *Abelmoschus esculentus*, and two varieties *viz.,* Susthira and AC5 of semi-domesticated species *Abelmoschus caillei* were crossed in all possible combinations. The parents and hybrids were evaluated for resistance against fruit and shoot borer, *Earias vittella* Fab. Differential response to fruit and shoot borer infestation was observed in all genotypes. The study revealed that the parents AC 5 and KL 9 are the potential donors of shoot and fruit borer resistance. Selection of genotypes with short growth habit, short flowering period and short fruit length will help to minimize the shoot and fruit borer infestation. Arka Anamika was identified as a good donor parent in breeding for fruit number, fruit weight and fruit length. The hybrid obtained by Sel 2 x AC5 cross was identified with high marketable fruit yield and resistance to fruit and shoot borer, and field tolerance to Yellow Vein Mosaic Virus.

Keywords: *Abelmoschus esculentus, Earias vitella,* okra, host plant resistance

INTRODUCTION

Shoot and fruit borer, *Earias vittella* is a major pest of okra which causes severe damage affecting both quantity and quality of yield. As okra is grown in an area of three lakh ha with an annual production of 32 lakh tonnes in India (NHB, 2005), it is of a prime importance to reduce the damage caused by this pest. Resistant varieties are preferred, because the use of pesticides will cause the residual problems as it is harvested and consumed as tender pods. The regular and indiscriminate pesticide usage will results in the development of resistance in insects, resurgence and environmental pollution (Suneetha *et al.*, 2007). Because of these factors an emphasis is always being given to develop insect resistant varieties. The improvement of the varieties is an effective way to reduce the losses in the crop. For a successful breeding programme, screening the available germplasm for resistance source and to combine the identified resistance to the existing high yielding varieties is necessary. Therefore, the present study was undertaken to introgress the resistance contributing traits with high yield and also to study the genetics behind fruit and shoot borer resistance attributes.

MATERIALS AND METHODS

The present research work was conducted at the Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Vellanikkara. The experimental materials included three high yielding varieties (Arka Anamika, Salkeerthy and Susthira)and three resistant genotypes (KL 9, Sel 2 and AC 5) which were previously evaluated (Karuppaiyan, 2006) and were crossed in all possible combinations. The whole research work was conducted to effect crosses between resistant and high yielding parent to develop advanced generations like F_1s, F_2, BC_1 and BC_2. Six generation materials (Parents, F_1, F_2, BC_1 and BC_2) selected F_2 lines of other crosses were evaluated against shoot and fruit borer and other yield attributes along with check variety. The parents and F_1 s were raised in green houses and the selfing and backcrossing of the F_1s were done with corresponding parents. F_2s and six generations of two selected crosses (Sel 2 x AC5 and KL9 x Salkeerthy) were raised in open field condition in randomized block design (RBD) with four replications. Recommended package of practices of KAU was followed to grow a successful crop of okra. The crop was left open for natural infestation by fruit and shoot borer and no pesticides were sprayed. Fruit borer susceptible variety Salkeerthy was raised in border rows.

Observations were recorded from five randomly selected plants from each replication and they were evaluated for yield attributes and resistance to fruit and shoot borer when there was maximum damage observed in the susceptible check variety. Biochemical and statistical analysis were employed to understand the genetics of various characters.The relative degree of resistance to shoot and fruit borer infestation was judged on the basis of percentage shoot and fruit infestation in each genotype. The genotypes were grouped into five resistance classes based on their shoot and fruit

infestation *i.e*, i) immune (0 per cent infestation), ii) highly resistant (1-10 per cent shoot or fruit infestation), iii) moderately resistant (11-20 per cent infestation), iv) susceptible (21-30 per cent infestation) and v) highly susceptible (>31 per cent infestation) (Kumbhar *et al*.,1991) and it was adopted in the present study.

RESULTS AND DISCUSSION

The donor parents used in the study were previously reported to have fruit and shoot borer resistance by Karuppaiyan (2006). Considering the cross compatibility of cultivated high yielding varieties, the resistant donor are preferably selected over wild resistant genotypes. The performances of genotypes were assessed in the open field condition, comparing the percentage of shoot infestation, fruit infestation, days to first flowering, plant height, internode length, fruit number pet plant, fruit weight, fruit length, fruit yield per plant and marketable fruit yield (Tables1). High variability was observed for shoot infestation and fruit infestation in F_2 generation of intervarietal crosses. High genetic advance, genetic gain and heritability were recorded for shoot infestation and fruit infestation indicated that selection can be resorted for the improvement of these characters. Positive association of fruit yield with number of fruits, number of internodes, fruit weight and fruit length leads to the conclusion that selection of these traits along with short growth habit, short flowering period and short fruit length will minimize the economic damage.

Generation mean analysis of Sel 2 x AC5 indicated the presence of complementary epistasis for fruit infestation. As these traits with high dominance variance are non fixable, selection for this traits is ineffective. Therefore heterosis breeding programme will be useful for improving these traits. In the inter varietal cross KL9 x Salkeerthy it was observed that duplicate epistasis govern the fruit borer resistance and digenic non-allelic interaction model was inadequate to explain shoot borer infestation. These results are in agreement with those of Akhtar *et al*. (2010) and Sogalad *et al.,* (2012). The selected F_2s and genotypes of generation mean analysis of Sel 2 x AC 5 and KL9 x Salkeerthy were screened for pest infestation in open field conditions with susceptible variety in border rows to raise the pest population for better screening. The mean infestation data was used for the classification. Based on the rating scale given by Kumbhar *et al*. (1991) the genotypes were classified for degree of resistance. Twenty seven genotypes of different crosses and different generations are screened in the present study. The study revealed that F_1s of Susthira x Sel 2, Susthira x AC 5, Sel 2 x Arka Anamika, Arka Anamika x Sel 2, Arka Anamika x Sal and Arka Anamika x KL9 were found immune to shoot borer but none of them were immune to fruit borer (Table.2). AC5 and KL9 x AC5 were highly resistant to shoot borer and F_1 of Sel 2 x AC5 was highly resistant to fruit borer.

The F_1, F_2 and BC_1 generation of Sel2 x AC 5 were also moderately resistant to shoot borer. No genotype was immune to fruit borer. AC5 has shown minimum shoot infestation among the parents. In case of fruit infestation F_1 of Sel 2 x AC 5, F2 s of KL9 x Sel 2 and Susthira x Sel 2 shown high resistance or low infestation. KL 9 x Sel 2 (11.76 per cent), Susthira x Sel 2 (14.2 per cent) and KL 9 x Susthira (20 per cent) showed less than 20 per cent fruit infestation hence treated as moderately resistant to fruit borer. Among the F_2 s of Arka Anamika x KL9, Arka Anamika x Sel 2, Sel 2 x Arka Anamika, Susthira x AC5 and Salkeerthy was found highly susceptible for both shoot borer and fruit borer infestation. Susthira x Sel 2. In case of marketable fruit yield KL9 x Susthira secured the highest. The susceptibility of many cultivated varieties to fruit borer was previously reported by Vyas and Patel (1991), Srinivasa and Sugeetha (2001), and Neeraja *et al*. (2004).

It is evident from the result that the progenies of resistant parents have shown preferable traits for further selection. These results are consistent with the findings of workers like Bairwa *et al*. (2005) and Karuppaiyan (2006). It was observed that there is a differential response to fruit and shoot borer infestation in all genotypes while the pest causing the damage was same. It may be because of some morphological and biochemical variation between the fruit and shoot of the same genotype and due to variation in insect preference and their interactions. The F_1 of Sel 2 x AC 5 was found having high resistance to both fruit borer (highly resistant) and shoot borer (moderately resistant) compared to all other genotypes under study. This genotype scored comparatively high fruit yield (163.11 g) and marketable fruit yield (152.41g) (Table.3) and its F_1 has shown field resistant to yellow vein mosaic virus also. Therefore this cross was identified as an elite genotype with both high yield and pest resistance and advanced to further generations. Even the fruit yield was highest in *A.esculentus* genotypes but the yield loss was minimum in *A. caillei* due to less susceptibility to pest and diseases. The F_2 generations of interspecific crosses of *A. caillei* and *A. esculentus* have shown less coefficient of infection for Yellow Vein Mosaic Virus and Salkeerthy and its progenies were highly susceptible to

Table 1. Mean performance of F_2 progenies and the parents in natural field condition

F2	Pht	FN	Nr	FW	FY	FL	SI	FI	FG	Flp	MFY
P3 x P2	49.20	**5.80**	5.00	35.00	203.00	17.80	18.18	27.50	**8.50**	28.44	147.17
P3 x P1	40.50	5.60	5.00	42.50	238.00	16.20	18.18	31.50	6.00	31.49	163.03
P3 x P5	56.24	4.80	6.00	30.00	144.00	20.00	16.66	20.80	6.00	29.85	114.04
P2 x P4	38.80	3.40	7.00	50.00	170.00	21.90	20.00	11.76	7.50	26.27	150.08
P2 x P6	42.20	3.80	8.00	60.00	228.00	15.20	8.33	36.84	7.50	26.70	144.04
P2 x P5	59.60	5.00	5.00	47.50	237.50	17.66	25.00	20.00	8.00	25.96	190.00
P1 x P2	69.20	5.00	5.00	42.50	212.50	21.60	0.00	24.00	8.00	27.60	161.50
P1 x P3	65.00	5.40	5.00	45.00	243.00	20.00	0.00	29.60	7.00	31.49	171.07
P1 x P5	61.20	5.00	5.00	42.50	212.50	18.00	30.00	36.00	6.10	29.02	136.00
P1 x P4	**63.00**	5.20	5.00	45.00	234.00	18.20	0.00	34.60	6.50	29.33	153.03
P4 x P3	**70.20**	3.80	5.00	42.50	161.50	17.50	11.00	42.00	6.50	30.16	93.67
P4 x P1	**74.60**	5.40	5.00	40.00	216.00	20.20	0.00	33.33	6.00	29.33	144.07
P5 x P6	**36.20**	3.80	5.00	40.00	152.00	19.20	0.00	21.05	7.50	28.12	120.04
P5 x P4	**25.60**	**1.40**	6.00	25.00	35.00	17.20	0.00	14.20	6.50	27.69	30.03
P3	**39.60**	3.80	5.00	50.00	190.00	15.60	28.57	80.00	7.50	32.33	38.00
MEAN	52.74	4.48	5.46	42.50	191.80	17.21	11.72	30.87	7.01	28.91	130.38
SD	14.85	1.15	0.91	8.39	54.35	4.59	11.36	16.12	0.83	1.93	45.63
VARIANCE	220.76	1.33	0.83	70.53	2953.95	21.14	129.07	259.94	0.70	3.74	2082.78
CD at 5 %	21.49	1.67	1.32	12.15	78.63	6.65	16.43	23.32	1.21	2.79	66.02
CD at 1 %	16.81	1.30	1.04	9.50	61.49	5.20	12.85	18.24	0.94	2.18	51.63

P1- Arka Anamika (A.A), **P2** - KL9, **P3**– Salkeerthy(Sal), **P4** - Sel 2, **P5**-Susthira (Sus), **P6** - AC5

Dff-Days to first flowering, , Pht-Plant height (cm), length, FN-Fruit number / plant, FW-Average fruit weight (g), FL-Fruit length (cm), FG-Fruit girth (mm), FY-Fruit yield (g)/ plant, MFY- Marketable fruit yeield (g), Nr – No. of ridges per fruit, SI – Shoot infestation (%), FI – Fruit infestation (%), Flp- flowering period (days). Values in bold and underlined refers to the minimum and maximum respectively

Table 2. Classification of germplasm based on their relative degree of resistance

Category	In terms of shoot damage	In terms of fruit damage
Immune (0% infestation)	Susthira x Sel 2, Susthira x AC 5, Sel2 x Arka Anamika, Arka Anamika x Sel2, Arka AnamikaxSal, Arka AnamikaxKL9	Nil
Highly resistant (1-10%)	AC5, KL9xAC5	Sel 2xAC5 (F_1)
Moderately resistant (11-20%)	Sel 2xSal, KL9xSel 2, SalxKL9, SalxArka Anamika, SalxSusthira, Sel 2xAC5 (F_1, F_2, BC_1), Kl 9 x Sal (F_1, BC_2)	KL 9 x Sel 2, Sal x Susthira, KL 9 x Susthira, Susthira x Sel 2, AC 5, Sel 2 x AC 5 (F2, BC_2), AC 5
Susceptible (21-30%)	KL 9 x Susthira, KL 9, KL 9 x Sal (BC_1), Sel 2 x AC 5 (BC_2)	Sel 2, KL9, SalxKL9, Arka Anamika xKL9, Arka Anamika x Sal, SusthiraxAC5, Sel 2xAC5(BC_1), Kl 9 x Sal (F_1, F_2, BC_1)
Highly susceptible (>30%)	Salkeerthy	Salkeerthy, SalxArka Anamika, KL9xAC5, Arka Anamika x Susthira, Arka AnamikaxSel 2, Sel 2xSal, Sel 2xArka Anamika, Kl 9 x Sal(BC_2)

Table 3. The resistance and yield related traits of genotypes

Genotype	%SI	%FI	FY	MFY	Genotype	%SI	%FI	FY	MFY
Salkeerthy x KL9	18.18	27.50	203.00	147.17	**Sel2 x AC 5**				
Salkeerthy x Arka Anamika	18.18	31.50	238.00	163.03	P_1	31.92	26.80	101.03	74.43
Salkeerthy x Susthira	16.66	20.80	144.00	114.04	P_2	2.87	19.88	36.86	29.24
KL9 x Sel 2	20.00	11.76	170.00	150.08	F_1	12.78	4.25	163.11	152.41
KL9 x AC5	8.33	36.84	228.00	144.04	F_2	14.75	18.88	115.58	21.13
KL9 x Susthira	25.00	20.00	237.50	190.00	BC_1	15.88	24.13	75.98	56.95
Arka AnamikaxKL9	0.00	24.00	212.50	161.50	BC_2	29.40	17.83	54.37	45.12
Arka Anamikax Salkeerthy	0.00	29.60	243.00	171.07	**KL9xSalkeerthy**				
Arka AnamikaxSusthira	30.00	36.00	212.50	136.00	P_1	29.68	27.93	174.20	124.07
Arka AnamikaxSel 2	0.00	34.60	234.00	153.03	P_2	27.01	27.02	205.04	156.00
Sel 2xSalkeerthy	11.00	42.00	161.50	93.67	F_1	15.86	22.37	197.58	157.03
Sel 2xArka Anamika	0.00	33.33	216.00	144.07	F_2	26.03	26.58	209.57	150.72
SusthiraxAC5	0.00	21.05	152.00	120.04	BC_1	28.42	28.71	108.81	77.33
SusthiraxSel 2	0.00	14.20	35.00	30.03	BC_2	19.70	33.64	120.92	82.35
Salkeerthy	28.57	80.00	190.00	38.00					

Per cent shoot infestation (%SI), Per cent fruit infestation (%FI), Marketable fruit yield (MFY), fruit yield (FY)

YVMV. These were previously observed by and Karuppaiyan (2006) and Kousalya *et al*. (2006) and they reported that *A. caillei* was highly resistant to YVMV. Presence of biochemical factors like high phenol and tannin content in the fruits and shoots of resistant genotypes compared to susceptible genotypes indicated that the biochemical constituents play a role in fruit and shoot borer resistance. The study revealed that The F_1 hybrid of Sel 2 x AC5 identified as the best hybrid for both high marketable fruit yield and resistance to fruit and shoot borer, and it also showed field tolerance to Yellow Vein Mosaic Virus. The present research work reveals that the parents AC 5 and KL 9 are the potential donors of shoot and fruit borer resistance. But many of the crosses using parent AC 5 (*Abelmoschus caillei*) genotype were not successful. Crossing barriers like cross incompatibility and hybrid sterility was found in the generation of F_1 plants of interspecific crosses involving *A. caillei* and *A. esculentus* genotypes. Embryo culture and genetic engineering methods are suggested in future to minimize the constrains in the gene transfer between these species and this will open new avenues for further research. Secondly promising F_1s like Sel 2 x AC 5, KL 9 x Salkeerthy (resistant high yielding genotypes) and other outstanding genotypes from the breeding lines can be further advanced to obtain elite varieties with desirable economical characters.

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Comparative efficacy of neem products, essential oils and synthetic insecticides for the management of onion thrips, *Thrips tabaci* Lindeman

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ABSTRACT : Field experiments were conducted at Indian Institute of Horticultural Research, Bengaluru, India to study the bio-efficacy of neem seed powder extract (NSPE) (4%), neem soap (NS) (1%), essential oils of Basil or Tulsi *(Ocimum tenuiflorum* syn. *O. sanctum*), (0.2%) and scented *Geranium,* (*Pelargonium graveolens*), (0.2%) along with commonly used synthetic insecticides *viz.*, dimethoate (0.06%), acephate (0.075%) and fipronil (0.05%) against onion thrips, *Thrips tabaci* Lind. during summer 2011 and 2012. All the treatments of botanicals, essential oils and insecticides significantly reduced thrips incidence during both the seasons. Fipronil was the most effective treatment in reducing thrips and also in increasing yield during both the seasons. However, *Basil* was at par with fipronil in reducing pest incidence and increasing yield. The NS and NSPE also reduced thrips incidence and were at par with acephate and dimethoate. When yields were considered, plots treated with NSPE, NS, *Basil* and *Geranium* and dimethoate were at par during both seasons. This study clearly illustrated that neem products and essential oils can be used as components of thrips IPM.

Keywords : Essential oils, *Geranium,* neem, *Ocimum,* onion thrips, *Thrips tabaci*

INTRODUCTION

Onion thrips (*Thrips tabaci* Lindeman) is a very serious pest of onion causing more than 50 per cent yield loss. Thrips are effectively managed by synthetic insecticides worldwide. However, efforts to develop alternative strategies to manage this pest by alternative technologies have been continuing. Neem has been found to be a very good alternative to synthetic insecticides (Schumeterer, 2002) and neem seed kernel extract (NSKE) has been found effective against this pest (Vijayalakshmi *et al*., 1995 ; Krishna Kumar, 2008). As an alternative to NSKE, the Indian Institute of Horticultural Research, Bengaluru has developed pulverised neem seed powder extract (NSPE) and neem soap (NS) for managing insect pests (Krishna Moorthy and Krishna Kumar, 2002 and 2011). Essential oils are also finding increased attention as alternatives to the use of synthetic insecticides in recent years (Isman, 2000). Laboratory studies by us have shown that spray of commercially available essential oils at 0.2 per cent concentration kills early instar caterpillars of diamondback moth (*Plutella xylostella* L.) (Unpublished work by the authors). So far very little information is available on their bio-efficacy under field conditions. Therefore, field experiments were conducted to test the bio-efficacy of the NS, NSPE and essential oils of *Basil*, *Ocimum tenuiflorum* (syn. *O. sanctum*) commonly called as *Tulsi,* and scented *Geranium* (*Pelargonium graveolens*) against onion thrips during summer season of 2011 and 2012. The results of the two trials are reported here.

MATERIALS AND METHODS

Field experiments were conducted during summer 2011 and 2012 at the experimental farm of the Indian Institute of Horticultural Research, Bengaluru. Onion variety *Arka Kalayan* was transplanted in ridges with a spacing of 50 x 10 cm on 25th January and 12th February during 2011 and 2012 respectively. During first season, first spray was given at 30 days after planting (DAP). However, during the second season spray was given only after 40 DAP when the pest incidence was high at around 100/plant. This was done to know whether botanicals and essential oils can be used as alternatives to synthetic insecticides under such conditions. Spray treatments of NSPE (4%), NS (1%), *Basil* (0.2%), *Geranium* (0.2%), dimethoate (0.06%), acephate (0.075%) and fipronil (0.08%) were given using a gator sprayer delivering 1000 l/ha. Before spraying, a commercially available sticker (@0.5 ml/litre of spray fluid) was mixed. A pulveriser was used to powder dry cleaned whole seeds of neem to a very fine consistency before the start of the experiments during both the seasons and stored in a cool dry place at room temperature in a gunny bag. Forty grams of pulverized neem seed powder was soaked in 200 ml water

overnight, filtered through double layered nylon mesh to prevent the powdered neem seed particles from clogging the nozzle and volume was made to 1 L to prepare 4 per cent solution for spraying. To prepare 100 L of spray fluid, 4 kg of pulverized neem seed powder is required. Neem soap was prepared in the laboratory. Essential oils were supplied by M/S Aroma Trading Co., Bengaluru, India. Sprays were given at 10 days interval, four times during 2011 and three times during 2012. The experiment design was Randomised Block Design (RBD) with three replications. Observations were recorded by using the non-destructive method by carefully examining the inner leaf sheaths and growing parts of the plants. For this purpose, 10 plants were selected in random in each plot and carefully observed. The data on the thrips incidence in different plots on individual dates were tabulated treatment wise and yield was converted to tonnes per hectare and subjected to analysis of variance (ANOVA) to separate the means. Data on thrips incidence were transformed to Log (X+1) transformation before analysis.

RESULTS AND DISCUSSION

The pest incidence under different treatments and corresponding yield during the two years are provided in Tables 1 and 2. During 2011, the results indicate that fipronil was the best treatment by recording 7.93 to 28.33 thrips/plant as compared to 38.87 to 83.33 thrips/plant on different observation dates recorded in control. Sprays of *Basil* and NSPE were at par with fipronil on three observation dates (50, 60 and 70 DAP). While plots treated with *Basil* recorded thrips incidence of 12.07 to 30.67/plant, NSPE recorded 12.7 to 35.00 /plant. Sprays of *Geranium* was at par with fipronil on two observation dates (60 and 70 DAP). The treatments of NSPE, *Basil* and *Geranium* were at par with each other on all observation dates. However, sprays of NS, acephate and dimethoate were at par with fipronil on one date of observation only and these treatments are at par with each other on three observation dates (50, 60 and 70 DAP). As far as yield is considered, though fipronil recorded highest yield (31.25 t/ha), all other treatments, namely, *Basil*, NSPE, Neem soap and acephate were at par with fipronil. These treatments recorded 30.66, 30.38, 29.54 and 29.78 t/ha, respectively. However, *Geranium* treated plots recorded 28.17 t/ha and were at par with NS, NSPE, *Basil* treated plots.

In the following season, thrips incidence was moderate (73.33 to 101.33/plant) (Table 2). Very high incidence of thrips was observed on 40 DAP when first spray was given. The incidence of thrips in different treatments was found to be significantly different at 50 and 60 DAP only. Again during this season, *Basil* and NS were as effective as fipronil in reducing thrips incidence on these two dates. Treatments of NSPE and dimethoate were on par with fipronil one date of observation only when thrips incidence was considered. During this season also *Basil*, *Geranium*, acephate and fipronil treated plots were on par with each other in increasing yield which recorded 36.87, 33.59, 32.65 and 36.53 t/ha respectively. NS and NSPE treated plots recorded 30.90 and 31.91 t/ha and were on par with dimethoate, *Basil* and *Geranium* treated plots. Control plot recorded 20.59 t/ha. The studies conducted for two seasons showed that *Basil* was on par with fipronil in reducing thrips incidence on all dates except one (25-3-2011). During season II, in spite of the high incidence of thrips before start of spray the essential oils of both *Basil* and *Geranium* increased the yield and were on par with fipronil and acephate (Table 2). NS and NSPE were also effective in reducing thrips incidence and increasing the yield as compared to control and recorded yield on par with basil. Homemade neem extract was found to reduce onion thrips (Vijayalakshmi, 1995 ; Krishna Kumar, 2008). Present study has shown that neem products like NSPE, neem soap can be effective alternatives to synthetic insecticides like dimethoate, acephate and fipronil and can be used in IPM programmes. This is also in conformity of our earlier finding that NSPE and NS are effective in reducing okra fruit borer *Erias vitella* F. (Krishna Moorthy and Krishna Kumar, 2012). Though NSKE and NSPE are effective, they have not become very popular to expected level among growers due to the difficulty in their preparation and difficulty in timely availability of seeds. Therefore, we developed neem soap and now this is made available to farmers commercially by transferring the technology to many *Krishi Vigyan Kendras* (KVK) and a few private firms in Karnataka, Tamil Nadu, Kerala, Andhra Pradesh and Maharastra. Hence, Neem soap can be used as alternative to synthetic insecticides. Further, essential oils like *Basil* and *Geranium* also have good potential as components in thrips IPM programmes. Not much information is available on the use of essential oils in insect pest management. Most of the research in essential oils is confined to the laboratory studies. However, Reitz *et al.* (2008) have found that spray of essential oils have reduced spotted wilt disease in tomato, though it did not reduce vector thrips (*Frankliniella* spp.) population. They also found that mixing kaolin (an inert adjuvant) with essential oils before spray has increased plant yield. Now we are reporting the field efficacy of essential oils on onion thrips as compared to neem products and

Table 1. Thrips incidence in onion as affected by different treatments (2011)

Treatment	40 DAP	50 DAP	60 DAP	70 DAP	Yield (t/ha)
Neem soap (1.0%)	20.13^{e}(3.05)	43.33^{b}(3.75)	43.67^{b}(3.79)	26.33^{ab}(3.30)	29.54^{ab}
Pulverised neem seed powder extract (4.0%)	12.87^{c}(2.62)	29.33^{ab}(3.40)	35.00^{a}(3.58)	24.00^{ab}(3.18)	30.38^{ab}
Essential oil Basil (0.2%)	12.07^{bc}(2.57)	21.67^{ab}(3.10)	30.67^{a}(3.45)	25.00^{ab}(3.26)	30.66^{ab}
Essential oil Geranium (0.2%)	15.53^{cd}(2.80)	35.00^{b}(3.56)	28.67^{a}(3.39)	25.00^{ab}(3.25)	26.78^{b}
Dimethoate (0.06%)	18.67^{de}(2.97)	31.67^{ab}(3.48)	42.00^{b}(3.76)	30.00^{b}(3.42)	28.17^{b}
Acephate (0.075%)	10.20^{b}(2.41)	20.67^{ab}(3.07)	38.67^{b}(3.67)	29.33^{b}(3.41)	29.78^{ab}
Fipronil (0.05%)	7.93^{a}(2.19)	19.33^{a}(2.96)	28.33^{a}(3.37)	20.67^{a}(3.07)	31.25^{a}
Control	38.87^{f}(3.66)	73.33^{c}(4.30)	83.33^{c}(4.43)	56.00^{c}(4.04)	15.58^{c}
CD (P=0.05)	0.21	0.52	0.21	0.27	4.23
CV%	4.44	8.99	3.35	4.79	9.05

Figures in parenthesis are log (x+1) transformed values

Table 2. Thrips incidence in onion as affected by different treatments (2012)

Treatment	40 DAP	50 DAP	60 DAP	70 DAP	Yield (t/ha)
Neem soap (1.0%)	94.67 (4.56)	57.50^{abc}(4.01)	43.00^{ab}(3.76)	6.80(1.98)	30.90^{b}
Pulverised neem seed powder extract (4.0%)	98.00(4.60)	38.67^{ab}(3.68)	49.00^{bc}(3.91)	7.67(2.10)	31.91^{b}
Essential oil Basil (0.2%)	97.67(4.59)	58.75^{abc}(4.04)	45.33^{ab}(3.80)	6.67(1.98)	33.59^{ab}
Essential oil Geranium (0.2%)	99.67(4.61)	69.17^{bc}(4.25)	51.33^{bc}(3.93)	9.67(2.27)	32.65^{ab}
Dimethoate (0.06%)	95.67(4.57)	72.50^{bc}(4.28)	42.00^{ab}(3.74)	10.33(2.42)	29.90^{b}
Acephate (0.075%)	101.33(4.63)	55.63^{ab}(3.68)	38.67^{ab}(3.68)	2.33(1.00)	36.53^{a}
Fipronil (0.05%)	95.33(4.56)	32.75^{a}(3.47)	32.75^{a}(3.47)	4.60(1.71)	36.87^{a}
Control	96.00(4.57)	84.38^{c}(4.45)	68.83^{c}(4.24)	7.67 (2.12)	20.59^{c}
CD (P=0.05)	NS	0.63	0.46	NS	4.55
CV%	2.37	6.47	6.47	26.48	8.22

Figures in parenthesis are log (x+1) transformed values

synthetic insectiides. However, further studies have to be done to reduce the dosage of essential oils to bring down the cost by developing suitable eco-friendly formulations.

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Efficacy of botanicals against major insect pests of cabbage (*Brassica oleracea* var *capitata*)

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ABSTRACT: A study was conducted to test the efficacy of different neem products, *viz.*, pulverised neem seed powder extract (PNSPE), pulverised neem seed powder formulation (PNSPF), neem soap and neem cake petrol-water extract (NCPE), and synthetic insecticides against the insect pests of cabbage at the Indian Institute of Horticultural Research (IIHR), Bangalore during *rabi* 2012-13. It was found that PNSPE and PNSPF treatments recorded significantly less diamond back moth (DBM) than other botanicals and chemical insecticides except spinosad. On other hand, botanicals such as PNSPF, PNSPE and NCPE were found to be on par with the chemical insecticides in reducing leaf webber, recording very low incidence. In case of *H. erysimi,* PNSPF recorded the lowest incidence (4.67 aphids/8 plants) than any other treatment plots, with a significant difference over the synthetic chemical insecticides such as indoxacarb (41.33 aphids/8 plants), spinosad (75.00 aphids/8 plants) and flubendiamide (61.33 aphids/8 plants). Whereas, incidence of *Brevicoryne brassicae* was found to be more in chemical treated plots such as spinosad and flubendiamide than those received PNSPF and PNSPE. Population of *Cotesia plutellae*, larval parasitoid of DBM was ranged from zero and 7.67 in spinosad, but there was no statistical significant difference found among different treatments. All the treatments were superior to control plots in terms of yield and marketable heads. Although highest yield was recorded in PNSPE (76.17 t/ha), PNSPF (73.15 t/ha) and other insecticide treatments, like spinosad (76.37 t/ha), flubendiamide (61.97 t/ha), there was no significant difference among them. This study showed that PNSPF and PNSPE can be used as alternatives to the synthetic insecticides in managing DBM and aphids.

Keywords: Botanicals, cabbage, diamondback moth, neem

INTRODUCTION

The major constraint in the production of cabbage is pest complex right from germination till harvest. About 51 insect pests have been reported on cruciferous crops throughout the world (Maison, 1965). In India, a total of 37 insect pests have been recorded on cabbage (Lal, 1975). Aphids and defoliating insects are the most destructive to the crop. Of these, two species of aphids, *Brevicoryne brassicae* and *Hydaphis erysimi*, four species of defoliators *viz.*, diamondback moth (DBM), *Plutella xylostella* (Linnaeus); cabbage butterfly *Pieris brassicae* L. (in Himalaya and Himalayan plain); cabbage semilooper *Trichoplusia ni* Hubner; tobacco caterpillar, *Spodoptera litura* (Fabricius) and head borer *viz*., *Hellula undalis* Fabricius are of economic importance in Karnataka (Mallapur, 1988). The extent of damage due to these pests in India is reported to be from 7 to 90 per cent with consequent reduction in yield from 20 to 80 per cent (Prasad, 1963).

Since cabbage is a highly remunerative, intensive plant protection measures involving a number of insecticides are common. In spite of large scale and indiscriminate applications of insecticides, the pest has been found to occur in severe form in all cabbage growing areas. This has resulted in several problems *viz*., pesticide resistance (Sannaveerappanavar and Viraktamath, 1997; Lingappa *et al*., 2000; Vastrad *et al*., 2004), resurgence (Nemato *et al*., 1984), residue problems, death of natural enemies due to effect of chemicals, environmental pollution *etc.* Besides, cabbage is consumed raw as salad, the presence of insecticide residues is dangerous health hazard to consumers. These have encouraged the development of non-chemical methods of pest management in cabbage and other crops. Recently, plant products, especially neem based products have gained worldwide attention (Schumeterer, 2002; Srivastava, 2001). They are not only effective against crop pests but also safer to natural enemies (Patel *et al*., 2003). Further in recent years, there is a lot of awareness and preference for organically produced foodstuff in the country. At Indian Institute Horticultural Research (IIHR), Bangalore, an IPM of using Indian mustard as a trap crop was developed (Srinivasan and Krishnamoorthy, 1991) under this two rows of Indian mustard has to be sown for every 25 rows of cabbage

and cabbage was sprayed with Neem seed kernel extract (NSKE). Whereas, the mustard foliage has to be protected by spraying Dichlorovas (DDVP). However, this IPM did not become popular due to resistance by farmers to take two rows of mustard and also due to drudgery in preparing NSKE and non availability of neem seeds when required. Hence, refinements were made in the IPM and this had led to the development of pulverised neem seed powder extract (PNSPE). Another two alternatives developed on NSKE by IIHR were neem soap and pongamia soap (Krishna Moorthy and Krishna Kumar, 2002). These are prepared from their respective oils. Another recent finding of IIHR is the use of petroleum extract of neem cake (NCPE), an alternative to synthetic insecticides which may be sustainable at village level and was found effective on aphids in cabbage and also in capsicum. Hence, these botanicals were compared with the popular synthetic insecticides like spinosad, indoxacarb and flubendiamide for their bio-efficacy in field conditions against major insect pests of cabbage like, DBM, aphids, leaf webber and also their impact on natural parasitoid like *Cotesia plutellae*. Results of the study have been presented in this paper.

MATERIALS AND METHODS

The experiment was carried out at IIHR Experimental farm, Bangalore (13° 5'N 77° 35'E) during *rabi* 2012-13. The most popular cabbage hybrid 'Unnathi' seedlings were transplanted on 26-11-2012 in thirty plots, each measuring 4 m x 3 m with a spacing of 50 cm x 30 cm. FYM (25 t/ha) was incorporated during land preparation, while the recommended fertilizer 150:125:100 NPK was also supplemented. The N was applied as urea in two split doses as 50% basal dose i.e. 7.5 kg and remaining 50% as top dressing; whereas, full dose of P_2O_5 and K_2O were applied as a basal dose. There were nine treatments viz., Pulverised Neem Seed Powder Extract (PNSPE 4%), Neem Seed Powder Formulation (PNSPF 3%), Neem Cake Petrol Extract (NCPE) (5% neem cake + 0.1% petrol), Neem soap (1%), Pongamia soap (1%); synthetic insecticides like indoxacarb 14.5 SC (0.01%), spinosad 45 SC (0.013%), flubendiamide 39.35 SC (0.0039%) along with an unsprayed, control. Each treatment was replicated thrice in a randomised complete block design (RCBD). The Neem seed powder extract was prepared by soaking the pulverised neem seed powder overnight in water and filtering through 2-3 layers of nylon mesh next day before spray. Similarly the neem seed powdered formulations also soaked overnight and sprayed next day after filtering. Likewise, neem cake petrol extract was obtained by soaking the neem cake in water and with known quantity of petrol over night (50g neem cake + 1ml of petrol/litre). The extract thus obtained is made upto required spray volume.

The treatments were given at the rate of 7-10 days intervals starting at 20 days after transplanting (DAT). Observation on number of DBM, aphids such as, *H. erysimi, B. brassicae,* leaf webber, number of parasitoid, *C. plutellae* pupae were recorded on randomly selected eight plants/plot at the rate of ten days interval. As the incidence of aphids was high in insecticide treated plots when the crop reached harvesting stage, one spray of dimethoate (0.06%), an effective and safer systemic insecticide was given to such insecticide plots only. The data was converted into Log (x+1) before subjecting to two way ANOVA for statistical interpretation.

RESULTS

Diamondback moth (DBM), *P. xyloxtella*

Pre-treatment count of DBM under different treatments was found to be at par with each other (Table 1), but spinosad found to be significantly superior (0.00 DBM/plant) over the rest of the treatments up to 50 DAT. Afterwards, botanicals like PNSPE and PNSPF were at par with it. When botanical sprays were compared, it was found that PNSPF and PNSPE treatments recorded significantly less DBM than other botanicals and chemical insecticides except spinosad. And at the end of the cropping period all botanicals were found to be at par with spinosad in reducing DBM (Table 1). On other hand, *C. plutellae* a known parasitoid of DBM was noticed in all treatments except in spinosad treated plot. Its incidence was ranged from zero to 7.67 in spinosad and neem soap received plots, respectively. Similar trend was observed even in untreated plots. However, there was no statistical significant difference found among the treatments, hence data has not been presented.

Leaf webber, *C. binotalis*

Incidence of leaf webber was found to differ significantly among the treatments at 50, 60 and 70 DAT. The high incidence of webber was recorded in untreated plots of cabbage than treated plots. There was no incidence of leaf webber in three chemical sprayed plots. However, botanical PNSPF, PNSPE and neem cake petrol extract were found to be on par with the chemical insecticides in reducing leaf webber, recording very low incidence (Table 2).

Table 1. Incidence of diamondback moth in cabbage under different treatments during *rabi,* 2012-13

Treatment	Dose	(Pre Treatment)	30 DAT	50 DAT	70 DAT
Neem seed powder formulation	40g/l	9.33(2.33)	4.67(1.66)^{b}	1.33(0.83)^{b,c,d}	0.00(0.00)^{a}
Neem Soap	10g/l	5.33(1.79)	8.67(2.26)^{c}	2.00(1.00)^{c,d,e}	2.33(0.96)^{a}
Pongamia Soap	10g/l	13.33(2.65)	12.33(2.59)^{d}	9.33(2.33)^{g}	1.00(0.60)^{a}
Neem seed powder extract	40g/l	11.67(2.51)	4.33(1.67)^{b}	1.00(0.60)^{a}	0.33(0.23)^{a}
Neem cake petrol extract	50g/l +1ml/ l petrol	6.00(1.73)	9.00(2.16)^{b}	4.33(1.60)^{e,f}	0.33(0.23)^{a}
Indoxacarb	(0.75ml/l)	9.67(2.36)	5.33(1.80)^{b}	0.33(0.23)^{a}	0.33(0.23)^{a}
Spinosad	0.3ml/l	4.00(1.52)	0.00(0.00)^{a}	0.00(0.00)^{a}	0.33(0.23)^{a}
Flubendiamide	(0.1ml/l)	6.00(1.92)	10.00(2.40)^{d}	4.00(1.56)^{d,e,f}	3.67(1.43)^{b,c}
Control	-	8.33(2.17)	14.00(2.65)^{d}	8.00(2.11)^{f,g}	4.67(1.71)^{c}
CD (P=0.05)		NS	0.54	0.74	0.99
CV		20.76	15.69	33.94	82.01

Values in the parenthesis are Log (x+1) transformed; DAT: Days after transplantation;

Table 2. Incidence of leaf webber, *Crocidolomia binotalis* on cabbage after different insecticidal sprays during *rabi,* 2012-13

Treatment	Dose	30 DAT	50 DAT	70 DAT	Yield (t/ha)
PNSPF	30g/l	0.00 (0.00)	0.67 (0.46)^{b}	0.00 (0.00)^{a}	73.15^{a}
Neem Soap	10g/l	0.00 (0.00)	2.67 (1.23)^{c}	1.67 (0.96)^{b,c}	61.28^{a}
Pongamia Soap	10g/l	7.33 (1.38)	5.00 (1.73)^{c,d}	1.00 (0.46)^{a}	57.89^{a}
PNSPE	40g/l	0.00 (0.00)	0.67 (0.46)^{b}	0.00 (0.00)^{a}	77.17^{a}
NCPE	50g/l +1ml/l petrol	1.00 (0.46)	4.00 (1.61)^{c,d}	0.00 (0.00)^{a}	53.10^{a}
Indoxacarb	(0.75ml/l)	0.00 (0.00)	0.33 (0.23)^{b}	0.00 (0.00)^{a}	65.96^{a}
Spinosad	0.3ml/l	0.00 (0.00)	0.00 (0.00)^{a}	0.00 (0.00)^{a}	76.37^{a}
Flubendiamide	(0.1ml/l)	0.00 (0.00)	0.33 (0.23)^{b}	0.00 (0.00)^{a}	61.97^{a}
Control	untreated	2.67 (1.06)	6.00 (1.92)^{d}	5.67 (1.50)^{c}	27.23^{b}
CD (P=0.05)	-	NS	0.62	0.83	28.34

PNSPF: Pellatised neem seed powder formulation; PNSPE: Neem seed powder extract; NCPE: Neem cake petrol extract;Values in the parenthesis are Log (x+1) transformed; DAT: Days after transplantation

Aphids (*H. erysimi* and *B. brassicae)*

There was no significant difference among treatments after first spray (Table 3). However after second spray, PNSPF recorded the lowest incidence (4.67 aphids/8 plants) of *H. erysimi* than any other treatment plots, with a significant difference over the synthetic chemical insecticides such as indoxacarb (41.33 aphids/8 plants), spinosad (75.00 aphids/8 plants) and flubendiamide (61.33 aphids/8 plants). Whereas, in case of *B. brassicae* none of them were found to differ significantly. Likewise, after third and subsequent sprays also PNSPF and PNSPE proved the best by recording lowest aphids than control (Table 3). On other hand, chemical insecticides such as spinosad and indoxacarb treated plots had more incidence of aphids than botanicals even after receiving a blanket spray of dimethoate (Table3). Results clearly suggested that botanicals, particularly PNSPF and PNSPE have good potential in managing aphids in cabbage.

Yield

Although highest yield was recorded in PNSPE (76.17 t/ha) and percent marketable yield in PNSPE (96.79 %), PNSPF (95.51 %) and chemicals such as spinosad (97.44 %), flubendiamide (96.79 %) sprayed plots (Table 4). There was no significant difference among botanicals and chemical insecticides in terms of yield and per cent marketable cabbage heads. But, all the treatments were superior to control plots (65.35%) as far as yield and marketable heads are concerned.

DISCUSSION

The superiority of neem seed powder formulations (PNSPF), neem seed powder extract (PNSPE) and neem cake petrol extract over the synthetic insecticides in managing major pests of cabbage were proved successful in this study. Products derived from neem seed (PNSPF, NSPE, Neem cake + petrol extract) have to be soaked overnight to enhance extraction of insecticidal compounds present in them. These compounds play a significant role in managing the pests of cabbage (Ishaaya *et al.,* 2007). Further, multiple mode of actions of these products such as repellent, antifeedant, ovipositional deterrent, growth regulator etc., have been attributed in controlling both sucking as well as foliage feeders (Xia *et al.,* 1995; Paterson, 2009; Boadu *et al.,* 2011). As the organic synthetic insecticides are more dangerous, leave toxic residues in food not readily biodegradable (Peshin and Dhawan, 2009) and can harm beneficial pests, Neem based formulations offers a sound alternative in these aspects.

Diamondback moth, leaf webber and aphids, (*B. brassicae* and *H. erysimi*) are the major insect pests of cabbage (Krishna Moorthy and Krishna Kumar, 2001). Use of pulverised neem seed powder and neem soap were found to be effective and economically viable (Gajanana *et al.,* 2004; Srinivasan and Krishna Moorthy, 1991). Results on DBM incidence under different treatment showed that DBM population gradually declined among the plots received different treatments. It was observed that spinosad was the most effective insecticide in reducing DBM on different dates of observation. Whereas, indoxacarb was at par with spinosad in the last four observations, though it did not bring down DBM to zero on any dates.

The present study has also shown that although, botanicals could not reduce DBM as effectively as spinosad initially, but were able to reduce the pest population at the end of cropping period significantly. PNSPF brought down the DBM to zero on the last two observations, whereas PNSPE and pongamia soap also brought down DBM population during the last observation. High leaf webber incidence was observed during the experimental period (Table 2) as observed in control plots on 40 DAT. Spinosad treated plots had significantly lower leaf webber incidence as compared to other plots. At 50 DAT, the incidence of leaf webber in botanicals and insecticide treated plots were significantly lower as compared to control, except neem soap treated plot on 70 DAT. This clearly states that mechanical control can be used effectively in tackling leaf webber. This will be very useful when cabbage or cauliflower raised organically.

The incidence of *B. brassicae* was initially lower in all the plots and increased later substantially, particularly in spinosad treated plots. Incidence of this aphid in flubendiamide, neem soap, and pongamia soap was at par and was > 30 aphid/ 8 plants. The plots treated with PNSPF and PNSPF had significantly reduced *B. brassicae* incidence (Table 3) as compared to other treatments. Almost similar trend was observed for another aphid, *H. erysimi* (Table 3). There was no statistical significant difference found interms of yield and per cent marketable yield as well among the treatments. Although yield in the botanicals treated plots were at par with chemical insecticide treated plots. Marketable yield (%) in PNSPE (96.79%), PNSPF (95.51%) were almost same as that of spinosad (97.44%), flubendiamide (97.44%), and Indoxacarb (94.23%) treated plots and significantly differ from control (65.38%). Results from study clearly suggest that botanicals not only control the

Table 3. Incidence of cabbage aphids, *H. erysimi* and *B. brassicae* in cabbage under different treatments during rabi, 2012-13

Treatment	Dose	30 DAT		50 DAT		70 DAT	
		H. erysimi	*B. brassicae*	*H. erysimi*	*B. brassicae*	*H. erysimi*	*B. brassicae*
PNSPF	30g/l	4.67 (1.39)[a]	10.67 (1.51)	5.00 (1.32)[a]	8.00 (1.07)[a,b]	6.67 (1.01)[a,b]	0.00 (0.00)
Neem Soap	10g/l	62.33 (4.07)[e]	9.33 (1.78)	49.0 (3.82)[b,c]	56.67 (3.71)[b,c]	10.0 (1.14)[a,b]	23.33 (2.38)
Pongamia Soap	10g/l	45.0 (3.78)[b,c]	1.33 (0.54)	80.0 (4.34)[b,c]	39.0 (3.40)[a,b,c]	5.0 (1.40)[a,b,c]	113.33 (2.73)
PNSPE	40g/l	22.67 (2.45)[a,b]	32.0 (2.56)	1.67 (0.60)[a]	1.33 (0.54)[a]	0.00 (0.00)[a]	0.00 (0.00)
NCPE	50g/l + 1ml/l petrol	48.0 (3.85)[b,c]	102.0 (4.24)	43.33 (3.57)[b]	110.8 (4.97)[c]	0.00 (0.00)[a]	17.0 (2.27)
Indoxacarb	(0.75ml/l)	41.33 (3.44)[b,c]	26.00 (2.77)	256.67 (3.88)[b,c]	534.0 (5.65)[c]	16.67 (2.16)[a,b,c,d]	152.0 (3.85)
Spinosad	0.3ml/l	75.00 (4.12)[e]	7.0 (2.07)	622.67 (5.81)[c]	615.67 (5.87)[c]	173.33 (4.16)[d]	54.0 (1.70)
Flubendiamide	(0.1ml/l)	61.33 (4.12)[e]	9.67 (1.95)	89.67 (4.37)[b,c]	52.0 (3.48)[c]	24.67 (2.80)[b,c,d]	65.0 (2.80)
Control	-	61.00 (3.96)[c]	22.67 (2.77)	116.67 (4.73)[b,c]	73.67 (4.26)[c]	180.33 (4.08)[d]	358.67 (5.35)
CD (P=0.05)		1.4	NS	2.16	2.95	2.53	NS
CV		23.35	63.56	33.87	46.23	71.45	87.65

pests of cabbage but also increase the yield by reducing the losses caused due to pest incidence.

Over all, the present experiment showed that PNSPF and PNSPE can be used as alternatives to the new and popular synthetic insecticides like spinosad, indoxacarb and flubendiamide in managing DBM and aphids. Mechanical control was also employed effectively in control leaf webber without using synthetic insecticides. Earlier, Krishnamurthy *et al.* (1998) have successfully demonstrated the use of neem seed kernel extract (NSKE) to control insecticide resistant diamondback moth under mechanised farming conditions. Later, extensive demonstration of IPM of cabbage and cauliflower were demonstrated in and around Bangalore and it was found economical (Gajanana *et al.*, 2004). They have opined that the availability of bio-pesticides as the major constraint in adoption of IPM. Therefore, IIHR has developed new formulations of PNSPF. It was found effective on all the insect pests of cabbage and also it can also be stored and commercialized. Hence, this can marketed by private firms, NGOs etc., and can be used as alternative to synthetic insecticides.

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Behavior of oxydemeton-methyl in/on citrus fruits and soil in the hilly zone of Karnataka, India

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ABSTRACT: Experiments were conducted to study the residue persistence and dissipation kinetics of oxydemeton-methyl **(**ODM) in/on citrus fruits following at the standard (0.025) and double dose (0.05%). The methodology included extraction with acetone, partitioning into chloroform, oxidation with 0.1 N potassium permanganate and partitioning into chloroform. The ODM sulfone residues were analyzed by gas liquid chromatograph (GLC). The initial deposit residues of oxydemeton methyl on citrus peel was 1.23 and 2.66 mg/kg from treatment at the standard and double doses, respectively. The residues persisted for 15 and 20 days and dissipated with the half-life of 2.2 and 4 days. Residue levels in citrus pulp reached highest of 0.018 and 0.057 mg/kg from standard and double treatments, which reached below detectable level after 20 days. The pre-harvest interval (PHI) of ODM on citrus was worked out to be 14 and 26 days for treatment at standard and double doses, respectively. The residues of ODM were below the detectable limit of 0.01 mg/kg in soil and citrus fruit at harvest.

Keywords: Citrus, oxydemeton-methyl, pre-harvest interval, residue

INTRODUCTION

India ranks sixth in the production of citrus fruit in the world. Citrus occupies third position after mango and banana in terms of production. Of the various types of citrus fruits grown in India, mandarins are of commercial importance and also popularly known as Santras in India. Mandarin (*Citrus reticulata Blanco*) occupies nearly 50 percent of the total citrus area and grows in all tropical and sub tropical regions. Specific cultivars of mandarins are cultivated in different regions of India. For example, Coorg mandarin is typical to Kodagu region of Karnataka and it is considered as one of the most important commercial variety in south India. It is particularly grown in semi arid tropical climatic conditions of Kodagu, Karnataka, as an inter-crop with coffee. Coorg mandarin is attacked by a number of insect pests and among them citrus psylla, *Diaphorina citri* Kuwayama, is an important pest in this region. Psylla is also a vector of citrus greening disease. Oxydemeton-methyl, (*S*-2-ethylsulfinylethyl *O,O*-dimethyl phosphorothioate) is a broad spectrum, systemic insecticide/miticide registered for use against mites, thrips, aphids and whitefly of fruits, vegetables, cotton, ornamentals and forestry (Anonymous, 1998a; Safaeian *et al.,* 2008). It belongs to aliphatic organo thiophosphate family of chemicals and widely used to control sucking pests. It is absorbed and translocated within the plant to kill insects that feeds on the plant. Oxydemeton-methyl is the primary thio-oxidation metabolite of demeton-S-methyl. It is metabolized similarly in plants and mammalian systems to form demeton-S-methyl sulfone, which is commonly known as oxydemeton-methyl sulfone. Oxydemeton-methyl is effectively used for control of citrus psylla (Shivankar *et al.,* 2000). Though it is widely used on citrus, no information is available on its residue persistence on citrus. This study was therefore conducted to evaluate the residue persistence of oxydemeton-methyl on citrus fruits and soil under the high rainfall hilly areas of Karnataka, India.

MATERIALS AND METHODS

Reference standards of oxydemeton-methyl (purity 85.5%) and the formulation Metasystox 25 EC were obtained from Bayer Crop Science Limited, India. All reagents and chemicals used were of analytical grade, procured locally. Oxydemeton-methyl standard solution (1000 ppm) was prepared with distilled acetone. Known quantity of oxydemeton-methyl standards were oxidized to the sulfone form as per the method described below and the same was used as working standard. Chemical structure of oxydemeton-methyl and its sulfone derivative is given in Fig.1. Field experiment of oxydemeton-methyl on citrus was carried out at Central Horticultural

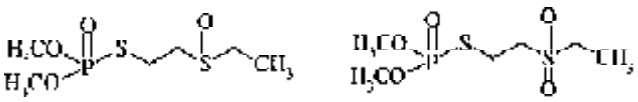

Fig. 1. Chemical structure of (a) oxydemeton-methyl, (b) oxydemeton-methyl sulfone

Experimental Station (CHES), Chettalli, Kodagu, Karnataka, India on Coorg mandarins. Treatments included recommended dose (0.025%) and double the recommended dose (0.05%) and a control. For every treatment, ten trees were selected. The spray volume was 1000 L/ha. Untreated control plots were sprayed with water. Two treatment sprays of oxydemeton-methyl formulation (Metasystox 25 EC) were given to the citrus crop at the 2 concentrations (0.025% and 0.05%) at 10 day intervals. The first spray application was given at the fruit development stage, further spray was given 10 days later using triple action/hollow cone nozzle sprayer. After the second spray, residue analysis of oxydemeton-methyl on citrus pulp and peel was carried out on 0 (1 h), 1, 3, 5, 7, 10, 15, 20, 25, 30 day and at harvest. Soil samples from the treated field was collected and analyzed at the time of harvest. On every sampling day approximately 250 g citrus fruits were collected from each tree and pooled together. A total of 2.5 kg was collected from each treatment. The fruits were packed in dry ice and transported in air conditioned vehicle to the laboratory for processing. The citrus fruits were peeled, the peel and pulp samples were cut into small pieces. The samples were mixed thoroughly and a representative 50 g pulp and 25 g peel sample (in triplicates) was taken for analysis of oxydemeton-methyl residues. From each plot, soil sample was collected from 3 x 3 grid and 30 cm depth with a total of ten sampling sites. From each treatment, soil samples (5 kg) were collected pooled together, air dried and passed through 2 mm sieve. A representative 100 g soil sample in triplicate was taken for analysis and processed in a similar manner like citrus peel and pulp.

Extraction and clean-up of oxydemeton-methyl in citrus was carried out as per (John *et al.*, 1977). A 50 g portion of representative citrus pulp sample was homogenized in a Waring blender with 100 mL acetone and filtered under vacuum through a Buchner funnel. The container and the filter cakes were washed twice with 100 mL acetone and the combined extracts were collected in a 500 mL flask. The combined extracts were evaporated in a flash evaporator and the aqueous fraction was partitioned with 100 + 50 + 50 mL chloroform after adding 35 mL saturated sodium chloride solution. The combined chloroform fraction was dried over anhydrous sodium sulphate and evaporated to dryness and the residues were redissolved in 5 mL of acetone. The oxydemeton-methyl residues in acetone were oxidized to the sulfone derivative using potassium permanganate. Twenty five mL of 0.1 N potassium permanganate was added to the sample and kept for 25 min with occasional shaking. The oxidized part was partitioned into 50 + 25 + 25 mL of chloroform. The combined chloroform extracts were concentrated and redissolved with 5 mL of distilled acetone for analysis by gas liquid chromatograph (GLC). Citrus peel (25 g) and soil samples (100 g) were processed similarly. The final volume of peel and soil samples were made to 10 mL with distilled acetone.

Before analysis of field sample, a recovery experiment was conducted by spiking untreated samples (citrus peel, pulp and soil) with analytical grade oxydemeton-methyl at the rate of 0.01, 0.05, 0.1 and 0.5 mg/kg. The spiked samples in five replicates were processed as per the analytical method described above to obtain the percent recovery. Citrus peel, pulp and soil samples without oxydemeton-methyl served as control. A gas chromatograph (Varian CP 3800) equipped with a thermionic specific detector (TSD) was used for analysis of oxydemeton-methyl. A capillary column, fused silica 30 m X 0.53 mm i.d. was used. Ultra pure nitrogen was used as carrier gas with a flow rate of 1.0 mL/min. Hydrogen and air flow rates were maintained at 4.0 and 175 mL/min, respectively. The oven temperature was initially maintained as 210^0C with a hold time of 5 min and programmed at the rate of 4^0C/min to 250^0 C with a hold time of 10 min. Injector and detector temperatures were maintained at 250^0C and 280^0C, respectively. Under the above conditions the retention time of oxydemeton-methyl (as sulfone) was 10.9 min. The residue data was subjected to statistical analysis according to Hoskins (1961) to compute the residual half-life ($t_{1/2}$) and pre-harvest interval (PHI).

Confirmatory studies were carried out with gas chromatograph mass spectrometry (GC-MS). For analysis by GC-MS a Shimadzu QP2010 Plus equipment, with a QP2010 Plus detector operated in full scan mode (40-450 m/z) was used. The gas chromatograph was operated in split less mode with injector temperature of 250^0C and ion source temperature of 200^0C. A capillary column, Agilent DB-5 (30 m X 0.25 mm i.d.) was used for analysis. The column oven temperature programme was same as with gas chromatograph analysis. Helium was used as carrier gas with a column flow rate of 1 mL/min. The initial solvent cut time was 4 min. The retention time of oxydemeton-methyl (as sulfone) was 8.6 min with the above parameters. The MS spectrum was compared with NIST mass spectra data base and found to match more than (94%) for each compound.

Table 1. Recovery of oxydemeton-methyl (as sulfone) residues on citrus pulp, peel and soil at various spiked levels

Spiked concentration (mg/kg)	Mean recovery* (%) ± SD		
	Pulp	Peel	Soil
0.01	85.43 ± 3.56	89.36 ± 2.31	86.75 ± 2.83
0.05	86.03 ± 2.21	91.46 ± 3.03	86.68 ± 3.58
0.10	86.56 ± 4.05	92.12 ± 3.21	87.34 ± 2.84
0.50	87.68 ± 2.44	93.96 ± 2.86	88.23 ± 1.23

*Average of five replicates± standard deviation

RESULTS AND DISCUSSION

Recovery study carried out by spiking citrus peel, pulp and soil with oxydemeton-methyl is presented in Table 1. In citrus pulp recovery of oxydemeton-methyl was in the range of 85.43 –87.68 percent, in peel the recovery was in the range of 89.36-93.96 percent and in soil the recovery was 86.68 – 88.23 percent. The limit of quantification (LOQ) of oxydemeton-methyl sulfone on citrus peel, pulp and soil using the above method was 0.01 mg/kg. Residues of oxydemeton-methyl (as oxydemeton-methyl sulfone) were estimated in citrus pulp and peel separately over a period of 30 days and at harvest. Residue deposits of oxydemeton-methyl on fruit peel immediately after the second application was 1.237 and 2.656 mg/kg from treatment at the recommended and double the recommended dose of 0.025 percent and 0.05 percent, respectively (Table 2). Oxydemeton-methyl residues dissipation was quite fast from citrus fruit. The residue dissipation over time is presented in Fig.2. A day after the treatment nearly 30 percent residues were lost from both the treatments. After 5 days 81 and 73 percent residues were lost from treatment at the recommended and double doses, respectively. Oxydemeton-methyl degraded with the half-life of 2 and 4 days. The residues on the fruit peel persisted for 10 and 20 days from treatment at the recommended and double the recommended doses, respectively. Movement of oxydemeton-methyl residues from the surface of the fruit to the pulp was a very slow process. The residues in citrus fruit pulp were initially detected after 7 days from treatment at recommended dose and after 5 days from treatment at double the recommended dose. From recommended dose of treatment the residues were 0.018 mg/kg on the 7th day which reduced to 0.011 mg/kg by 15 days and reached below detectable level (BDL) by 20 days (Table 2). From double dose treatment the residues were first detected on the 5th day, reached a maximum of 0.056 mg/kg on the 7th day. Thereafter the residue level reduced and reached below detectable level by 25 days. Confirmatory studies were carried out with GC-MS showed the presence of oxydemeton methyl reported in citrus sample. The maximum residue limit (MRL) of oxydemeton-methyl in citrus is recommended at 0.02 mg/kg by European Union (Anonymous 2003). Based on this value the pre-harvest interval (PHI) for safe consumption of citrus fruits after treatment with oxydemeton-methyl is recommended as 14 and 26 days, for treatment at the recommended and double the recommended dose of 0.025 percent and 0.05 percent. From the recommended dose of treatment, residues remained for a longer period of time in the fruit pulp compared to the fruit surface, but the level was lower than the MRL prescribed.

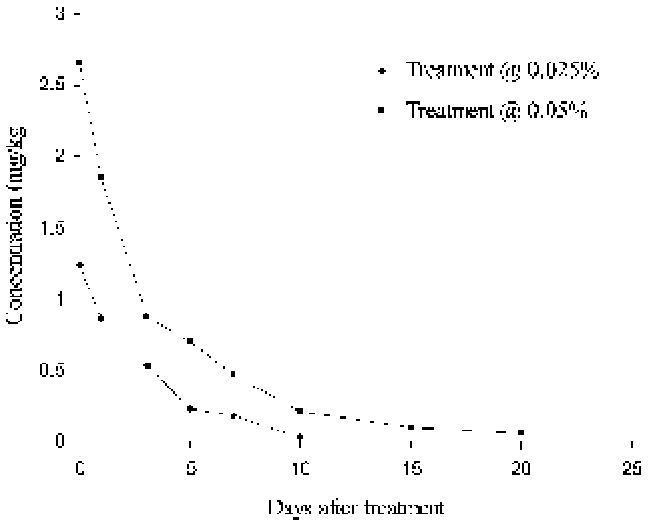

Fig. 2. Dissipation of oxydemeton-methyl on citrus

Residue trial of oxydemeton-methyl on grapes treated at the rate of 0.38 and 0.76 kg a.i./ha resulted in

Table 2. Residues of oxydemeton-methyl (as sulfone) in/on citrus pulp, peel and soil

Days after treatment	Residues recovered (mg/kg)*				
	Treatment @ 0.025%			Treatment @ 0.05%	
	Untreated control	Pulp	Peel	Pulp	Peel
0	ND	BDL	1.237 ± 0.080	BDL	2.656 ± 0.054
1	ND	BDL	0.867 ± 0.028 (29.91)	BDL	1.846 ± 0.106 (30.50)
3	ND	BDL	0.534 ± 0.010 (56.83)	BDL	0.878 ± 0.021 (66.94)
5	ND	BDL	0.234 ± 0.022 (81.08)	0.011 ± 0.001	0.699 ± 0.003 (73.68)
7	ND	0.031 ± 0.002	0.185 ± 0.015 (85.04)	0.057 ± 0.002	0.468 ± 0.025 (82.38)
10	ND	0.018 ± 0.001	0.035 ± 0.002 (97.17)	0.042 ± 0.002	0.217 ± 0.011 (91.83)
15	ND	0.011 ± 0.001	BDL	0.022 ± 0.001	0.101 ± 0.010 (96.20)
20	ND	BDL	BDL	0.015 ± 0.001	0.061 ± 0.002 (97.70)
25	ND	BDL	BDL	BDL	BDL
Citrus peel (at harvest)	ND	BDL	BDL	BDL	BDL
Citrus pulp (at harvest)	ND	BDL	BDL	BDL	BDL
Soil (at harvest)	ND	BDL	BDL	BDL	BDL

*****Average of three replicates± standard deviation.
The values in the parenthesis show the percent dissipation of residues.
Limit of quantification (LOQ) - 0.01 mg/kg
Below detectable limit (BDL) > 0.01 mg/kg, ND- Not detected

residue levels below the MRL of 0.2 mg/kg in 10 and 14 days, respectively (Rao *et al.*, 1990). Supervised field trials conducted to evaluate residues of oxydemeton-methyl on various crops showed that it degraded faster on vegetables but persisted longer on fruit crops (Anonymous 1998b). The residues could be found upto 90 days on apple depending on the stage of application, whereas on cabbage it could reach <0.01 mg/kg by 7 days. In the present study it was observed that residues of oxydemeton-methyl dissipated faster from the surface of citrus fruits. A small portion of the surface residue penetrated to the fruit pulp. Once it moved to the fruit pulp it dissipated very slowly. However the residues remained on the fruits only for 20 days.

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Management of nematodes in banana through bio-rationale approaches

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Abstract: Studies were undertaken to determine the efficacy of different bio-rationale approaches in managing the incidence of burrowing nematode, *Radopholus similis* and spiral nematode, *Helicotylenchus multicinctus* in banana under field conditions. The bio-rationale treatments included sucker dip with neem oil (1.5 %), soil application of pressmud at the rate of 5 kg/plant, neem cake at the rate of 500 g/plant, vermicompost at the rate of 250 g/plant, growing marigold (*Tagetes errecta*) around the basin and growing coriander (*Coriandrum sativum*) around the basin which were compared with chemical check carbofuran 3G at the rate of 40 g/plant and untreated control. The study revealed the superiority of the marigold and neem cake treatments in reducing nematode population significantly and increasing banana bunch yield on par with chemical check with 18.3 - 24.6 per cent over the untreated control. High rate of multiplication of bacterial feeding nematodes *Rhabditis* spp. and predatory nematode, *Mononchus* spp. were observed in these treatments. The economic returns were also higher with incremental benefit- cost ratio of 16.8 and 12.5 in marigold and neem cake treatments respectively.

Keywords: Banana nematode, bio-rationale, management

INTRODUCTION

Banana is one of the important fruit crops in India cultivated over 0.83 million ha, constituting about 44.3 per cent of total fruit production. In recent years, the intensive cultivation of banana on a commercial scale is threatened by several pests and diseases. Among the major biotic stresses, plant parasitic nematodes pose a daunting challenge for banana production. The nematode of major concern in banana production throughout the world is the burrowing nematode, *Radopholus similis* (Cobb) Thorne. Other nematode species that frequently cohabit in mixed populations with *R. similis*, such as lesion nematode (*Pratylenchus coffea*), spiral nematode (*Helicotylenchus multicinctus*) and *Meloidogyne* spp. are also regarded as a contributing factor to overall nematode losses. Nematodes attack root and corm tissues causing damage that can result in lengthening of the vegetative growth cycle, production of small bunches, shortened life of the production unit and toppling of the plants. In addition, nematode induced root lesions provide an entry of fungi and bacteria, which contribute to further damage the root system (Gowen and Queneherve, 1990).

The management of nematode pests relies mainly on the repeated use of nematicides which give 50 per cent higher yields than untreated plantations (Gowen and Queneherve, 1990). However, the use of chemical nematicides has many drawbacks such as the potential residue in fruits, groundwater contamination, effect on non target organisms, and toxicity to applicators. From an ecological safety point of view, use of bio-rationale nematode control technique is a better option to keep nematode population level at a minimum, thereby preventing major loss to the bunch yield. For nematode management, application of organic amendments and use of antagonistic plants can be a major strategy that can be exploited under field conditions. In the present study we evaluated the potential of soil amendments with neem cake, vermicompost and press mud, intercropping with marigold (*Tagetus erecta*) and coriander (*Coriandrum sativum*) for the management of banana nematodes in comparison with conventional chemical check.

MATERIALS AND METHODS

Two trials were conducted in the fields predominantly populated with *R. similis* and *H. multicinctus* at the experimental farm, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The first trial was initiated during November 2009 in the field having mean population of *H. multicinctus* at the rate of 104.4/ 250g soil and *R. similis at the rate of* 32.5/250g soil. The second confirmation trial was initiated afresh during January 2011 in a field having mean population of *H. multicinctus* (167.0/ 250 g soil) and *R. similis* (39.0/ 250 g soil). The experiments were laid out in a randomized complete block design with three replications of the treatments *viz.*, (i) sucker dip with neem oil (1.5%), (ii) pressmud at the rate of 5 kg/plant, (iii) neem cake at the rate of 500 g/plant, (iv) vermicompost at the rate of 250 g/plant, (v) growing marigold (*T. errecta*) around the basin, (vi) growing coriander (*C. sativum*) around

the basin, (vii) carbofuran at the rate of 40 g/plant and (viii) untreated control. Uniform sized tissue culture banana plants of cv. Grand Naine planted at 1.8 m x 1.8 m spacing were used for the study. For sucker dip treatment, 25L of neem oil (1.5%) was prepared and the suckers were dipped for one minute and planted immediately. The remaining suspensions were drenched around the sucker after planting in each replication. For intercrop treatment, seeds of marigold cv. MDU 1 and coriander cv. CO 4 were sown around the banana plants in basin. Sixty days after planting (DAP), inter cropped plants were uprooted and incorporated around banana plants. Neem cake, vermicompost, pressmud and carbofuran were applied in plating pits and mixed with soil before planting the suckers. Standard horticultural practices for banana were followed as per TNAU recommendation (TNAU, 2004). The nematode population in soil and root was determined at planting, vegetative phase (100 days after planting), flowering phase (200 days after planting) and at harvesting phase (300 days after planting). Each sample consisting of 10 cores was randomly collected at a depth of 15-20 cm in rhizosphere of plants from each plot. These cores were pooled together into a composite sample (250g/plot). Samples were processed for extraction of nematodes by Cobb's sieving and decanting technique, followed by modified Baermann funnel technique (Southey, 1986). The nematodes collected were killed by heat and fixed by adding equal volume of 4 per cent formalin. The number of different genera of nematodes in suspension was counted under a stereoscope microscope at 40x magnification. Roots collected randomly from three plants in each plot were pooled and 5 g was collected from that composite. Nematode population in roots was determined by the method of Carlier *et al*. (2003). Observations were recorded on pseudo stem height, pseudo stem girth, number of leaves and bunch weight during harvesting stage. Cost effectiveness of each treatment was assessed based on incremental benefit cost ratio i.e. the ratio of incremental return to the incremental cost of by taking the existing market price of banana bunches and application charges. Data from two experiments were pooled and treatment differences were inferred through analysis of variance (ANOVA) followed by comparison of differences using Duncan multiple range test (Panse and Sukhatme, 1989).

RESULTS AND DISCUSSION

The results revealed that the application of all the bio-rationale treatments reduced the plant parasitic nematode population density in soil and infestation in roots compared to untreated control (Table 1). However, the level of nematode infestation varied among the bio-rationale treatments. At vegetative stage, the total plant parasitic nematode population was significantly less (61.3/ 250g soil and 4.2/5g root) in carbofuran treatment followed by neem cake and *Tagetes* treatments which were on par. But at flowering stage, plants from the neem cake and *Tagetes* treatments recorded significantly lesser nematode population (50.1-52.6/250 g soil and 8.8 - 10.8/ 5g root) than other treatments including carbofuran chemical check. A similar trend was maintained up to the harvesting stage in these treatments. The over all mean nematode population of *R. similis* and *H. multicinctus* together was significantly less in the treatments *viz*., *Tagetes* treatment (86.9/250g soil and 89.6/5g root) and neem cake at the rate of 500 g/plant (89.6/250g soil and 16.3/5g root). The population reductions in these treatments were 44.8 -46.5 per cent in soil and 72.3 - 84.0 per cent in roots as compared to untreated control. Effect of these treatments is significantly better than carbofuran treatment which recorded nematode population of 115.7/250g soil and 17.1/5g root.

The population of bacterial feeding nematode (*Rhabditis* spp.) was significantly higher in all bio-rationale treatments than control and carbofuran treatment (Table 2). The population registered an increase of 9-10 fold than initial population in marigold and neem cake treatments whereas it was only three fold in control plots. The predatory nematode population (*Mononchus* spp.*)* was also significantly higher in marigold (44.7/ 250g soil) and neem cake (43.5/250g soil) treated plots compared to control. The carbofuran treated plots had least population (2.6/255g soil) followed by control plot (10.0/ 250g soil). The plants treated with neem cake and *Tagetes* were also significantly taller (226.7-235.3 cm), thicker (61.0-63.3 cm pseudo-stem girth) and with more number (14.7-15.7) of leaves (Table 3). The banana yield was significantly higher (30.0-31.6 kg bunch weight) in plants treated with neem cake and *Tagetes*. These treatments increased the banana yield by 15.6 – 19.9 per cent than control plants. The chemical check registered a bunch yield of 30.2 kg/plant. The results on economics revealed that marigold treatment to record higher (16.8) incremental benefit cost ratio followed by the neem cake application (12.5).

It is apparent that different bio-rationale treatments *viz*., application of neem cake, vermicompost and press mud, coriander intercrop and marigold intercrop in banana resulted in a general reduction in the population of plant parasitic nematodes. The nematicidal effect of neem cake, vermicompost, pressmud, marigold and

Table 1. Effect of bio-rationale treatments on plant parasitic nematode population

Treatment	Pre treatment Population/ 250g soil	nematode population/250 g soil				nematode population/5g root			
		VS	FS	HS	Mean	VS	FS	HS	Mean
Sucker dip neem oil @ 1.5%	170.0	83.2 ^{b}	103.7 ^{cd}	212.8 ^{c}	133.2 ^{c}	8.3 ^{d}	21.1 ^{d}	44.4 ^{d}	24.6 ^{d}
Pressmud @ 5 kg/plant	175.3	94.3 ^{c}	77.0 ^{b}	164.4 ^{b}	111.9 ^{b}	9.5 ^{e}	14.1 ^{b}	34.8 ^{c}	19.4 ^{c}
Neem cake @ 500 g/plant	170.9	78.6 ^{a}	58.7 ^{a}	96.9 ^{a}	78.0 ^{a}	7.3 ^{c}	12.8 ^{b}	23.0 ^{b}	14.3 ^{b}
Vermicompost @ 250 g/plant	177.1	104.1 ^{d}	91.6 ^{c}	179.8 ^{bc}	125.1 ^{c}	13.9 ^{f}	17.9 ^{c}	41.1 ^{d}	24.3 ^{d}
Growing Tagetes around the basin	173.8	80.1 ^{b}	61.8 ^{a}	84.8 ^{a}	75.6 ^{a}	6.3 ^{b}	‘10.4 ^{a}	16.5 ^{a}	9.2 ^{a}
Growing coriander around the basin	168.7	107.0 ^{d}	94.6 ^{c}	197.1 ^{c}	132.9 ^{c}	14.5 ^{g}	18.5 ^{c}	42.9 ^{d}	25.3 ^{d}
Carbofuran @ 40 g/plant	168.0	72.0 ^{a}	90.3 ^{c}	143.1 ^{b}	101.8 ^{b}	5.0 ^{a}	17.4 ^{c}	23.0 ^{b}	15.1 ^{b}
Untreated control	168.1	196.9 ^{e}	212.2 ^{e}	424.3 ^{d}	196.5 ^{d}	19.8 ^{h}	30.0 ^{e}	101.6 ^{e}	50.4 ^{e}
S Ed	NS	3.69	4.98	12.8	7.1	0.14	0.57	1.95	0.88
CD at 5%	NS	7.60	10.60	24.7	15.2	0.38	1.27	4.25	1.92

VS: vegetative stage (100 DAP); FS: flowering stage (200 DAP); HS: harvesting stage (300 DAP); Column letter followed by different letters are significantly different at P=0.05 level by DMRT

Table 2. Effect of bio-rationale treatments on free living and predatory nematode population

Treatment	Population of *Rhabditis* spp./250 g soil					Population of *Mononchus* spp./250 g soil				
	PT	VS	FS	HS	Mean*	PT	VS	FS	HS	Mean*
Sucker dip with neem oil @ 1.5%	88.3	270.7 [d]	400.0 [e]	482.0 [e]	384.2 [e]	6.0	9.0 [d]	16.3 [e]	19.3 [e]	14.9 [d]
Pressmud @ 5 kg/plant	93.7	571.0 [b]	659.0 [c]	724.3 [c]	651.4 [c]	5.7	15.3 [b]	24.3 [d]	29.3 [d]	23.0 [c]
Neem cake @ 500 g/plant	83.0	691.0 [a]	741.3 [b]	856.3 [b]	762.9 [b]	7.0	28.3 [a]	49.3 [b]	53.0 [b]	43.5 [a]
Vermicompost @ 250 g/plant	91.7	431.3 [c]	514.3 [d]	652.7 [d]	532.8 [d]	6.3	12.3 [c]	25.0 [d]	27.3	21.5 [c]
Growing Tagetes around the basin	85.3	258.1 [d]	1013.3 [a]	1243.7 [a]	838.4 [a]	7.1	9.0 [d]	58.0 [a]	67.0 [a]	44.7 [a]
Growing coriander around the basin	94.7	246.0 [d]	732.3 [b]	842.7 [b]	607.0 [c]	6.3	8.3 [d]	31.7 [c]	37.3 [c]	25.8 [b]
Carbofuran @ 40 g/plant	90.3	31.7 [f]	50.3 [g]	61.7 [g]	47.9 [g]	7.3	2.0 [e]	2.7 [g]	3.0 [g]	2.6 [f]
Untreated control	84.0	180.3 [e]	264.0 [f]	296.3 [f]	246.9 [f]	6.7	7.7 [d]	10.0 [f]	12.3 [f]	10.0 [e]
S Ed	NS	16.6	25.9	30.8	24.4	NS	0.6	1.3	1.5	1.1
CD at 5%	NS	35.3	55.7	65.4	52.1	NS	1.3	2.8	3.3	2.5

VS: vegetative stage (100 DAP); FS: flowering stage (200 DAP); HS: harvesting stage (300 DAP); Column letter followed by different letters are significantly different at P=0.05 level by DMRT

Table 3. Effect of biorational modules on growth parameters and yield of banana Cv. Grand Naine

Treatment	Plant height (cm) at harvest	Plant girth (cm) at harvest	No. of leaves at harvest	Bunch weight (kg/plant)	IBCR
Sucker dip with neem oil @ 1.5%	237.8 [a]	57.8 [ab]	13.3 [b]	27.6 [c]	1:10.6
Pressmud @ 5 kg/plant	214.1 [ab]	58.5 [ab]	13.5 [b]	28.7 [bc]	1: 4.9
Neem cake @ 500 g/plant	229.0 [a]	62.1 [a]	15.0 [a]	30.3 [b]	1:12.5
Vermicompost @ 250 g/plant	214.0 [ab]	58.3 [ab]	13.3 [b]	28.4 [c]	1:11.2
Growing *Tagetus* around the basin	237.8 [a]	64.5 [a]	16.0 [a]	31.9 [a]	1:16.8
Growing coriander around the basin	211.5 [ab]	57.8 [ab]	13.5 [b]	28.0 [c]	1: 6.4
Carbofuran @ 40 g/plant	228.1 [a]	61.8 [a]	14.6 [ab]	30.2 [b]	1:12.2
Untreated control	193.7 [b]	53.3 [b]	11.6 [c]	25.6 [d]	
S Ed	8.2	2.1	0.6	0.67	
CD at 5%	15.4	4.0	1.2	1.5	

IBCR-Incremental benefit cost ratio; Column letter followed by different letters are significantly different at P=0.05 level by DMRT

coriander have been reported earlier (Seenivasan, 2010; Jonathan *et al*. 2000; Seenivasan, 2011; Kim *et al*. 2008). Among the various bio-rationales studied, neem cake and marigold treatments proved to be most effective and consistently registered better results in nematode reduction. The significant nematicidal efficacy of marigold observed in the present study could be inferred due to the release of biologically active toxic principles like á-terthienyl and 5-(3-buten-1-enyl)-2,2'–bithienyl from roots (Uhlenbroek and Bijloo (1958), generation of singlet oxygen by photoactivated á-terthienyl (Gommers and Bakker, 1988), dodecanoic acid, myristic acid, palmitic acid and steric acid from flowers (Debprasad *et al*., 2000). Above ground plant parts of marigold have also been found toxic to nematodes (Siddiqui and Alam, 1988). The reasons for improvement in nematode control in marigold treatment in the present study can be summarized as deterrence to early penetration and development of *R. similis* and *H. multicinctus* by the nematicidal principles of root exudates and release of toxic principles by incorporation of their foliage. The significant nematicidal efficacy of neem cake is inferred as a result of potency of biologically active toxic principles like azadirachtin, nimbicidic acid, nimbin, kaimpferol, quercetin and thionemone. Apart from the direct nematicidal effect of these active compounds, some of the organic molecules of these bitter principles might be absorbed by the banana plants bringing about acquired resistance and changes in chemical composition of plant and such roots may exert a broad spectrum of influence on pathogenesis of parasitic nematodes. The increased multiplication and heavy population of bacterial feeding nematodes observed in these treatments may exert antagonism by means of competition for space in the rhizosphere which might lead to reduction of plant parasitic nematodes. Similar antagonistic competition effect of free living bacterial feeding nematode populations against the plant parasitic nematode was reported by Stirling (1991) supports our findings. Soil incorporation of chopped neem leaves resulted in increasing the population of the predatory nematode *Mononchus aquaticus* and thereby reducing the development of root-knot nematode populations by 67.5 % (Akthar and Mamood, 1993). Similarly, in our study more predatory nematode population was observed in neem cake and marigold incorporated plots which resulted in least population of the plant parasitic nematodes. The higher reduction of plant parasitic nematodes in neem cake and marigold treatments reflected on significantly increased bunch yield.

Decline in the efficacy of carbofuran against banana nematodes 100 days after planting was observed in this study. Similarly, Musabyimana *et al*. (2000) also reported that carbofuran was inadequate for long-term control of banana nematodes. However, the efficacy of neem cake and marigold treatments in bananas against nematodes was clearly validated in the present study. It is evident that neem cake and marigold treatments decomposes slowly and remains effective against banana nematodes in the soil for up to harvesting stage. It is concluded that among the different bio-rationale treatments studied neem cake application at the rate of 500g/plant at planting and growing marigold (*T. errecta*) around the basin and incorporation of their bio-mass 60 days after sowing was the most effective treatments as revealed by nematode control potential, growth promotion and profitable returns in banana. These treatments can effectively replace or reduce the use of toxic and expensive chemicals for the management of banana nematodes.

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Identification of *Alternaria* species associated with leaf blights of fruit, vegetable and oil seed crops based on protein and multi-locus enzyme finger prints

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ABSTRACT: Total proteins (both native and SDS dissociated) and four isoenzyme systems (superoxide dismutase, malate dehydrogenase, glutamate dehydrogenase and glucose phosphate isomarase) were analysed for 65 isolates belonging to 13 *Alternaria* species isolated from vegetable, fruits and oil yielding crops. The results allowed the discrimination of *Alternaria solani, A. porri, A.dauci, A. macrospora, A. brassicicola, A. brassicae, A.sesami, A. ricini, A. carthami, A.helianthi, A.alternata, A. mali* and *A. burnsii.* The results obtained with electrophoresis support the use of these macromolecular criteria as an aid in differentiating the species of *Alternaria encountered* on vegetable, fruits and oil yielding crops.

Keywords: *Alternaria*, electrophoresis, protein, isozyme

INTRODUCTION

The *Alternaria* is cosmopolitan genus found worldwide in association with a wide variety of substrates. Many species are saprophytes commonly found in soil, decaying plant tissues, wood, wood pulp, compost and unusual substrates such as sewage or jet fuel (Rotem, 1994). Some species cause a wide range of diseases with huge crop losses on a large variety of cereal, vegetable, fruit, oil–yielding and ornamental crops and also cause extensive spoilage of agricultural output as post harvest pathogens (Thomma, 2003). In addition, they are gaining prominence as air borne allergens (Montemurro and Visconti, 1992), causative agent of phaeohyphomycosis in immunocompromised patients (Rossmann *et al*., 1996) and producers of powerful toxic secondary metabolites (Ostry, 2008) that have been implicated in the development of cancer in mammals (Brugger *et al*., 2006). In view of their wide distribution and potential threat to range of crop plants, animals and humans, information on the extent of intra- and inter-specific diversity is essential for development of rapid and reliable identification systems and effective management strategies (Rotem, 1994; Thomma, 2003).

The identification and classification of *Alternaria* species had been mainly based on their morphological characteristics such as color, size, shape of conidia and conidiation patterns as well as pigmentation (Simmons, 1992). Based on these morphological criteria, a number of attempts have been made to organize taxa into subgeneric groupings (Elliot, 1917; Neergaard, 1945; Joly, 1964), Later, Simmons (1992) placed morpho species into 14 morphological groups. Morphological criteria often overlap among the closely related species that led to taxonomic confusion (Rotem, 1994), making identification and classification very difficult at species level. Subsequent karyotype analysis (Akamatsu *et al*., 1999), molecular approaches based on RAPD fingerprinting (Weir *et al*., 1998; Morris *et al*., 2000; Roberts *et al*., 2000; Pryor and Michailides, 2002), RFLP analysis of rDNA (Adachi *et al*., 1993; Aradhya *et al*., 2001**;** Pryor and Michailides, 2002), and sequence data of the ITS, mt SSU, mt LSU, glyceraldehyde 3-phosphate dehydrogenase, β-tubulin, endo-polygalacturonase (endo-PG), and anonymous genomic regions (OPA1 and OPA2, actin, calmodulin, chitin synthase, translation elongation factor alpha and 1,3,8-trihydroxynaphthalene reductase (Pryor and Gilbertson, 2000; Chou and Wu, 2002; de Hoog and Horre, 2002; Pryor and Bigelow, 2003; Peever *et al*., 2004) and IGS (Hong *et al*., 2005) have supported the placement of *Alternaria* species into distinct species-groups but species within the same species group were not separated due to lack of sufficient genetic information. Chemotaxonomic approach based on known and unknown secondary metabolites, has been used for classification and identification of certain *Alternaria* species with limited success (Anderson *et al*., 2001; 2002; 2005; 2008).

Isozyme analysis has been shown to be useful to assess the intra and inter specific variation and to clarify taxonomic relationships in wide range of fungal

pathogens (Snider, 1973; Burdon and Marshall. 1981; Tooley *et al*., 1985; Micales *et al*., 1986; Leung and Willams, 1986; Nygaard *et al*., 1989; Coetzee *et al*., 2009). Polyacrylamide gel electrophoresis of soluble proteins has also been widely used for delineation of genera, species and sub species of various fungi (Hall, 1973; Schechter, 1973; Snider, 1973; Hansen *et al*., 1986; Bielenin *et al*., 1988; Chowdappa and ChandraMohanan, 1995; El-Din *et al*., 1994). Recently, proteomic study based on matrix assisted laser desorption ionization time-of-flight intact cell mass spectrometry (MALDI-TOF ICMS) was shown to be useful for differentiation of 12 plant pathogenic *Alternaria* species, which exhibited characteristic protein finger prints at species level (Chowdappa *et al*., 2013). Although the analysis of polypeptides/proteins using MALDI-TOF MS has been successful for rapid and reliable identification of *Alternaria* spp., the availability of equipment is limited to sophisticated laboratories and cost prohibitive. Thus, cost effective, simple and rapid techniques for analyses of proteins are required. The objective of the present study was to assess whether single gel native and dissociated protein electrophoresis and isozyme analysis could be used for rapid identification of 13 species of *Alternaria* isolated from vegetable, fruits and oil-yielding crops.

MATERIALS AND METHODS

Fungal isolates

Sixty five morphologically and genetically well characterized isolates belonging to 13 species of *Alternaria* collected from different geographical locations in India were used for analysis. Table 1 provides details of the isolates, geographical origin, host source and GenBank accession numbers for ITS region of r DNA. Isolates were maintained on slants of potato dextrose agar (PDA) at 4°C.

Extraction of soluble protein

Isolates were grown in 250 ml conical flasks containing 100 ml of potato dextrose broth. Each flask was inoculated with three mycelial disks, each of five mm diameter cut from the advancing margin of seven-day-old cultures grown on PDA at 24±1°C in dark. The inoculated flasks were incubated in dark at 24±1°C for seven days· Then, the mycelial mat was harvested by filtering through Whatman No. 1 filter paper, washed with phosphate buffer (pH 7·0) and damp dried and frozen overnight at -20 °C.

The buffer soluble proteins were extracted by grinding 1 g of freeze-dried mycelium by using pestle and mortar in liquid nitrogen with 2 ml of 0.1M Tris-HCl buffer (pH 8.0). The mixture was centrifuged for 45 min at 10,000 rpm and the supernatant was collected. The protein content in supernatant was precipitated by adding equal volume of chilled acetone and kept it for overnight at -20^{0}C. Then, centrifuged the acetone precipitated solution for 30 min at 10,000 rpm, discarded the acetone layer and collected protein precipitate and air dried. Later, dissolved the precipitated protein by adding 1 ml of extraction buffer and centrifuged for 30 min at 10,000 rpm. The supernatant was used for protein and isozyme electrophoresis. The protein content in supernatant was estimated (Bradford, 1976) with bovine serum albumin as standard protein.

Separation of native protein

Electrophoresis of native protein preparations was carried out on a discontinuous system using (5%) stacking gel and (12%) separating gel in a vertical slab (15 × 7.5 ×1.5 cm) according to the method of Laemmli (1970). Gels were fixed, stained and destained according to Bielenin *et al*., (1988). Tris-gylcine buffer at pH 8.3 was used as electrophoresis buffer. Sucrose and bromophenol blue dye were added to soluble protein preparations and aliquots containing 80 µg of fungal protein were placed into wells in the gel. Standard proteins for molecular weight determination (Merck Biosciences, Bangalore, India) were run parallel to the samples. Electrophoresis was carried out at 10 mA for the stacking gel, and at 15mA for the separating gel in cold room at 4°C. The samples were run till the marker dye reached the bottom of the gel plate. The protein patterns were visualized by staining the gels for 7 h with Coomassie brilliant blue G in water: methanol: perchloric acid (15:1:4) mixture and destained with several changes of mixture of water: methanol: acetic acid (7:2:1). The stained gels were imaged and analysed by Alpha imager EP (Alpha Innotech Corporation, San Leandro, CA, USA). The molecular weights of the protein bands in the samples were calculated based on molecular weight standards using the molecular weight analysis software of this system.

Separation of dissociated protein

Sodium dodecyl sulfate (SDS) discontinuous polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970) and slightly modified by Bielenin *et al*. (1988). Soluble protein extracts were mixed with disruption buffer (0.5MTris-HCl at pH 6.8,glycerol, 4% SDS and 10% 2-mercaptoethanol) in the ration of 1:3 and boiled in water bath for 5 min. then 50 µg of dissociated protein samples

Table 1. *Alternaria* isolates used in the present study

Fungal isolates	Host	State	NCBI accession No
Alternaria solani			
OTA 8OTA 78	Tomato	Karnataka	JF491209
OTA 22	Tomato	Karnataka	HQ270459
OTA 66	Tomato	Uttara Pradesh	JF796063
OTA 73	Tomato	Karnataka	JF491204
OTA 81	Tomato	Karnataka	JF491212
Alternaria porri			
OOA 2	Onion	Karnataka	JF710495
OOA 6	Onion	KarnatakaAndhra	JF710488
OOA 8	Onion	Karnataka	JF710494
OOA 12	Onion	Karnataka	JF710497
OOA 13	Onion	Karnataka	JF710491
Alternaria brassicicola			
OCA 7	Cauliflower	Karnataka	JF710522
OCA 8	Cauliflower	Meghalaya	JF710521
OCA 11	Cauliflower	Sikkim	JF710517
OCA 10	Cauliflower	Sikkim	JF710518
OCA 12	Cauliflower	Assam	JF710519
Alternaria brassicae			
OCA 2	Rape seed mustard	New Delhi,	JF710515
OCA 3	Rape seed mustard	Himachal Pradesh	—
OCA 4	Rape seed mustard	Assam	JF710516
OCA 5	Rape seed mustard	Meghalaya	—
OCA 15	Rape seed mustard	Delhi	—
Alternaria sesami			
OSA 08	Sesame	Karnataka	JF710585
OSA 09	Sesame	Tamil Nadu	JF710584
OSA 11	Sesame	Karnataka	JF710582
OSA 12	Sesame	Andhra Pradesh	JF710583
OSA18	Sesame	Karnataka	—
Alternaria ricini			
OAR 1	Castor	Andhra Pradesh	JF710543
OAR 2	Castor	Karnataka	JF710544
OAR 3	Castor	Tamil Nadu	—
OAR 4	Castor	Andhra Pradesh	—
OAR 5	Castor	Andhra Pradesh	—
Alternaria carthami			
OAcr 1	Safflower	Andhra Pradesh	JF 710541
OAcr 2	Safflower	Andhra Pradesh	JF710542
OAcr 3	Safflower	Karnataka	—
OAcr 4	Safflower	Maharashtra	—
OAcr 5	Safflower	Karnataka	—

Alternariaster helianthi			
OSFA 33	Sunflower	Maharashtra	
OSFA 35	Sunflower	Maharashtra	
OSFA 36	Sunflower	Maharashtra	
OSFA 37	Sunflower	Maharashtra	
OSFA 39	Sunflower	Rajsthan	
Alternaria alternata			
OTA 11	Tomato	Andhra Pradesh	HQ270456
OTA29	Tomato	Karnataka	JF710500
OTA48	Tomato	Jammu and Kshmir	JF796070
OTA56	Tomato	Himachal Pradesh	JF796071
OTA 65	Tomato	Uttara Pradesh	JF796077
Alternaria burnsii			
OCuAb1	Cumin	Rajasthan	—
OCuAb4	Cumin	Gujarat	—
OCuAb3	Cumin	Rajasthan	—
OCuAb4	Cumin	Rajasthan	—
OCuAb5	Cumin	Rajasthan	—
Alternaria macrospora			
Am 1	Cotton	Karnataka	—
Am 2	Cotton	Andhra Pradesh	—
Am 3	Cotton	Tamil Nadu	—
Am 7	Cotton	Karnataka	—
Am 9	Cotton	Andhra Pradesh	—
Alternaria mali			
OAAM1	Apple	Jammu and Kashmir	—
OAAM2	Apple	Jammu and Kashmir	—
OAAM3	Apple	Himachal Pradesh	—
OAAM4	Apple	Himachal Pradesh	—
OAAM5	Apple	Himachal Pradesh	—
Alternaria dauci			
OCAD1	Carrot	Tamil Nadu	—
OCAD2	Carrot	Tamil Nadu	—
OCAD3	Carrot	Tamil Nadu	—
OCAD4	Carrot	Tamil Nadu	—
OCAD5	Carrot	Tamil Nadu	—

were loaded into wells in the polyacrylamide gel containing (10%) SDS. Standard proteins for molecular weight determination (Merck Biosciences, Bangalore, India) were run parallel to the samples. Electrophoresis was carried out at 50 V for the stacking gel, and at 80 V for the separating gel at room temperature. The samples were run till the marker dye reached the bottom of the gel plate. After electrophoresis, the proteins were visualized and analyzed as described for native proteins.

Multi-locus enzyme analysis

Isozyme electrophoresis was performed in vertical polyacrylamide gels with a discontinous buffer system according to Laemmli (1970) as described for native proteins. The staining procedure for superoxide dismutase (SOD,EC 1.15.1.1), malate dehydrogenase (MDH, EC 1.1.1.37) and glutamate dehydrogenase (GDH, EC 1.4.1.2) was identical to that of Sadasivam and Manickam (1996), and for glucose phosphate isomerase (GPI, EC 5.3.1.9) was to that of Goodwin

et al. (1995). The experiments including mycelium production, protein extraction, and isozyme analyses were repeated five times to ensure reliability of results. Isolate banding patterns (zymograms) were scored for each enzyme system according to the method of Lebot *et al*., (2003). Different letters were used to identify polymorphic zymograms for each enzyme system. Based on an isozyme pattern, distinct zymotypes were identified by seven coded zymograms. No interpretation of the genetic similarities and genetic distances of fungal isolates based on the banding patterns were attempted.

Computing numerical data

The images of the gels were captured using an Alpha imager EP (Alpha Innotech Corporation, San Leandro, CA, USA) and the molecular weight of each protein band was determined by molecular weight analysis software of this system. The presence or absence of protein band in the zymogram was scored as 1 and 0, respectively. Similarly, bands in the isozyme gels of each isolate were scored by their presence or absence to generate a multilocus isozyme genotype. The combined data set for SOD, MDH, GDH and GPI were analyzed. These data made it possible to construct a binary value matrix that was analyzed by Jaccard's similarity co-efficients in DARWin. The binary matrix data were subjected to cluster analysis using DARWin version 5.0 (Perrier *et al*., 2003; Perrier and Jacquemoud-Collet, 2006) using the unweighted neighbor joining method as clustering method.

RESULTS

The native and dissociated protein profile of the species of *Alternaria* is shown in Fig.1 and 2. Banding patterns of native as well as dissociated proteins between in the range of 20 -205 KDa were recorded (Table 2 and 3). A total of 50 native and 68 dissociated protein bands were observed. Unique protein mas from both native and SDS-PAGE profile was obtained for isolates belonging to *A.solani, A.porri, A.macrospora, A.dauci, A.alternata, A.brassicae, A.brassicicola, A.sesami, A.helianthi, A.ricini, A.carthami, A.brunsii,* and *A.mali* (Fig. 1 and 2, Table 2 and 3). For example *A.solani* shared the native protein masses (82.746 KDa, 154.953 KDa) and dissociated protein masses (22.450 KDa, 25.809 KDa and 28.328 KDa) which were not present in the other *Alternaria* species including closely related species like *A.porri* (native,50.016 KDa, 73.675 KDa, 112.414 KDa, 205.00 KDa and dissociated,23.962 KDa, 25.473 KDa, 30.696 KDa, 41.515 KDa), *A.macrospora* (native,35.580 KDa, 46.118 KDa, 172.469 KDa and

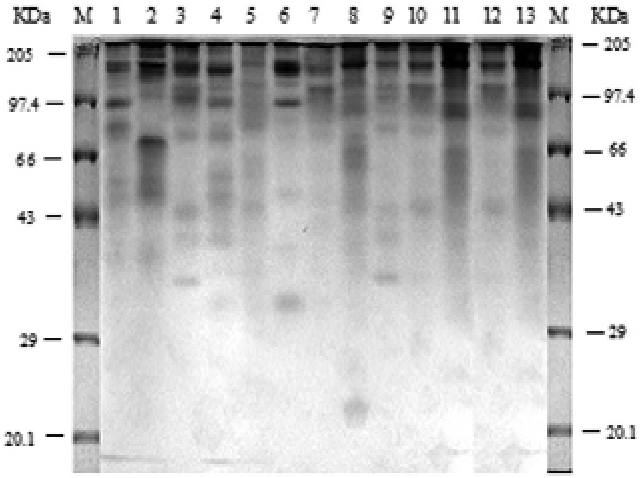

Fig. 1 Representative protein profiles for 13 *Alternaria* species as separated by polyacrylaimde gel electrophoresis of native proteins

M. Marker, Lane 1. *A.solani*, Lane 2. *A.porri*, Lane 3,.*A.macrospora*, Lane 4. *A.dauci*, Lane 5. *A.alternata*, Lane 6,.*A.brassicicola*, Lane 7. *A.brassicae*, Lane 8. *A.sesami*, Lane 9. *A.helianthi*, Lane 10. *A.ricini*, Lane 11. *A.carthami*, Lane 12. *A.burnsii* and Lane 13. *A. mali*

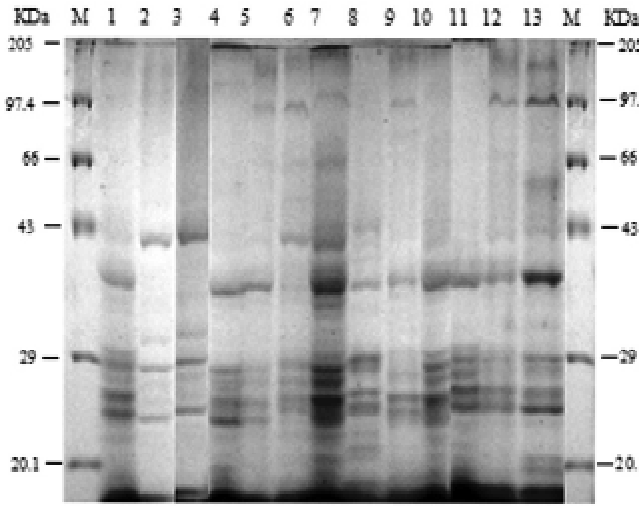

Fig. 2 Representative protein profiles for 13 *Alternaria* species as separated by polyacrylaimde gel electrophoresis of SDS-dissociated proteins

M. Marker, Lane 1. *A.solani*, Lane 2. *A.porri*, Lane 3,.*A.macrospora*, Lane 4. *A.dauci*, Lane 5. *A.alternata*, Lane 6,.*A.brassicicola*, Lane 7. *A.brassicae*, Lane 8. *A.sesami*, Lane 9. *A.helianthi*, Lane 10. *A.ricini*, Lane 11. *A.carthami*, Lane 12. *A.burnsii* and Lane 13. *A. mali*

Table 2. Protein mass of 13 *Alternaria* species as revealed by electrophoresis of native proteins

Species	**Protein mass (KDa)**							
A.solani (OTA22)	56.254	82.746*	97.40	139.939	154.953*	167.465	187.483	
A.porri (OOA2)	50.016*	56.254	73.675*	112.414*	139.939	167.465	182.479	205.00*
A.macrospora (Am1)	35.580*	41.040	46.118*	77.862	104.907	134.934	172.469*	
A.dauci (OCAd1)	41.040	50.796*	57.813*	77.862	96.702	127.427	164.962*	
*A.alternata (*Aa4)	47.288	84.142*	96.,702	117.418*	139.939	187.483		
A.brassicicola (OCA1)	33.060*	52.355*	97.400	137.437	179.976			
A.brassicae (OCA3)	77.164*	104.907	127.427	167.465				
A.sesami (OSA12)	22.153*	48.457*	67.395*	91.817*	142.441*	187.483		
A.helianthi (OSFA33)	36.140*	41.180*	83.444*	104.907	134.934	184.981*		
A.ricini (OAR2)	47.288	79.257*	107.409*	134.934	179.976			
A.carthami (OACr2)	43.779*	68.093*	89.724*	96.702	137.437	177.474*		
*A.burnsii (*OCuAb1)	47.288	97.400	132.432*	182.479				
A.mali (OAAM1)	66.6978*	92.515*	179.976					

*Denotes species-specific protein mass

Table 3. Peptide masses of 13 *Alternaria* species as revealed by electrophoresis of SDS-dissociated proteins

Alternaria species	Peptide mass (KDa)												
A.solani (OTA22)	22.450*	24.466	25.809*	27.320	28.328*	29.636	37.484	42.363					
A.porri (OOA2)	23.962*	25.473*	28.160	30.696*	41.515*								
A.macrospora (Am1)	24.466	28.160	38.121*	41.727									
A.dauci (OCAd1)	23.626*	25.137*	26.481	28.160	36.00*	127.52*							
*A.alternata (*Aa4)	23.794*	25.305*	27.320	28.832	36.636	93.348	161.96*						
A.brassicicola (OCA1)	24.298	25.641	27.320	28.832	36.636	41.303	93.348	174.87*					
A.brassicae (OCA3)	20.603	22.618	24.130*	25.641	27.320	29.000	34.727*	36.636	38.969*	41.303	43.696	66.00*	106.00*
A.sesami (OSA12)	20.771*	22.618	24.633	26.313*	28.664	29.212*	37.060	40.878*	43.696				
A.helianthi (OSFA33)	24.298	25.977	27.656*	29.424	37.0601	41.727	78.154*	95.374*					
A.ricini (OAR2)	20.603	24.298	25.641	27.488*	29.424	37.272*							
A.carthami (OACr2)	21.443*	24.633	26.481	27.992*	29.000	30.272*	37.484	39.818*					
*A.burnsii (*OCuAb1)	24.633	25.977	27.824	29.636	37.696	39.606*	42.151*	96.387	183.480*				
A.mali (OAAM1)	20.603	24.80*	26.481	27.824	29.636	37.696	42.363	52.757*	59.727*	96.387	1,57,656.00*		

*Denotes species – specific peptide mass

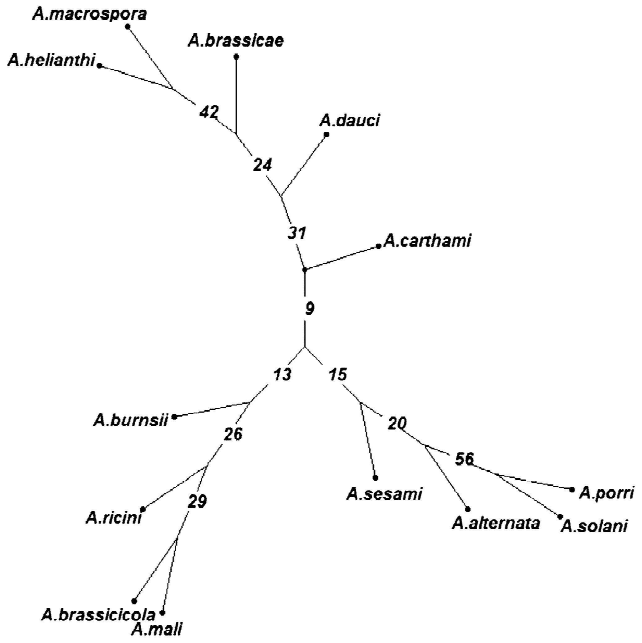

Fig. 3. Cladogram of *Alternaria* species as revealed by electrophoresis of native protein profile

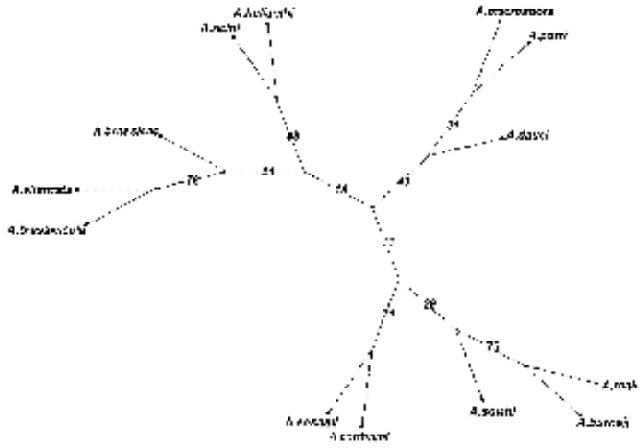

Fig. 4. Cladogram of *Alternaria* species as revealed by electrophoresis of dissociated protein profiles

dissociated, 38.121 KDa) and *A.dauci* (native, 50.796 KDa, 57.813 KDa, 164.962 KDa and dissociated, 23.626 KDa, 25.137 KDa, 36.00 KDa, 127.52 KDa). *A.burnsii* (native, 132.432 KDa and 39.606 KDa, 42.151 KDa, 183.48 KDa of dissociated) and *A. mali*, (native,66.697 KDa, 92.515 KDa and dissociated 24.8 KDa, 59.727 KDa, 157.656 KDa) which has been referred to as a special forms of *A. alternata* (native,84.142 KDa, 117.418 KDa and dissociated 23.794 KDa, 25.305 KDa, 161.96 KDa), has specific protein masses that are distinct from *A. alternata*.

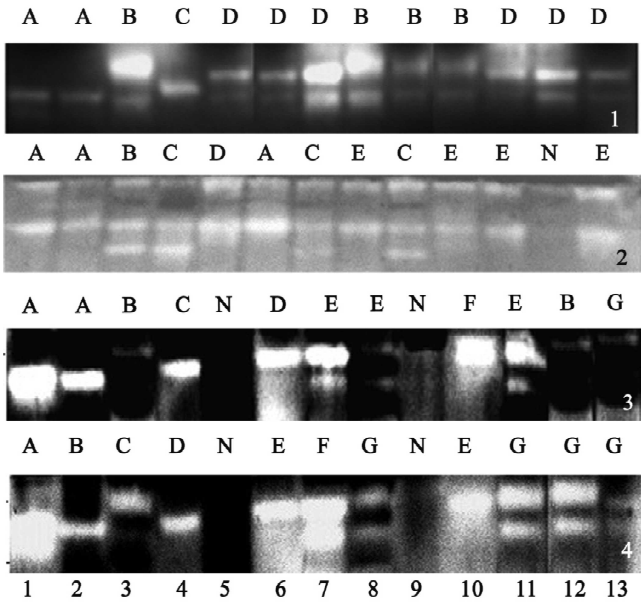

Fig. 5. Zymograms (denoted alphabetically by upper case letters) observed for each enzyme system of *Alternaria* species.

1. Superoxide dismutase, 2. Glucose phosphate isomerase, 3. Malate dehydrogenase and 4. Glutamate dehydrogenase. Lane, 1. *A. solani,* Lane, *2. A. porri,* Lane, *3. A. macrospora,* Lane, *4. A.dauci*, Lane, *5. A. alternata,* Lane, *6. A. brassicicola,* Lane,*7. A.brassicae,* Lane, *8. A. sesami,* Lane,*9. A. helianthi,* Lane, *10. A.ricini*, Lane,*11. A. carthami,* Lane, *12. A. burnsii*, Lane, *13. A mali*

The isolates from oil-yielding crops, namely, *A.sesami* (native, 22.153 KDa, 48.457 KDa, 67.395 KDa, 91.817 KDa, 142.441 KDa and discociated, 20.771 KDa, 26.313 KDa, 29.212 KDa, 40.878 KDa), *A. carthami* (native, 43.779 KDa, 68.0093 KDa, 89.724 KDa, 177.474 KDa and dissociated 21.443 KDa, 27.992 KDa, 30.272 KDa, 39.818 KDa) and *A. ricini* (native,79.257 KDa, 107.409 KDa and dissociated 27.488 KDa, 37.272 KDa) had distinct protein finger prints. *A.brassicae* (native,77.164 KDa and dissociated 24.13 KDa, 34.727 KDa, 38.969 KDa, 66.00 KDa, 106.00 KDa) and *A.brassicicola* (native 33.060 KDa, 52.355 KDa and dissociated 174.87 KDa) from cole crops also had characteristic protein profiles. The characteristic native as well as dissociated protein masses were also observed in *A.helianthi* (native 36.14 KDa, 41.18 KDa, 83.444 KDa, 184.981 KDa and dissociated 27.656 KDa, 78.154 KDa, 95.374 KDa) isolates of sunflower. However, isolates within a species had identical protein patterns.

The cladogram based on native and SDS-PAGE protein patterns resolved isolates belonging to 13 different

species of *Alternaria* into different groups (Fig. 3 and 4), indicating the potential of the method for identification of *Alternaria* species according to their characteristic protein masses.

The zymograms obtained for the four enzyme systems and for the 13 distinct species of *Alternaria* are shown in Fig.5. MDH and GDH exhibited seven distinct zymograms. GPI exhibited five different zymograms and SOD showed four zymograms . The zymotypes obtained for 13 *Alternaria* species are presented in Table 4. Zymograms were species-specific and discriminated all the 13 *Alternaria* species. However, isozyme patterns of isolates within a species are identical. The cladogram based on combined data of four different enzymes resolved isolates belonging to 13 different species of *Alternaria* into different clades (Fig. 6).

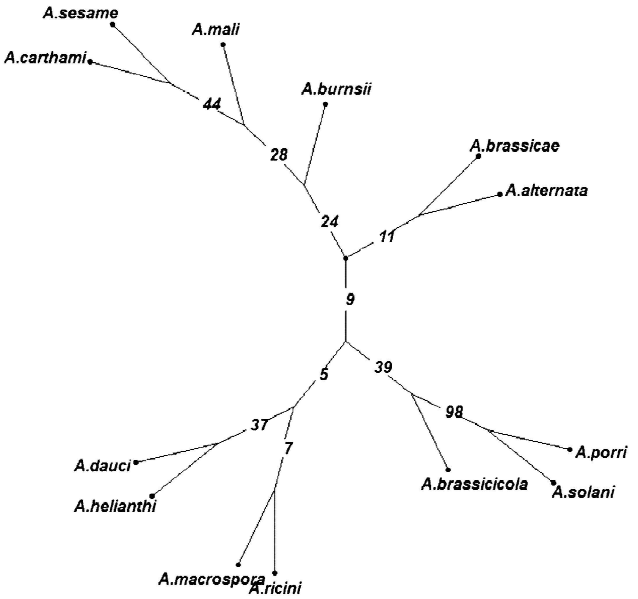

Fig. 6. Cladogram of *Alternaria* species based on combined data of isozyme profile of SOD, GDH, MDH, GPI.

DISCUSSION

The precise identification of *Alternaria* species is very crucial for implementing effective disease management strategies. As identification of *Alternaria* species based on morphological characters has limitations, the species were organized into species groups (Simmon and Roberts, 1993). For example *A.solani, A.porri, A.dauci* and *A.macrospora* are in the same clade, *A.porri* group (Rotem, 1994) and *A.longipes, A.gaisen, A.citri* and *A.mali* have been referred as special forms of *A.alternata* (Anderson *et al*., 2001) are placed in *A.alternata* group. In this study, we have shown that electrophoresis of native and dissociated proteins can be used to separate 13 species of *Alternaria* encountered

Table 4. Zymotypes of *Alternaria* species and species-specific zymograms

Fungal species	Zymograms				
	SOD	GPI	MDH	GDH	Zymotype
A.solani	A	A	A	A	1
A.porri	A	A	A	B	2
A.dauci	B	B	B	C	3
A.macrospora	C	C	C	D	4
A.alternata	D	D	N	N	5
A.brassicicola	D	A	D	E	6
A.brassicae	D	C	E	F	7
A.sesami	B	E	E	G	8
A.helianthi	B	C	N	N	9
A.ricini	B	E	F	E	10
A.carthami	D	E	E	G	11
A.burnsii	D	N	B	G	12
A.mali	D	E	G	G	13

N denotes the isozyme not detected

on vegetable, fruits and oil seed crops. Isolates in different species of *Alternaria* had distinctive and reproducible native and SDS–dissociated protein patterns, where as isolates within a single species produced largely homogenous banding patterns. Earlier studies on molecular approaches using different gene sequence phylogenies failed to distinguish *Alternaria* species within the species-groups due to lack of sufficient genetic information (Pryor and Bigelow, 2003; Peever *et al*., 2004; Andrew *et al*., 2009). Electrophoretic profiling of native and SDS-dissociated proteins have been successfully employed as an aid in distinguishing the species and subgroups within species of fungal pathogens such as *Phytophthora* (Bielenin *et al*., 1988 Chowdappa and Chandramohanan, 1995; Hansen *et al*., 1986) and *Fusarium* isolates (El-Din *et al*., 1994; Aly *et al*., 2003; Bhuvanendra Kumar *et al*., 2010).

The present study also demonstrated that combined SOD, GDH, MDH, GPI data can be used to differentiate *Alternaria* species. Through isozyme patterns of aconitase, malate dehydrogenase and isocitrate dehydrogenase, Petrunak and Christ (1992) reported that the populations of *Alternaria solani and Alternaria alternata* could be differentiated. On the basis of esterase isozymes, Hwang *et al*. (1987) showed that populations of *A.mali* within a geographic feature were more closely related than geographically separated isolates.

Electrophoretic patterns of soluble proteins and isozymes were shown to be more valuable as a diagnostic aids in distinguishing fungal isolates whose variability in morphology is indeterminate and overlapping (Coetzee *et al*., 2009; Hansen *et al*., 1986; Bielenin *et al*., 1988; Odumans and Coffey, 1991; Otrosina *et al*., 1992; Mchau and Coffey, 1995). While DNA based analyses may offer better resolution of isolate differences and phylogenetic relationships in many fungal pathogens other than *Alternaria* (Andrew *et al*., 2009), they are time consuming and expensive relative to protein and isozyme profiles. Protein and isozyme analyses are easier to interpret, can be performed in most laboratories, considerably less expensive than comparable molecular biological techniques and proven useful to differentiate morphologically similar fungal isolates (Micales and Bonde, 1995). Also isozymes can be used as markers in genetic studies (Oudemans and Coffey, 1991). Thus, polyacrylamide gel electrophoresis of soluble mycelial proteins and isozymes patterns obtained in this study can be used as a reproducible and sensitive finger print for thirteen species of *Alternaria* encountered on vegetable, fruit and oil seed crops for rapid identification.

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Bioefficacy of Azoxystrobin 25 SC along with bioagents against chilli anthracnose diseases under field conditions

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ABSTRACT : Field experiments were carried out to study the effect of azoxystrobin 25 SC at the rate of 100, 125, and 150g a.i ha^{-1} along with other fungicides on anthracnose disease of chilli caused by *Colletotrichum capsici.* Maximum control of *C. capsici* (PDI of 4.44 and 2.78 on leaves and fruit respectively) was recorded with 150 g a.i. The fruit yield also has significantly increased recording 59.88 and 55.30 per cent yield increase over control. No phytotoxic effects such as leaf tip/surface injury, wilting, vein clearing necrosis, epinasty, hyponasty,fruit injury of azoxystrobin 25 SC were observed at the doses of 100, 125, 150 and 250 g ai ha^{-1}.

Keywords: Azoxystrobin**,** anthracnose, bioefficacy, chilli, *Colletotrichum capsici,* phytotoxicity

INTRODUCTION

Chilli (*Capsicum annum*) is the fourth most important vegetable crop in the world and first in Asia, with world production f oapproximately 122.34 million tonnes of fresh chilli and 2.8 tonnes of dry chilli in 2010 (Indian Horticultural Database). Demand for chilli in the world is increasing every year. Chilli is a very remunerative spice crop of the Indian subcontinent (Sharma *et al.,* 2005) and occupies an area of about 0.81 million ha (Suthin Raj and Christopher, 2009) which accounts for 25per cent of the world production (Chandra Nayaka *et al*., 2009). In Tamil Nadu, chilli is cultivated on 49.0 thousand hectares with 31.8 thousand tonnes of production. Chilli is attacked by several fungal, bacterial and viral diseases. Among them, anthracnose and powdery mildew are the major diseases incurring heavy losses, if not cared. Anthracnose (fruit rot and die back) caused by *Colletotrichum capsici* (Syd. Butler and Bisby) is prevalent throughout the chilli growing areas of India (Jeyalakshmi, 1996). Several fungicides have been recommended against anthracnose but still there is a need to widen the choice by introducing new molecules. Azoxystrobin, produced by the Basidiomycetes fungus, *Strobilurus tenacellus* (Pers. ex Fr.) Singer, has a novel mode of action (Hewit, 1998). Its fungicidal activity results from the inhibiting mitochondrial respiration of higher fungi, which is achieved by the prevention of Electron transfer between cytochrome b & cytochrome c (Becker *et al.,* 1981). The present investigation was carried out using a new formulation viz., azoxystrobin 25 SC for its bio efficacy and phytotoxicity against chilli anthracnose disease.

MATERIALS AND METHODS

Field studies

A new formulation Azoxystrobin 25 SC w/w of United Phosphorus, Limited, Mumbai was used for all studies in the present investigation. The new formulation was compared with two fungicides *viz.*, azoxystrobin 23 SC, hexaconozole and chlorothalonil (75%) WP (2%) SC and two fungal pathogens *viz.*, *Pseudomonos fluorescens* and *Bacillus subtilis.* Two field trials were conducted during 2011 and 2012 at Kinathukadavu and Mathampatti using variety *viz.*, Sierra. The trials were laid out in a Randomized Block Design (RBD) with nine treatments and three replications in a plot size of 20 m^2 and with a spacing of 90 × 60 cm. The treatments comprised as follows:

T1 - Spraying of Azoxystrobin@ 100 g a.i ha^{-1} immediately after the first appearance of disease symptoms followed by two sprays at 15 days interval

T2 - Spraying of Azoxystrobin@ 125 g a.i ha^{-1} immediately after the first appearance of disease symptoms followed by two sprays at 15 days interval

T3 - Spraying of Azoxystrobin @150 g a.i ha^{-1}immediately after the first appearance of disease symptoms followed by two sprays at 15 days interval

T4 - Spraying of Azoxystrobin (Amrister) @ 125 g a.i ha^{-1}immediately after the first appearance of disease symptoms followed by two sprays at 15 days interval

T5 - Spraying of hexaconazole @60 g a.i ha^{-1}immediately afte*r* the first appearance of disease symptoms followed by two sprays at 15 days interval

T6 - Spraying of chlorothalonil @ 600 g a.i ha^{-1}immediately after the first appearance *of* disease symptoms followed by two sprays at 15 days interval

T7 - Spraying of *P. fluorescens* @ 0.2 per cent with 2.0 x 10^{8} CFU/g immediately after the first appearance of disease symptoms followed by two sprays at 15 days interval

T8 - Spraying of talc-based formulation of *B. subtilis.* @ 0.2 per cent after the first appearance of disease followed by two sprays at 15 days interval

T9 - Untreated control –water spray

The recommended package of practices was followed for the trial. The observation on the disease incidence was recorded before initiation of spray and after third spray.

The severity of anthracnose disease was recorded on 10 plants and in each plant 10 fruits were selected at random in each replication of the treatment.Percent Disease Index (PDI) was calculated usingstandard score chart as described earlier.

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of fruit observed}} \times \frac{100}{\text{Maximum category value}}$$

Similarly, the incidence of powdery mildew was also scored in 10 plants and 10 leaves were scored at random in each plant and PDI was worked out as per the standard formula.The yield details were also recorded.

Phytotoxicity

The fungicide was sprayed at the concentration of 250 g a.i/ha and compared with other doses as mentioned in the previous study. The phytotoxicity symptoms were recorded a week after last spray and observations on the following parameters *viz.*, leaf tip/surface injury, wilting, vein clearing necrosis, epinasty, hyponasty and fruit injury were recorded (Archana, 2009).

RESULTS AND DISCUSSION

Efficacy of Azoxystrobin 25 SC against anthracnose of chilli

Field studies

In order to confirm the results from the glasshouse studies two field trials were conducted during 2011 and 2012 at two locations in Coimbatore district. Three doses of Azoxystrobinat 150g ai/ha, 125 g ai/ha and 100 g ai/ha were compared with azoxystrobin,hexaconazole and chlorothalonil and the two bioagents *P.fluorescens* and *B.subtilis* strains. Three sprays were given, starting the first spray on the initiation of the disease. The disease incidences were record just before spray and subsequent observations were taken week after each spray.

Anthracnose

The results from two season trails clearly revealed that Azoxystrobin 150 g a.i/ha provided the maximum control of the anthracnose disease, followed by Azoxystrobin at 125 g a.i/ha. The effect was noticed both in leaves and fruits when compared to other fungicides. In addition, the Azoxystrobin at the rate of 150 g a.i /ha recorded the maximum yield of27.18 t/ha which was on par with the spray treatment of Azoxystrobin at the rate of 125 g a.i/ha with yield of 26.57 t/ha. Untreated control yielded only 17 t/ha. From this study, it is evident that Azoxystrobin at the rate of 150 g a.i /ha was considered as the optimum dose to combat the disease.The similar results were also available in the literatures.

Chemicals are the most common and practical method to control anthracnose diseases. However, fungicide tolerance often arises quickly, if a single compound is relied upon too heavily (Staub, 1991). The fungicide traditionally recommended for anthracnose management in chilli is Manganese ethylenebis dithiocarbamate (Maneb) (Smith, 2000) although it does not consistently control the severe from of anthracnose on chilli fruit. The strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio) have recently been labeled for the control of anthracnose on chilli, but only preliminary reports are available on the efficacy of these fungicides against the severe form of the disease (Alexander, 2002). Anand *et al* ., 2010 found that Pf1 at 2.5 kg ha^{-1} tested in combination with reduced concentration of azoxystrobin at the rate of 250 ml ha^{-1} was highly efficient in management of chilli anthracnose .Dale, 1999 found that Amistar(Azoxystrobin) at 125-250 mg ai/l provided longer disease protection than benomyl against anthracnose disease of chili (*Colletotrichum capsici*).

The strobilurin fungicides represent important class of chemicals for the management of a broad range of fungal diseases in agricultural production systems. Sudaravadana *et al*. 2007 found that treating trees with these *viz.*, 1, 2 and 4 ml/l. concentrations provided 100

Table 1. Effect of different fungicides and bioagents on the incidence of anthracnose of chilli under field condition (Season I) (Kinathukadavu-Coimbatore)

Treatment	PDI on leaves		Per cent decrease over control	PDI on fruits		Per cent decrease over control	Yield t/ha	Yield increase over control
	Before Spray	After 3 rd Spray		Before Spray	After 3rd Spray			
Azoxystrobin@ 100 g a.i ha^{-1}	14.80 ^{b}	12.45 ^{c}	67.40	9.46 ^{b}	7.22 ^{b}	72.30	26.11 ^{abc}	53.58
Azoxystrobin@ 125 g a.i ha^{-1}	8.96 ^{a}	6.67 ^{b}	82.60	5.82 ^{a}	3.89 ^{a}	85.10	26.57 ^{ab}	56.29
Azoxystrobin@ 150g a.i ha^{-1}	6.84 ^{a}	4.44 ^{a}	88.40	5.91 ^{a}	2.78 ^{a}	89.40	27.18 ^{a}	59.88
Azoxystrobin @ 125 g a.i(Amrister)	8.46 ^{a}	6.22 ^{ab}	83.70	6.12 ^{a}	4.00 ^{a}	84.68	24.00 ^{bcd}	41.18
Hexaconazole @ 60 g a.i ha^{-1}	23.16 ^{c}	20.89 ^{d}	45.39	17.48 ^{d}	15.55 ^{d}	40.40	23.17 ^{cde}	36.29
Chlorothalonil @ 600 g a.i ha^{-1}	15.98 ^{b}	13.78 ^{c}	64.00	13.26 ^{c}	10.55 ^{c}	59.60	22.43^{de}	31.94
P. fluorescens@ 0.2%	25.23 ^{d}	23.24 ^{e}	39.19	19.22 ^{e}	17.24 ^{e}	33.97	22.00 ^{de}	29.41
B. subtilis@ 0.2%	27.16 ^{d}	25.32 ^{e}	33.75	21.36 ^{f}	19.64 ^{f}	24.77	21.04 ^{e}	23.76
Control	41.46 ^{f}	38.22 ^{f}	-	29.36 ^{g}	26.11 ^{g}	-	17.00 ^{g}	
SEd	1.09	0.99		0.83	0.71			1.39
D(0.05)	2.34	2.11		1.75	1.51			2.93
Cv%	7.39	7.29		7.15	7.38			7.35

Values are means of three replications

Figures in the parentheses represent arcsine transformed values

The common letters show non- significant differences among the treatments based on DMRT

Table 2. Effect of different fungicides and bioagents on the incidence of anthracnose of chilli under field condition (Season II) (Madampatti – Coimbatore)

Treatment	PDI on leaves			PDI on fruits				
	Before Spray	After 3 rd Spray	Per cent decrease over control	Before Spray	After 3rd Spray	Per cent decrease over control	Yield	Yield increase over control
Azoxystrobin@ 100 g a.i ha^{-1}	16.42 ^{b}	14.32 ^{c}	64.62	10.26 ^{c}	8.46 ^{b}	71.23	28.60^{ab}	47.27
Azoxystrobin@ 125 g a.i ha^{-1}	10.32 ^{a}	8.75 ^{b}	78.38	7.81 ^{ab}	5.32 ^{a}	81.91	30.12 ^{a}	55.09
Azoxystrobin@ 150g a.i ha^{-1}	8.88 ^{a}	6.12 ^{a}	84.88	6.48 ^{a}	4.71 ^{a}	83.98	30.16 ^{ab}	55.30
Azoxystrobin @ 125 g a.i ha^{-1} (Amrister)	11.26 ^{a}	8.74 ^{b}	78.40	9.54 ^{b}	6.86 ^{a}	76.67	26.18 ^{c}	34.80
Hexaconazole @ 60 g a.i ha^{-1}	24.84 ^{c}	22.68 ^{d}	43.97	19.26 ^{d}	17.12 ^{d}	41.78	26.00^{bc}	33.88
Chlorothalonil @ 600 g a.i ha^{-1}	17.32 ^{b}	15.16 ^{c}	62.54	14.86 ^{e}	12.53 ^{c}	57.39	24.80 ^{c}	27.70
P. fluorescens @ 0.2%	26.82 ^{cd}	24.48 ^{de}	39.52	21.26 ^{e}	19.66 ^{e}	33.15	22.00 ^{d}	13.28
B. subtilis@ 0.2%	28.44 ^{d}	26.32 ^{e}	34.98	22.47 ^{f}	20.48 ^{f}	30.36	20.00 ^{d}	12.98
Control	43.68^{e}	40.48 ^{f}	-	32.81^{g}	29.41 ^{g}		19.42 ^{d}	
SEd	1.19	1.07		0.93	0.82		1.26	
CD(0.05)	2.52	2.28		1.97	1.74		2.67	
Cv%	6.98	7.11		7.10	7.28		6.12	

Values are means of three replications

Figures in the parentheses represent arcsine transformed values

The common letters show non- significant differences among the treatments based on DMRT

and more than 60 per cent reduction of panicle and leaf anthracnose compared to untreated mango trees for which disease incidences were 27.73 and 53.68 PDI. This controlling effect was mainly due to translaminar and systemic movement of azoxystrobin, inside the tissues, azoxystrobin is widely distributed from the application side by diffusion (Vincelli, 2002).

Phytotoxicity of Azoxystrobin 25 SC

The observations on the leaf tip, surface injury, wilting, vein clearing, necrosis, epinasty, hyponasty and fruit injury were recorded during both the seasons. The observations showed that azoxystrobin 25 SC even at 250g a.i ha^{-1} did not show any phytotoxicity symptoms. Similarly other fungicides tested also did not exhibit any phytotoxicity symptoms. A number of fungicides are being routinely used for crop protection but their phytotoxic effects have been often ignored (Vyas, 1993). Curative and eradicant activity of strobilurins against several airborne pathogens have been reported (Reuveni, 2001; Anesiadis *et al.,* 2003). According to the fungicide resistance action committee (FRAC, 2004) preventive use and a limited number of applications of strobilurins are recommended (i.e., no more than six per season or up to three sequential applications) to reduce the risk of phytotoxicity and development of fungicide resistance pathogen strains (Affourtit *et al.,* 2000).

This was in accordance with the results of Nithyameenakshi *et al*. (2006), the fungicides azoxystrobin and difenoconazole were generally non phytotoxic at or below the recommended dose for field application (2.2 µg a.i ml^{-1}). But at higher concentration, both the fungicides exhibited concentration dependant phytotoxicity in *Vigna catjung* Walp. Sendhil Vel *et al.* (2004) and Sundaravadana (2005) reported that there were no phytotoxic symptoms throughout the cropping season of grapevine and mango due to azoxystrobin application. Findings of our field studies suggest that azoxystrobin 25 SC is effective in reducing anthracnose disease in fruits and leaves at the concentrations of 150 and 125 g a.i ha^{-1}. No phytotoxic symptoms were recorded after spraying on the plants even at highest dose. The azoxystrobin 25 SC on chilli anthracnose disease will increase the choice of fungicides.

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Bio-efficacy of endophytic actinomycetes for plant growth promotion and management of bacterial wilt in tomato

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ABSTRACT: Antagonistic effect of endophytic actinomycetes isolated from tomato plants collected from different locations in Kerala was evaluated against bacterial wilt pathogen under *in vivo* conditions. Maximum inhibition (44.63%) of the pathogen was observed with Cherumkuzhy isolate (EACK) which was on par with Ozhalapathy isolate (EAOP) (42.59%). All the isolates belonged to genus *Streptomyces* sp. Evaluation of endophytic actinomycetes against bacterial wilt pathogen under pot culture conditions revealed that minimum per cent wilt incidence (37.03%) was shown by plants treated with EAOP isolate. Observations on biometric characters showed that Ozhalapathy (EAOP) and Eruthenpathy (EAET) isolates were the most promising in plant growth promotion. It was concluded from the study that the Ozhalapathy isolate (EAOP) (*Streptomyces* sp.) was the best among the five endophytic actinomycetes isolates in plant growth promotion as well as in the management of bacterial wilt in tomato.

Keywords: Bacterial wilt, endophytic actinomycetes, *Ralstonia solanacearum*, tomato

INTRODUCTION

Tomato (*Solanum lycopersicum* Mill.) is one of the most popular protective foods because of its high lycopene content and is a widely grown vegetable in the world. Being versatile for culinary purposes, it is also one of the most commonly grown vegetable in the kitchen garden (Rana, 2008). Among the various diseases affecting the crop, bacterial wilt caused by *Ralstonia solanacearum* is the major one, and cent per cent yield loss has been reported among the susceptible varieties. Indiscriminate use of chemicals for the management of the disease may result in the development of resistant strains of pathogen as well as residual effect on the produce. So, biological control of disease is an alternative measure, because of its eco-friendly nature, cost effectiveness and long lasting effects. Endophytes are microorganisms that inhabit at least for a period of their lifecycle inside plant tissues without causing any apparent harm to the hosts. The use of endophytic actinomycetes as bioagents of soil borne root diseases is of interest through their ability to colonize healthy plant tissues and produce antibiotics *in situ*. de-Oliveira (2010) observed that *Streptomyces pluricolorescens*, an endophytic actinomycete isolated from tomato plants in Brazil showed (86.6%) antimicrobial activity against at least one pathogen in tomato. As plant growth promoters, endophytic actinomycetes enhance growth of plants including the formation of increased number of lateral roots and root hairs (Pillay and Nowak, 1997) in addition to an increase in plant height, shoot weight and shoot diameter (Yates *et al.*,1997). In this context, the present study was taken up to know more about the bioefficacy of endophytic actinomycetes on plant growth promotion and management of bacterial wilt in tomato.

MATERIALS AND METHODS

The present study was carried out at the Department of Plant Pathology, College of Horticulture, Thrissur, Kerala (India) during the years 2009-11. Endophytic actinomycetes were isolated from healthy tomato plants collected from high wilt incidence areas of Vellanikkara, Cherumkuzhy and Elanad (Thrissur district, Kerala, India) and low wilt incidence areas of Ozhalapathy and Eruthenpathy (Palakkad district, Kerala, India). Local cultivars of both young (less than two weeks) and old plants (more than two weeks) were obtained from different locations of the above mentioned areas. The pathogen, *Ralstonia solanacearum* causing bacterial wilt in tomato was isolated from the naturally infected tomato plants using Triphenyl Tetrazolium Chloride (TZC) agar medium. An *in vitro* experiment was conducted to find out the antagonistic effect of the isolated endophytic actinomycetes against the pathogen by dual culture method and the per cent inhibition was calculated by PI = (C – T)/C x 100 where PI = Per cent inhibition; C = Growth of the pathogen in control plates (cm); T = Growth of the pathogen in dual culture plates (cm).-

It was statistically analysed using MSTAT software followed by Duncans Multiple Range Test (DMRT). The

morphological and cultural characters of endophytic actinomycetes were studied based on standard keys given by Locci (1989).

Two pot culture experiments were conducted to find out the efficacy of endophytic actinomycetes isolates on plant growth promotion and management of bacterial wilt in tomato. Sterilized potting mixture consisting of sand: soil: cow dung at the rate of 1:1:1 was filled in earthen pots of size 12''x12''. Bacterial wilt susceptible variety Pusa Ruby was used for the study. All agronomic practices were performed as envisaged in the package of practices recommendations of the Kerala Agricultural University (KAU, 2007). Experiment I was laid out in CRD with 8 treatments and three replications, to find out the efficacy of endophytic actinomycetes in the management of bacterial wilt in tomato. Nine plants were maintained for each treatment. The application of treatments were started at the time of planting of seedlings, 45 days after planting and 60 days after planting as soil drenching. Challenge inoculation of pathogen (10^5 cfu/ml) was done at 30 DAP using fresh bacterial suspension (at the rate of 10 ml/plant). Experiment II was also laid out in CRD with 6 treatments and three replications, to find out the efficacy of endophytic actinomycetes in plant growth promotion without giving the challenge inoculation of the pathogen. Observations on per cent wilt incidence, days to flowering, days to first harvest, number of fruits per plant, per fruit weight and yield per plant were recorded and statistically analysed using MSTAT software followed by Duncans Multiple Range Test (DMRT).

After the evaluation of endophytic actinomycetes isolates against *Ralstonia solanacearum* under pot experiments, the most promising one was selected and 16S rRNA sequence analysis was carried out based on the amplification of 16S rRNA gene using Polymerase Chain Reaction (PCR) as described by Cook and Meyers (2003). The quality of isolated DNA was evaluated through agarose gel electrophoresis. The DNA bands separated by electrophoresis were views and photographed using Vision Works LS software and UVP GelDoc- IT™ imaging system. 20µl of the PCR product was purified using PCR purification kit (Bioserve) and the purified product was sequenced at Seigenome Pvt. Ltd. Cochin using the primers 27f and 1492r. The Blastn programme (http://www.ncbi.nlm.nih.gov/blast/) was used to find out the homology of the nucleotide sequences.

RESULTS AND DISCUSSION

Isolation of endophytic actinomycetes

The endophytic actinomycetes isolated from healthy tomato plants collected from five different locations were designated as EAVK (Vellanikkara), EACK (Cherumkuzhy), EAEN (Elanad), EAOP (Ozhalapathy) and EAET (Eruthenpathy). Only a single type of actinomycete isolate was obtained from each location. The maximum population was obtained from the roots of tomato plant collected from Cherumkuzhy (EACK) ($8x10^1$ cfu/g), followed by Eruthenpathy (EAET) ($3x10^1$ cfu/g). The least count was obtained from Vellanikkara (EAVK), Elanad (EAEN) and Ozhalapathy (EAOP) ($1x10^1$cfu/g). More number of actinomycetes was obtained from roots than from stem of tomato plants and it was in accordance with the results obtained by Shimizu *et al.* (2000).

In vitro evaluation of antagonistic effect of endophytic actinomycetes on *Ralstonia solanacearum*

Of the five isolates, maximum inhibition (44.63%) of pathogen was recorded with the Cherumkuzhy isolate (EACK) which was on par with the Ozhalapathy isolate (EAOP) (42.59%) (Table 1). Moura *et al.* (1998) observed that endophytic actinomycetes isolated from the root tissues of tomato showed cent percent control of the pathogen. Similarly, Moura and de-Romeiro (1999) noted that endophytic actinomycetes isolated from various hosts showed a high inhibitory activity against *R. solanacearum.*

Identification of endophytic actinomycetes

The colony size of all the isolates was medium. On the solid agar substratum, the branching network of hyphae developed by all the isolates grew both on the surface of the substratum and inside it forming a substrate mycelium. But chain of spores and sporangia were absent in the substrate mycelium of all the isolates. Fragmentation of substrate mycelium and motility of spores were absent for all the isolates. Later the colonies of all the isolates became covered with aerial mycelium bearing chain of arthrospores, which was initially white but assumed a range of colors when spore formation started. Color of the spore mass for all the isolates except Cherumkuzhy isolate was gray. Spiral spore chain morphology was observed for all the isolates and the spores were smooth surfaced. Red-orange colored, diffusible pigments were produced by Vellanikkara and Cherumkuzhy isolates which were absent for the other three isolates. The isolates from Vellanikkara and Cherumkuzhy produced red- orange colored pigmentation for the substrate mycelium whereas for the other three isolates distinctive pigmentation was absent. Based on these morphological and cultural characters, the isolates were tentatively identified as genus *Streptomyces*. This

was in agreement with the results of identification of endophytic actinomycetes from wheat roots from a range of sites across South Australia (Coombs and Franco, 2003) and tomato roots in South China (Cao, *et al.*, 2004).

Evaluation of endophytic actinomycetes on plant growth promotion and management of bacterial wilt in tomato under pot culture conditions

Experiment I

Evaluation of endophytic actinomycetes against bacterial wilt pathogen under pot culture conditions revealed that minimum per cent wilt incidence was shown by plants in pots drenched with urea (@ 44 g m^{-2}) and lime (@ 500 g m^{-2}) mixture [29.63%] and it differed significantly from the other treatments. The results were in accordance with the findings of Mathew (2004), who reported the effectiveness of urea and lime mixture for the management of *R. solanacearum* infecting solanaceous vegetables. Among the endophytes, the plants treated with EAOP isolate showed minimum per cent wilt incidence [37.03%] (Table 1). Consequently, the study confirmed that application of either a mixture of urea and lime or EAOP isolate were the best treatments in the management of bacterial wilt in tomato.

Experiment II

With respect to yield parameters such as days to flowering, days to first harvest, number of fruits per plant, per fruit weight and yield, plants treated with the endophytic actinomycetes were superior in performance when compared to the control (Table 2). The number of days taken for the emergence of first flower and also the number of days taken from fruit set to harvest should be less so that the total crop duration is not extended unduly. In the present study the plants treated with EAEN isolate recorded the minimum number of days (34.83) for flowering and the plants treated with EAVK isolate took the minimum number of days (81.66) to first harvest. Numbers of fruits per plant and per fruit weight are the two parameters having a direct effect on crop yield. In the present study, the plants treated with EAET isolate produced the maximum number of fruits per plant (17.57) followed by EAOP isolate (16.25) and they were found to be statistically on par. The data regarding per fruit weight indicated that the plants treated with EAOP isolate produced fruits with maximum weight (20.30 g) which was on par with EAET isolate (19.33 g) and was statistically superior to all other treatments. Highest yield (332.02 g) was recorded for the plants treated with EAET isolate followed by EAOP isolate (316.81 g). Hence, the observations on biometric characters revealed that Ozhalapathy (EAOP) and Eruthenpathy (EAET) isolates were the most promising in plant growth promotion. Hence, it is concluded from the study that the endophytic actinomycete *Streptomyces* sp. from Ozhalapathy (EAOP) showed superior performance among the five isolates in plant growth promotion as well as in the management of bacterial wilt in tomato. This promising endophytic actinomycete has a potential to be developed as a biocontrol agent for the management of bacterial wilt in tomato.

16S rRNA sequence analysis of the promising endophytic actinomycete isolate

Based on the results of pot experiments, the efficient endophytic actinomycete isolate, EAOP was identified by 16S rRNA sequence analysis using Polymerase Chain

Table 1. Effect of various treatments on bacterial wilt in tomato

Isolate/Treatment	Per cent inhibition (*in vitro*)	Per cent wilt incidence
EAVK	27.22b	66.67 (0.96)^{b}
EACK	44.63a	55.56 (0.84)^{bc}
EAEN	26.11b	66.67 (0.96)^{b}
EAOP	42.59a	37.03 (0.65)^{cd}
EAET	31.11b	48.15 (0.77)^{bcd}
Urea + lime mixture	–	29.63 (0.57)^{d}
Copper hydroxide	–	40.74 (0.69)^{cd}
Control	0.00	87.96 (1.24)^{a}
CD (p=0.05)	6.10	16.6

Figures in parenthesis are arc- sine transformed values.

Means followed by a common letter in a column are not significantly different at 5 % level by DMRT

Mean of 3 replications

Table 2. Effect of endophytic actinomycetes isolates on yield parameters of tomato

Isolate	Days to flowering	Days to first harvest	No. of fruits	Per fruit weight (g)	Fruit yield (g/plant)
EAVK	35.50^{ab}	81.66^{b}	11.26^{b}	15.46^{bc}	161.65^{c}
EACK	40.20^{ab}	83.93^{ab}	13.75^{ab}	18.21^{ab}	226.65^{bc}
EAEN	34.83^{b}	83.73^{ab}	13.57^{ab}	14.49^{cd}	182.67^{c}
EAOP	39.39^{ab}	85.01^{ab}	16.25^{ab}	20.30^{a}	316.81^{ab}
EAET	36.77^{ab}	82.45^{b}	17.57^{a}	19.33^{a}	332.02^{a}
Control	40.39^{a}	86.38^{a}	11.48^{b}	11.96^{d}	131.75^{c}
CD (p=0.05)	4.6	3.0	4.3	9.0	85.5

DAP - Days after transplanting
Means followed by a common letter in a column are not significantly different at 5 % level by DMRT
Mean of 3 replications

Reaction (PCR). Only one amplicon of about 1500bp was obtained. Since only a single band was obtained, PCR product was directly purified and sequenced. Homology search of nucleotide sequences obtained from the isolate with other reported 16S rRNA gene sequences revealed that EAOP showed homology with *Streptomyces thermodiastaticus* (95% query coverage and 97% identity).

Consequently, it was concluded from the study that the endophytic actinomycete *Streptomyces thermodiastaticus* from Ozhalapathy (EAOP) showed superior performance among the five isolates in plant growth promotion as well as in the management of bacterial wilt in tomato. This promising endophytic actinomycete has a potential to be developed as a biocontrol agent for the management of bacterial wilt in tomato.

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Strategic approaches for the management of pea nut bud necrosis virus disease of tomato

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ABSTRACT: The Pea nut bud Necrosis virus (PbNv) causes serious threat to tomato cultivation in the North Western Zone of Tamil Nadu comprising of the Dharmapuri and Krishnagiri districts where large area is covered under hybrid tomato cultivation. Field trials conducted at the Regional Research station, Paiyur with plant leaf extracts, *Pseudomonas fluorescens* (Pf-1) and insecticides showed that foliar spray with imidacloprid (0.0375%) at 7th day after transplanting (DAT) and 30th day (based on the pest incidence); *Pseudomonas fluorescens* (Pf-1) (2%) + butter milk (0.3%) on 15, 30 and 45 DAT was found to be highly effective as it recorded only 5.0 per cent incidence compared to control (24 percent disease incidence). The pooled mean of the three on farm trial conducted at MalaiPaiyur of Krishnagiri with US 618 tomato hybrid revealed that foliar spray with Imidacloprid (0.0375%) 7th and 30th DAT; *Pseudomonas fluorescens (Pf-1)* (2%) + butter milk (0.3%) on 15,30 and 45 DAT recorded minimum incidence of 0.9 per cent compared to the untreated control (21.0 per cent incidence). This management technology is practiced successfully in the North Western zone of Tamil Nadu district of India.

Keywords: Pea nut bud necrosis virus, *Pseudomonas fluorescens*, tomato

INTRODUCTION

The Pea nut bud necrosis virus (PbNv) is currently known to cause economic losses to many commercial crops like ground nut, chilli, potato, tomato, tobacco, and early maturing legumes such as mung bean and urd bean. In Tomato crop, a loss of 1.26 t in fruit yield per hectare due to infection by Peanut bud necrosis disease PbNvd caused by PbNv was reported. (Singh and Tripathi,1991). Until 1990, PbNvd in India was reported to be caused by Tomato spotted wilt virus (TSWV). Data from serological comparisons and subsequently from sequencing of nucleic acids revealed the existence of several distinct tospoviruses. The PbNvd was found to be associated with a distinct tospovirus and they named it as PbNvd as it was shown to be serologically distinct from TSWV and *Impatiens necrotic spot virus* (INSV) (Reddy *et al*.,1995). Though this virus is mechanically transmissible, in nature, it is transmitted by the vector *Thrips palmi* in India .However, the symptomatology varies depending on the strain, host species and genotype and is also influenced by environmental factors such as temperature. (Murai, 2000; Chaisuekul *et al*., 2003;Gitaitis, 2009). The Tomato hybrid crop is cultivated on a large scale in Dharmapuri and Krishnagiri districts of the North Western Zone of Tamil Nadu where the summer crop suffers huge loss due to infection by PbNv. Reducing the thrips populations using appropriate insecticides can help to reduce the virus spread. However, insecticides alone are of limited value in insect transmitted viral disease management , as virus spread from non-crop areas is an important source of infection and thrips require only limited feeding times for virus transmission. Frequent use of insecticides may also lead to development of insecticide resistance in thrips populations. Hence, strategic planning in the management of PbNv by using the biocontrol agents like Fluorescent pseudomonad's would offer great scope in the management of virus diseases by inducing resistance in plants. Perusal of reports show the use of *Pseudomonas fluorescens* on tomato plants against Tospovirus (Kandan *et al*., 2002) and *Fusarium oxysporum f. sp. lycopersici* (Asha *et al*.,2011). With this aim, the present study was conducted to manage the PbNv in tomato both by inducing resistance in plants through biological means as well as by managing the thrips population.

MATERIALS AND METHODS

Survey for PbNvD

Roving survey was conducted in major tomato growing areas *viz*., Pennagaram, Palacode and Harur blocks of Dharmapuri district and Kaveripattinam, Rayakottah, Thali , Hosur blocks of Krishnagiri districts of Tamil Nadu during 2006 for recording the pea nut bud necrosis virus disease incidence in Tomato hybrid crop

both in Summer/Masipattam (March-June) and Adipattam (July-October) which is the major season for tomato cultivation. In each block three villages were surveyed. In each village, three fields were surveyed.

Screening of plant extracts and *Pseudomonas fluorescens* against PbNvD

A field trial with Tomato hybrid US 618 (summer crop) was laid out in Regional Research Station of Paiyur, Tamil Nadu Agricultural University, Krishnagiri district during 2006 for the management of PbNvd with the following ten treatments. The treatments were replicated thrice in Randomized block design

The treatments included foliar spraying of plant extracts *viz*.,

T1 : *Adathoda vasica* @ 10 % concentration on 7, 15, 30 and 45 DAT

T2 : *Vitex negundo* @ 10 % concentration on 7, 15, 30 and 45 DAT

T3 : *Cocos nucifera* @ 10 % concentration on 7, 15, 30 and 45 DAT

T4 : *Azadirachta indica* @ 10 % concentration on 7, 15, 30 and 45 DAT

T5 : *Calotropis giantea* @ 10 % concentration on 7, 15, 30 and 45 DAT

T6 : *Prosophis juliflora* @ 10 per cent concentration on 7, 15, 30 and 45 DAT

T7 : Bacterial biocontrol agent *Pseudomonas fluorescens* @ 2 % on 7,15,30 and 45 DAT

T8 : Combination of Imidacloprid (0.0375 %) on 7 DAT; *P. fluorescens* (2%) + butter milk (0.3 %) on 15 DAT; Imidacloprid (0.0375 %) on 30 DAT (if needed based on pest incidence); *P. fluorescens* (2%) + butter milk (0.3 %) on 30 DAT and 45 DAT

T9 : Imidacloprid alone on 7, 15, 30 and 45 DAT

T10 : Control (water spray) on 7, 15, 30 and 45 DAT.

The per cent infection of PBND was calculated by counting the wilt affected plants from 15 DAT till 60 DAT. The fruit yield was recorded from till 120 DAT.

On farm testing trials on the management of PBND

Based on the results from the above experiments selected treatments were imposed on summer Tomato hybrid US 618 against PbNvd during 2007. Three on farm trials were carried out in farmers field at Malai Paiyur of Krishnagiri district .

T1 : Bacterial biocontrol agent *Pseudomonas fluorescens* @ 2 % on 7, 15, 30 and 45 DAT

T2 : Imidacloprid alone on 7, 15, 30 and 45 DAT

T3 : Combination of Imidacloprid (0.0375 %) on 7 DAT; *P. fluorescens* (2%) + butter milk (0.3 %) on 15 DAT; Imidacloprid (0.0375%) on 30 DAT (if needed based on pest incidence); *P. fluorescens* (2%) + butter milk (0.3 %) on 30 DAT and 45 DAT

T4 Butter milk (0.3%)

T5 : Control (water spray) on 7, 15, 30 and 45 DAT.

The per cent infection of PbNvd was calculated by counting the wilt affected plants from 15 DAT till 60 DAT. The fruit yield was recorded from till 120 DAT.

RESULTS AND DISCUSSION

PbNvD incidence in major tomato growing areas of Tamil Nadu

The Tomato hybrid crop US 618 is largely cultivated in the Krishnagiri and Dharmapuri districts of Tamil Nadu in two seasons March- June (summer/ Masi pattam) and July- October (Adipattam).The crop suffered heavy loss due to PBNvd in both seasons. Hence, the roving survey conducted for recording the PBNvd incidence revealed that the summer crop of Tomato showed maximum incidence of PBNvd which ranged from 18.8 to 30.6 per cent compared to Adipattam season where the disease recorded ranged from 11.9 to 15.9 per cent. Though the disease incidence recorded was higher (30.6 per cent) in Pennagaram block in summer season, however, in Adipattam season the disease incidence recorded was very much reduced (11.9 per cent). Such similar trend in disease incidence was observed in all the blocks in both the seasons (Table 1). The reason may be due to the heavy build up of the thrips population coupled with high relative humidity during summer season compared to other seasons. Singh and Tripathi (1991) also reported maximum occurrence of disease during March months.

Management of PbNvD under field conditions

Field trials were conducted by adopting botanical, biological and chemical strategies for the management of PbNvd in Tomato. The results from Table 2 showed that treatment T8 where spraying with combination of Imidacloprid (0.0375%) on 7 DAT; *P. fluorescens* (2%)

Table 1. Pea nut Bud Necrosis Disease incidence in major tomato growing regions of North Western zone of Tamil Nadu

District/Block	Villages	March- June (Summer/Masipattam)		July-October (Adipattam)	
		Incidence of PbNvd (%)	Mean incidence (%)	Incidence of PbNvd (%)	Mean incidence (%)
Dharmapuri district					
Pennagaram	Chinnampalli	30.0	30.6	15.8	11.9
	Bikampatti	32.0		9.2	
	Narasipuram	30.0		10.8	
Palacode	Somanahalli	28.5	28.1	15.1	14.3
	Belrampatti	27.4		12.1	
	Beumanahalli	28.6		15.8	
Harur	Erulapatti	29.3	22.4	17.2	15.7
	Jakkamapatti	20.0		15.0	
	Papampatti	18.0		15.0	
Krishnagiri district					
Kaveripattinam	Mahadevagonahalli	22.2	25.6	13.2	13.4
	Paiyur	28.0		13.4	
	Velampatti	27.1		13.5	
Rayakottah	Devasamudram	27.1	25.4	15.8	12.5
	Nagamanagalam	25.0		10.7	
	Karukanahalli	25.0		10.9	
Thalli	C.Kurupatti	18.6	18.4	17.9	15.9
	Eravandapalayam	18.0		15.0	
	Madhagondapalli	21.4		14.7	
Hosur	Bagalur	18.7	18.8	15.0	13.7
	Deiveranapalli	18.9		13.0	
	Bataverapalli	18.9		13.5	

+ butter milk (0.3%) on 15 DAT; Imidacloprid (0.0375%) on 30 DAT; *P. fluorescens* (2%) + butter milk (0.3%) on 30 DAT and 45 DAT was adopted, there was minimum disease incidence of 5.0 per cent on 75th day compared to control (24% incidence). Also higher yield of 42 t/ha with B:C ratio of 2.7 was recorded in this treatment compared to other treatments. The reason for effective control of PbNvd may be due to contribution of induced resistance by *P. fluorescens*, action of butter milk on the host by blocking the infection sites and altering the pH thereby reducing the uptake of virus by the insect and the direct effect of insecticide on the thrips population. Though the insecticide Imidacloprid recorded only 3.4% disease incidence the B:C ratio was 2.3. In our study, all plant extract treatments recorded disease incidence ranging from 9.5 to 14.8 per cent compared to control. The reason may be due to low persistence nature of the antiviral principle. However ,there are many reports on the effect of botanicals like *Glyricidia* leaf extract, *Clerodendron* leaf extract and also sorghum leaf extract in reducing the leaf curl disease incidence and whitefly population activity at early stage of the crop up to 45 days after transplanting in Tomato (Anjaneya *et al*., 2010), *Bougainvillea spectabilis* extract against tobamo viruses in tomato and bell pepper (Madhusudhan *et al*., 2011), extracts of *Mirabilis jalapa* and *Harpulia cupanoides* and the bacteria *Pseudomonas chlororaphis* was found highly effective in reducing the TSWV incidence besides the reduction of vector population (Renuka Devi, 2002). Karthikeyan *et al*. (2011) has

Table 2. Effect of plant products, animal products and biocontrol agents against PbNvd in Tomato

Treatment	Disease incidence (%)					Yield (t/ha)	B:C ratio
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT		
T1	1.1 [b] (6.02)	3.4 [c] (10.62)	5.7 [c] (13.81)	12.5 [f] (20.70)	12.5 [f] (20.70)	27.0 [f]	1.9
T2	0.0 [a] (0.00)	2.9 [cd] (9.80)	9.5 [d] (17.95)	9.5 [e] (17.95)	9.5 [e] (17.95)	28.0 [e]	1.9
T3	1.5 (7.03)	5.5 [f b] (13.56)	13.5 [e] (21.56)	14.8 [g] (22.63)	14.8 [g] (22.63)	29.0 [e]	2.0
T4	2.3 [c] (8.72)	8.6 [h] (17.05)	10.2 [d] (18.63)	12.5 [f] (20.70)	12.5 [f] (20.70)	30.0 [e]	2.0
T5	2.2 (8.53)	6.8 [g c] (15.12)	6.8 [c] (15.12)	11.6 [f] (19.91)	11.6 [f] (19.91)	32.0 [d]	1.9
T6	0.0 [a] (0.00)	4.4 [e] (12.11)	5.6 [c] (13.69)	7.8 [d] (16.22)	7.8 [d] (16.22)	34.0 [c]	2.0
T7	0.0 [a] (0.00)	2.4 [c] (8.91)	5.9 [c] (14.06)	5.9 [c] (14.05)	5.9 [c] (14.05)	35.0 [c]	2.4
T8	0.0 [a] (0.00)	5.0 [a] (12.92)	5.0 [a] (12.92)	5.0 [a] (12.92)	5.0 [b] (12.92)	42.0 [a]	2.7
T9	0.0 [a] (0.00)	1.2 [b] (6.29)	3.4 [b] (10.62)	3.4 [b] (10.64)	3.4 [a] (10.64)	37.0 [b]	2.3
T10	1.3 [b] (6.55)	12.7 [i] (20.88)	15.4 [f] (23.11)	20.6 [h] (26.99)	24.0 [h] (29.33)	23.0 [g]	-
SEd	0.267	0.3651	0.5188	0.5858	0.6344	0.8777	
CD (P=0.05)	0.562	0.7672	1.0899	1.2308	1.3329	1.8440	

Mean of three replications, Values in the parenthesis are transformed values.

reported abinav cultivar to be tolerant to PBNV and Tomato leaf curl virus. However, the mode of action of the plant products on the vector is still not clear and many scientists have reported the plant extracts to possess anti-feedant properties. Though plant products possess array of phytochemicals their stability under field conditions is limited.

Based on the above results, three on farm trials were conducted with spraying of *P. fluorescens*, Imidacloprid, butter milk and combination of all the three . Pooled mean of three trials conducted at Malaipaiyur (Table 3) showed that the treatment where spraying with Combination of Imidacloprid (0.0375 %) on 7 DAT; *P. fluorescens* (2%) + butter milk (0.3 %) on 15 DAT; Imidacloprid (0.0375%) on 30 DAT; *P. fluorescens* (2%) + butter milk (0.3%) on 30 DAT and 45 DAT was adopted was highly significant 0.9 per cent disease incidence was recorded until 75th day compared to control (21.0 per cent incidence). Kandan *et al*., (2002) reported that *P. fluorescens* effectively controlled TSWV incidence in Tomato. Sanjay Bandi and Sivasubramanian (2012) reported that the activity of defense related enzymes *viz*., Peroxidase (POD), Polyphenoloxidase (PPO) and Phenylalanine Ammonia Lyase (PAL) was enhanced in onion plants treated with foliar application of *Pseudomonas fluorescens*. The activity of these enzymes was negatively correlated with thrips population. This is in agreement with the findings of Sathish and Raguraman (2007) who reported that the activity of these defense related enzymes were negatively correlated to *Helicoverpa armigera* infestation in tomato. Murugesan and Kavitha (2009) reported the effectiveness of *P. fluorescens* against leafhopper, *Amrasca devastans* in

Table 3. On Farm Trials on the management of PbNvd at Malai Paiyur, Krishnagiri district (Pooled mean of three trials)

Treatment	Disease incidence (%)					Yield (t/ha)	B:C ratio
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT		
T1	0.0[a] (0.00)	2.6[b] (9.28)	76.9[c] (15.23)	8.6[c] (17.05)	9.4[c] (17.85)	32.8[c]	2.3
T2	0.0[a] (0.00)	2.6[b] (9.28)	3.4[b] (10.62)	5.9[b] (14.06)	5.9[b] (14.06)	32.0[b]	2.3
T3	0.0[a] (0.00)	0.0[a] (0.00)	0.9[a] (5.44)	0.9[a] (5.44)	0.9[a] (5.44)	40.9[a]	2.7
T4	4.3[b] (11.97)	5.9[d] (14.06)	10.0[d] (18.81)	16.4[d] (23.89)	16.4[d] (23.89)	27.8[d]	1.9
T5	5.1[c] (13.05)	5.0[c] (12.92)	12.6[d] (20.79)	19.3[e] (26.06)	21.0[e] (27.27)	22.9[e]	-
SEd	0.18	0.26	0.35	0.49	0.53	0.39	
CD (P=0.05)	0.39	0.570	0.77	1.08	1.16	0.86	

Values in the parenthesis are transformed values.

cotton through induced resistance. In our study also *P. fluorescens* application involved induction of defense enzymes in phenyl propanoid pathway and accumulation of phenolics might have contributed to induced resistance against thrips population in Tomato there by reducing the PbNvd disease incidence. Hence, it is clear from the study that the management of vector with insecticides at the initial stages contributes for reduction in vector population and spraying with *P. fluorescens* offer induced resistance for better control of PbNvd incidence in Tomato apart from increasing the yield. Currently, this management technology is practiced successfully for the management of PbNvd in the North Western zone of Tamil Nadu district of India.

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Molecular characterization of tobacco curly shoot virus infecting tomato (*Solanum lycopersicum L.*) in India

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ABSTRACT: Tomato is an important vegetable crop cultivated through out India and leaf curl disease is the major constraint for production. The disease has previously been shown to be associated with begomoviruses and betasatellites. A distinct monopartite begomovirus was found associated with tomato plants showing sever leaf curl, interveinal chlorosis and stunting symptoms in polyhouse grown tomatoes in Pantnagar, Uttarakhand, India. The complete DNA-A component was amplified through rolling circle amplification (RCA) using Phi29 DNA polymerase and characterized. The DNA-A of the four isolates as comprised of 2,758 nucleotides, encoding six open reading frames (ORFs) with genome organization typical of an old world monopartite begomovirus. The begomovirus showed highest sequence identity of (89.3 to 95.8%) to tobacco curly shoot virus (TbCSV) and some other begomoviruses (59.6 to 88.1%) reported from India in tomato. Based on sequence comparison and phylogenetic analysis these isolates are strains of tobacco curly shoot virus. Recombinant analysis of the four isolates revealed that some of the isolates are recombinants of Ageratum enation virus (AEV) and tobacco curly shoot virus isolates. This is new begomovirus infecting tomato in addition to the known 16 begomoviruses occurring in India. Information on the distribution and prevalence of the different begomovirus species and recombinant forms comprising the disease complex is crucial in guiding tomato breeding programs in the search for stable and durable sources of resistance.

Keywords: Rolling circle amplification, RCA, RDP, *Solanum lycopersicum,* Tobacco curly shoot virus

INTRODUCTION

Tomato (*Solanum lycopersicum* L) is one of the most economically important vegetable crops in India. Its production is constrained by tomato leaf curl disease (ToLCD). Although incidence of tomato leaf curl disease in India was first reported from northern region (Vasudeva and Sam Raj, 1948), this disease is now widely spread in almost all tomato growing regions of the country. Tomato leaf curl disease epidemics are increasing every year in intensive tomato growing areas causing (27-100%) yield loss, depending on the stage of infection (Muniappa *et al*., 2000). The disease is transmitted by the whitefly *Bemisia tabaci* (Homoptera:Aleyrodidae), with symptoms consisting of leaf curling, vein clearing, reduction in leaf lamina, vein enation and, overall stunting of plants (Chowda Reddy *et al*., 2012). The incidence of diseases caused by begomoviruses in tomato has increased dramatically in recent years in India. The first report of begomovirus infection on tomatoes in the country date back to the 1950's (Vasudeva and Sam Raj, 1948). However, in the late 1990's and early 2000's an extremely diverse begomovirus complex has emerged in India, coinciding with the introduction and spread of a new biotype of the whitefly vector *Bemisia tabaci* biotype B (Banks *et al*., 2001; Chowda Reddy *et al*., 2012; Rekha *et al*., 2005; Shankarappa *et al*., 2007). At least fifteen different begomovirus (family *Geminiviridae*, genus *Begomovirus*) species associated with ToLCD have been reported in India (Brown *et al*., 2012; Fauquet *et al*., 2008), and mixed infections in natural epidemics can be frequent (Delatte *et al*., 2005; García-Andrés *et al*., 2007). The existence of mixed infections is a cause of concern because it is a prerequisite for recombination to occur, a frequent phenomenon among geminiviruses (Padidam *et al*., 1999, Kirthi *et al*., 2002), with unpredictable pathological consequences.

The members of the genus Begomovirus have typical geminate particles encapsidating a single stranded circular DNA genome, infect dicotyledonous plants and are transmitted by the whitefly *B. tabaci*. They have either a bipartite genome with two single-stranded (ss) DNA components designated as DNA A and DNA B, each one about 2.7 kb in size, or a monopartite genome with one ssDNA component analogous to DNA A (Briddon *et al*., 2012). The genomes of monopartite (DNA A components of bipartite) begomoviruses are typically <2800 nucleotides in length and encode genes bidirectionally

from a non-coding intergenic region which contains promoter elements and the origin (ori) of virion-strand DNA replication. The virion-strand ori consists of a predicted hairpin structure containing the absolutely conserved (for geminiviruses) nonanucleotide (TAATATTA↓C) loop sequence and repeated motifs upstream known as iterons. The virion-sense strand encodes the coat protein (CP) that is required for insect transmission and movement in plants) and V2 protein that is to be involved in virus movement in plants. (Rojas *et al*., 2001). The complementary-sense strand encodes the replication-associated protein (Rep) the only virus-encoded gene product required for viral DNA replication, which is a rolling circle replication initiator protein that recognizes the iterons and nicks within the nonanucleotide sequence to initiate replication (Hanley-Bowdoin *et al*., 2004), the transcriptional activator protein (TrAP) which up-regulates the late [virion-sense] genes bipartite begomoviruses, is a suppressor of post-transcriptional gene silencing (PTGS; Yang *et al*., 2007) and also overcomes virus induced hypersensitive cell death (Hussain *et al*., 2007; Mubin *et al*., 2010), the replication enhancer protein (REn) that is involved in establishing an environment conducive for virus replication (Pedersen and Hanley-Bowdoin, 1994) and the C4 protein, the function of which remains unclear but for some viruses is a pathogenicity determinant and a suppressor of PTGS; Gopal *et al*., 2007; Saeed *et al*., 2008; Vanitharani *et al*., 2004). DNA B encodes two ORFs, nuclear shuttle protein (NSP, BV1) on the viral-sense strand and movement protein (MP, BC1) on the complementary-sense strand (Hanley-Bowdoion *et al*., 1999).

A small, circular, single-stranded DNA (1.3kb) referred to as DNA β is found to be associated with DNA A of old world monopartite begomoviruses. Betasatellites induce severe symptoms in plants and are dependent on DNA A for replication, encapsidation and transmission (Briddon *et al*., 2008; Briddon and Stanley, 2006; Sivalingam and Varma, 2012). They have a highly conserved structure, consisting of a single gene encoded in the complementary-sense (known as β C1), a region of sequence rich in adenine (A-rich) and an approximately 100–150 nucleotide sequence (known as the satellite conserved region) conserved between all betasatellites that contains a predicted hairpin structure containing a loop with the nonanucleotide sequence with similarity to the origin of virion-strand DNA replication of the geminiviruses (Briddon *et al*., 2003). The âC1 protein has DNA binding properties and is involved in overcoming host defenses (by suppression of PTGS), eliciting symptoms and possibly mediates virus movement in plants (Cui *et al*., 2005; Kon *et al*., 2007; Saeed *et al*., 2005, 2007). In addition to betasatellites, monopartite viruses are found to be associated with another type of satellite known as alphasatellite, which encodes one replication initiation protein similar to those of nanoviruses in the viral strand. How these alphasatellites are involved in pathogenesis has not been deciphered (Brown *et al*., 2012).

Despite the efforts to manage ToLCD, viral disease problems continue to emerge in tomato, invading new areas every year. During January 2013, a severe ToLCD was observed in a tomato grown in polyhouses near Pantnagar. Form this severely infected tomato plants, we cloned and characterized DNA-A and we report a new monopartite begomovirus in tomato prevalent in northern India based on comprehensive biological and molecular analyses and following International Committee on Taxonomy of Viruses (ICTV) norms.

MATERIALS AND METHODS

Sample collection and DNA isolation: Symptomatic young leaves were collected from infected tomato plant and total DNA was isolated by a modified CTAB method as follows. One gram of fresh leaf tissue was ground into a fine powder in liquid nitrogen using a mortar and pestle, 100mg of powder was added to a 2.0-ml screw cap microcentrifuge tube (Fisher Scientific, USA) and 1ml of CTAB buffer (2% CTAB, 2% PVP-40, 100mMTris-HCl, pH 8.0, 1.4MNaCl, 20mMEDTA, and 1.0% 2-mercaptoethanol) and vortexed thoroughly for few minutes. The homogenate was incubated at 65°C for 10 min, followed by addition of equal volume of chloroform-isoamyl alcohol (IAA) (24:1 v/v) mixed and centrifuged at 10,000*g* for 10 min at 4 °C. The supernatant (500µl) was transferred to a 1.5 ml microcentrifuge tube containing 3/4th volume of isopropanol, and the mixture was incubated at "20°C for 2 to 3 hours or overnight at 4 °C. After the incubation sample was centrifuged at 10,000*g* for 30min. The supernatant was discarded and pellet was air-dried and dissolved in 100µl of 20mMTris-HCl, pH 8.0. The pooled DNA samples were treated with RNase and incubate for 1 hr at 37°C. To the sample mixture, equal volume of chloroform-isoamyl alcohol (IAA) (24:1 v/v) was added, mixed and centrifuged at 10,000*g* for 10 min at 4°C. The supernatant was discarded and pellet was washed with 70% ethanol by centrifugation at 15,000*g* for 5 min, air-dried and dissolved in 100µl of 20mM Tris-HCl, pH 8.0.

The final elute was adjusted to 80-100µl with sterile water and stored at "20 °C for short term (d"2 months) or "80°C for long term(e"3months) for further use. The quality of the extracts was measured using a NanoDrop® ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and visualized by electrophoresis through 1.5% agarose gels.

Rolling circle amplification and cloning of viral genome: The viral genomic DNA was amplified through rolling-circle amplification (RCA) (Haible *et al*., 2006). Amplification of circular DNA was performed using 10-20ng of total nucleic acid as template. The reaction mixture contained 2µl of reaction buffer (10X), 2µl of random hexamer primers (500 mM Fermentas catalog no. SO142) and 2µl of dNTPs (10 mM). The mixture was denatured for 3 min at 94°C and was then cooled down to room temperature for 5 min, after which 4 µl of pyrophosphatase (0.1 U/ µl, Fermentas catalog no. EF 0221) and 0.7µl of ϕ29 DNA polymerase 10 U/µl (Fermentas catalog no. EP0092) was added. The reaction mixture was incubated for 18-20 h at 30°C and was inactivated at 65°C for 10 min. Concatamers obtained through RCA were digested with restriction endonucleases *BamHI, EcoRI, HindIII* and *KpnI* to release 2.7kb fragments of full-length DNA A and which were cloned in the vector pUC18 following standard protocol (Sambrook *et al*., 1989). The clones were confirmed by restriction digestion with *BamHI, EcoRI, HindIII*, and *KpnI* and positive clones were confirmed by sequencing. The universal degenerate primers for DNA-B (Rojas *et al*., 1993), and specific primers based on DNA-A intergenic region were used to detect presence of DNA-B in infected plant sample.

Sequence analysis of viral genome DNA-A

Three clones in each case were sequenced by automated DNA sequencer ABI PRISM3730 (Applied Biosystems) at Eurofins India Ltd, Bangalore, India. Full-length sequences of DNA A were analyzed using BioEdit version 7.0.9. Open reading frames (ORF) were predicted using GENERUNNER (Hartings Software Inc., Hastings, NY, USA; http://www.generunner.net). The sequence results were analyzed using NCBI (www.ncbi.nlm.nih.gov) blast search, followed by multiple sequence alignments using ClustalX (Thompson *et al*. 1997) and Bioedit Sequence Alignment Editor (version 5.0.9) (Hall, 1999), to determine percentage sequence similarities with other species, which showed maximum similarities in the blast search. Full-length genome of selected Begomovirus species, phylogenetic trees were generated by MEGA 5.0 software (Tamura *et al*. 2011), using the neighbor joining method with 1000 bootstrapped replications, to estimate evolutionary distances between all pairs of sequences simultaneously. Phylogenetic trees were constructed using the neighbour joining algorithm of Clustal X and displayed, manipulated and printed using Tree view (Page, 1996).

Detection of recombination events

To unmask probability of natural recombination as cause of origin of begomovirus associated with tomato leaf curl disease, the viral sequence was analyzed using the recombination detection program, RDP version 3 (Martin *et al*., 2010). Defaults RDP settings were used with a P-value cut off of 0.05 and the standard Bonferroni correction. Only internal references were allowed with a parental cutoff 70–100%. For general recombination detection, sequences were considered circular, consensus daughters were found and sequences were polished. For bootscan analysis, 200 boot replicates with 95% cutoff percentage was taken and for GENECONV analysis g-scale parameter was set to 1 (Martin *et al*., 2010).

RESULTS

Sample collection, detection and cloning of viral genome

Fifty one leaf samples collected from polyhouse grown tomatoes at Pantnagar, Uttarakand, India, showing severe leaf curling, vein clearing, yellowing, reduced leaf size and stunting were analyzed, Of the 51 samples tested, 48 samples gave 1.2-bp amplicons with PAR1v722 and PAL1c1960 primers (Deng *et al*., 1994, Rojas et al., 1993). From partial sequencing data, PCR amplicons were identified as belonging to AEV, TbCSV. No amplification was seen with specific primers for ToLCNDV, ToLCGuV, ToLCBaV or ToLCKaV. The begomovirus species detected in tomato grown in polyhouse indicated 31 samples of TbCSV (60.78%), 18 samples of AEV (35.3%) with the incidence of 27.6 to 68.5% tomato leaf curl disease in five different polyhouses. Viral genomic DNA of infected plants was enriched by rolling-circle amplification (RCA), and the RCA product was digested individually with *BamHI, EcoRI, HindIII* and *KpnI*. Clear bands of 2.7 kb, the expected unit genome length of begomoviruses were detected in BamHI and HindIII treated samples (Fig. 1). Of the twelve samples analyzed, only eight showed clear RCA products. These 2.7- kb fragments were purified and cloned in the pUC18 vector and linearized with the corresponding enzymes. The cloned components were

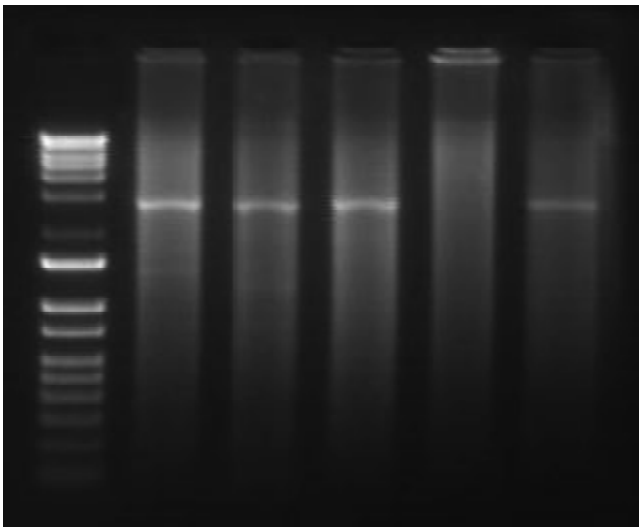

Fig. 1. Gel photograph showing restriction digests of the RCA products by different enzymes. Lane 1: BamHI, 2: HindIII, 3: EcoRI, 4: XbaI, 5:Kpn1, M:10 kb DNA Ladder (Fermentas).

confirmed by restriction digestion and further confirmed by partial sequencing.

Genome organization of TbCSV

The full-length nucleotide sequences of begomovirus genome (DNA A component) obtained from isolates TCb1, TCb8, TCh8 and TCh82 were determined to be 2758 bp in length (NCBI accessions JX457341, JX457342, JX467692 and JX467693). Attempts to amplify a begomovirus genome DNA B component from these isolates using the two primer pair's specific to DNA-B were uniformly negative. This may indicate that the virus associated with these isolates is distinct monopartite virus. Analysis of the two begomovirus components showed the presence of a predicted hairpin structure with the sequence TAATATTA↓C forming part of the loop. This structure is typically part of the origin of virion-strand replication of geminiviruses. The final adenine nucleotide of the nonanucleotide sequence was by convention, used to start nucleotide numbering. Further examination of the sequences using ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) showed the presence of six predicted genes with a coding capacity greater than 12kDa, two in the virion-sense (encoding the coat protein (CP) and precoat protein AV2) and four in the complementary sense (encoding the replication associated protein (Rep), the transcriptional activator protein (TrAP), the replication enhancer protein (REn) and AC4 (PTGS Suppressor), diverging from a non-coding sequence (the intergenic region) that contains the predicted hairpin structure. This arrangement of genes is typical of the genomes (or DNA A components) of begomoviruses originating from the Old World.

Sequence comparisons and analysis

The complete nucleotide sequences of the begomoviruses cloned from isolates TCb1, TCb8, TCh8 and TCh82, have 99.4 to 99.8% sequence identity among these isolates. An initial comparison of the four sequences obtained with sequences in the databases using BLAST indicated them to be more similar to viruses previously identified in tomato originating from china. Multiple alignment and pair-wise sequence comparison of complete nucleotide sequences of the 27 begomoviruses infecting occurring in India has shown highest nucleotide similarities of 89.3 to 95.8% with tobacco curly shoot virus isolates. Based on the presently applicable species demarcation threshold 89% for begomoviruses (Fauquet *et al*., 2008), It is concluded that that the four begomoviruses are isolates of the same species. Among the tomato infecting begomoviruses in India, isolates TCb1, TCb8, TCh8 and TCh82 have 74.6 to 88.1% homology, where as with other begomoviruses they have nucleotide sequence homology of 59.7 to 75.5% (Table 1). The highest level of identity of 89.3 to 95.8% with tobacco curly shoot virus indicates that present isolates are a strains of TbCSV infecting tomato in North India. Multiple alignment and Comparison of individual proteins with begomoviruses has indicated that TCb1, TCb8, TCh8 and TCh82 have highest nucleotide similarities of 97.2 to 100% with TbCSV coat protein (CP), 93.5 to 100% with replication associated protein (Rep), 89.7 to 100% with transcriptional activator protein (TrAP), and 87.3 to 100% with replication enhancer protein (REn). Where as with the non coding IR sequence has 97.2 to 100% (Table 1).

Phylogenetic relationships of TbCSV

A phylogenetic analysis of the begomovirus sequences obtained in the study known sequences of begomoviruses associated with ToLCD and selected other sequences are shown in Fig. 2. A phylogenetic tree, based upon an alignment of the full-length sequences of DNA A component of selected begomoviruses present in India, shows that sequences of TCb1, TCb8, TCh8 and TCh82 to be most closely related to isolates of TbCSV, but the tomato isolates from India formed a separate sub group along with TbCSV(Y1)(AF240675) from china infecting tobacco. The relative positions of these isolates are well supported by boot -strapping.

Detection of recombination

Table 1 Pairwise comparisons of percent nucleotide identities between the genomic components and amino acid sequence identities of encoded genes four isolates of TBCSV infecting tomato with selected begomoviruses from NCBI the database

Begomovirus species	Full genome	IR	AV1	AV2	AC1	AC2	AC3	AC4
1. HG00365	93.1-93.4	78.5-78.9	99.2-99.6	96.8	96.8	91.9-91.6	93.2-97	89.8-90.1
2. JX457342	99.4-100	76.7-77.0	99.6-100	100	100	100	100	42.0-100
3. JX457341	99.4-99.8	99.6-100	99.6-100	100	100	100-99.7	94.0-100	94.2-100
4. JX467693	99.4-99.8	99.6-100	99.6-100	100	100	100-99.7	92.5-100	95.0-100
5. JX467692	99.4-99.6	99.6-100	99.6-100	100	100	99.7-100	92.5-100	94.2-100
6. HQ407395	89.3- 92.2	75.1-75.5	98.8-99.2	96.8	96.8	96.7	87.3-89.5	90.1-92.4
7. GU001879	95.6-95-8	85.7-86.0	98.8-99.2	97.4	97.4	95.0-94.7	93.2-95.5	95.0-98.9
8. GU199584	92.4-92.6	78.2-92.6	98.0-98.4	93.5	93.5	91.4-91.1	93.2-95.5	89.6-94.2
9. GU199583	93.0-93.1	78.5-78.9	97.6-98.0	95.4	95.4	92.5-92.2	90.2-91.0	84.2-88.6
10. AF240675	93.5-93.7	85.3-85.7	98.8-99.2	94.1	94.1	90.0-89.7	87.3-91.7	95.0-98.9
11. AJ420318	95.5-95.7	85.7-86.0	99.2-99.6	97.1	97.1	95.2-95.0	93.2-95.5	95.0-98.9
12. AJ971266	95.6-95.7	85.7-86.0	99.2-99.6	96.8	96.8	94.7-94.4	93.2-95.5	95.7-97.9
13. AJ457986	93.5-93.7	80.2-80.6	98.4-98.8	97.4	97.4	92.2-91.9	92.5-94.7	90.7-94.2
14. JN387045	94.4-94.7	86.3-86.7	97.2-97.6	92.7	92.7	96.1-95.8	90.2-95.5	95.0-97.9
15. JN176565	84.2-84.4	72.0-72.3	97.6-98.0	28.9	28.9	83.6-83.3	73.8-76.8	75.0-75.7
16. AM884015	71.2-71.3	50.4-50.8	89.8-100	70.4	70.4	63.8-64.1	74.4-75.8	74.2-78.1
17. GU112084	72.6-72.7	52.1-52.4	91.7-92.1	75.5	75.5	75.0 -74.7	75.8-76.6	75.0-76.4
18. EU862323	74.6-74.8	61.8-62.2	61.7	70.9	70.9	77.5-77.2	65.6-68.6	65.7-67.0
19. U38239	84.7-84.8	71.7-72.0	96.0-96.4	90.7	90.7	85.5-85.3	82.8-85.8	85.0-87.4
20. GU732204	83.7-83.9	62.7-63.1	97.2-97.6	88.7	88.7	86.0-87.8	83.5-85	85.7-88.4
21. AY190290	81.4-81.5	71-71.4	80.0-80.4	88.2	88.2	85.8-85.5	76.8-79.1	75.0-83.2
22. EF068246	72.7-72.8	50.4-50.8	91.7-92.1	74.7	74.7	75-74.7	74.4-75.8	74.2-75.1
23. GQ994095	89.9-90.0	70.5-70.9	98.0-98.4	94.5	94.5	88.3-88	92.5-94.7	82.4-83.5
24. DQ339117	77.1-77.2	68.4-68.7	96.8-97.2	92.4	92.4	76.7-76.4	75.1-76.6	74.9-75.2
25. AM992618	63.3-63.4	49.1-51.4	50.1	52.5	52.5	71.8-71.5	41.7-41	53.5-54.6
26. HM461863	78.6-78.8	48.5-48.9	97.6-98.0	88.5	88.5	79-78.7	78.3-77.6	76.477.5
27. DQ629103	82.4-82.6	70.4-70.8	93.3-93.7	87.1	87.1	84.4-84.2	70.1-73.1	75.7-77.3
28. JN135234	76.2-76.3	71.1-71.5	95.3-95.7	89.3	89.3	72.5-72.2	67.1-70.8	76.4-78.9
29. JX436472	87.5-87.8	76.7-77.0	99.2-99.6	94.1	94.1	81.4-81.1	88.8-92.5	86.4-87.2
30. AY754814	78.9-79.1	56.0-56.3	90.6-91.0	74.2	74.2	83.9-83.6	76.1-79.8	75.0-76.0
31. EU910141	80.7-80.9	61.8-62.1	96.0-96.4	90.4	90.4	86.6-86.7	82.8-84.3	83.5-84.4
32. AF188481	88.0-88.1	68.1-68.5	98.8-99.2	91.8	91.8	87.5-87.2	83.5-85.8	85.0-87.2
33. AJ575819	73.2-73.4	48.1-48.3	91.4-91.7	77.8	77.8	77.8-77.5	65.6-67.9	75.7-76.0
34. JN663865	81.0-81.1	60.1-60.5	96.4-96.8	86.6	86.6	83.3-83.1	74.6-76.8	84.2-85.2
35. HQ588901	59.6-59.7	34.0-34.3	36.6	37.5	36.5	41.2-41.2	35.8-36.5	36.0-43.2
36. FN645902	78.2-78.4	70.9-71.2	82.4-82.8	83.3	90.4	74.5-74.2	82.8-83.5	85.0-86.8
37. HQ698591	85.1-85.4	64.9-65.2	98.4-98.8	72.1	89	86.2	76.1-79.8	86.0-87.4
38. GQ220850	75.4-75.5	40.7-40.9	95.7-96.0	74.0	73.3	76.6	70.8-73.1	75.0-75.6
39. GU111999	71.1-71.2	45.9-46.0	41.4-41.7	87.1	72.1	86.3	67.9-70.1	83.5-84.1
40. AF241479	72.4-71.5	8.5-48.7	93.3-93.7	48.5	74.0	86.6	70.1-71.6	73.1-74.0
41. HM007104	83.0-83.1	72.2-72.6	95.7-96.0	96.8	87.1	85.8-85.5	70.8-73.1	85.0-86.7

Table 2. Break point analysis of TbCSV and their putative parental sequences

DNA-A	Break point			P-Values					
	begin-end	Major Parent	Minor parent	RDP	GENECOV	Max Chi	Chimera	Si Scan	3Seq
TbCSV.TCb1	93-932	ToLCRnV [GQ994095]	TbCSV.WSF1[HQ407395]	$1.241X10^{-6}$	$2.935X10^{-5}$	$5.91X10^{-7}$	$1.436X10^{-8}$	$2.573X10^{-7}$	$1.769X10^{-8}$
	2358-2707	TbCSV.Y-T8 [HG00365]	TbCSV[IN:var:FB:12].JQ733557	$8.14X10^{-15}$	$1.22X10^{-4}$	$1.523X10^{-9}$	$3.401X10^{-10}$	$1.607X10^{-11}$	$6.198X10^{-16}$
TbCSV.TCh82	2358-2707	ToLCRnV [GQ994095]	TbCSV.WSF1[HQ407395]	$1.241X10^{-6}$	$2.935X10^{-5}$	$5.91X10^{-7}$	$1.436X10^{-8}$	$2.573X10^{-7}$	$1.769X10^{-8}$
	93-932	TbCSV.Y-T8 [HG00365]	TbCSV[IN:var:FB:12].JQ733557	$8.14X10^{-15}$	$1.22X10^{-4}$	$1.523X10^{-9}$	$3.401X10^{-10}$	$1.607X10^{-11}$	$6.198X10^{-16}$
TbCSV.TCb8	93-932	ToLCRnV [GQ994095]	TbCSV.WSF1[HQ407395]	$1.241X10^{-6}$	$2.935X10^{-5}$	$5.91X10^{-7}$	$1.436X10^{-8}$	$2.573X10^{-7}$	$1.769X10^{-8}$
	2358-2707	TbCSV.Y-T8 [HG00365]	TbCSV[IN:var:FB:12].JQ733557	$8.14X10^{-15}$	$1.22X10^{-4}$	$1.523X10^{-9}$	$3.401X10^{-10}$	$1.607X10^{-11}$	$6.198X10^{-16}$
TbCSV.TCh8	93-932	TbCSV.WSF1[HQ407395]	TbCSV.WSF1[HQ407395]	$1.241X10^{-6}$	$2.935X10^{-5}$	$5.91X10^{-7}$	$1.436X10^{-8}$	$2.573X10^{-7}$	$1.769X10^{-8}$
2358-2707		TbCSV.Y-T8[HG00365]	TbCSV[IN:var:FB:12].JQ733557	$8.14X10^{-15}$	$1.22X10^{-4}$	$1.523X10^{-9}$	$3.401X10^{-10}$	$1.607X10^{-11}$	$6.198X10^{-16}$

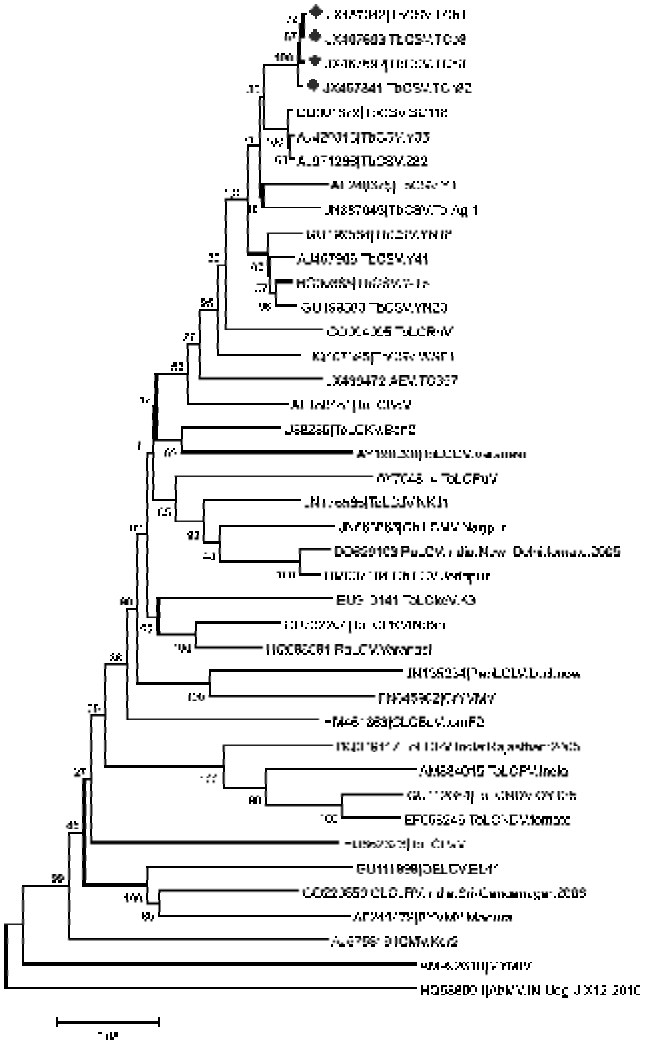

Fig. 2. Phylogenetic tree of complete nucleotide sequences of the DNA-A of the Tobacco curly shoot virus (TBCSV) isolates originating from Pantnagar with selected other begomoviruses available in the databases. The TBCSV isolates originating from pantnagar is highlighted. The number at the nodes indicates the bootstrap confidence values (1000 replicates). For each isolate the database accession numbers are given.

Recombination is a common feature in the evolutionary history of many begomoviruses. To determine whether the begomovirus identified here shows an evidence of recombination, RDP3 analysis was conducted based on alignments with full-length sequences of selected begomoviruses and the closest identity with two begomoviruses such as Ageratum enation virus and tomato leaf curl Ranchi virus along with other available in the NCBI database. The results of this are shown in (Fig. 3) with the details, including p-values, given in Table

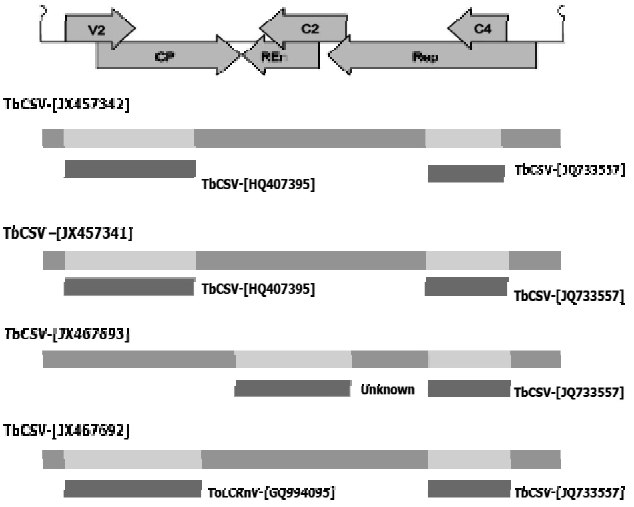

Fig. 3. Analysis for recombination among selected begomoviruses associated with ToLCD. Recombinant fragments in the sequences of TbCSV isolates were identified using RDP3 analysis of a Clustal W alignment. The default X-Over settings was used for recombination analysis. Recombinant sequences are identified as dark lines below the sequence (identified above each full-length sequence line).

3. The analysis showed TbCSV isolates from tomato to consist of the virion-sense sequences of ToLCRnV and the complementary-sense sequences of AEV and other isolates of TbCSV covering in the C1 region and C4 region. The isolate TbCSV-(JX467692) was found to be recombinant with ToLCRnV-(GQ994095) as major and TbCSV-(JQ733557) as minor parents. The recombination breakpoints were determined are shown in the (Table 2).

DISCUSSION

The tomato leaf curl disease (ToLCD) is one of the most devastating diseases of tomato (*Solanum lycopersicum* L.) and mixed infections in natural epidemics can be frequent (Delatte *et al*., 2005; García-Andrés *et al*., 2007). The existence of mixed infections is a cause of concern because it is a prerequisite for recombination to occur, a frequent phenomenon among geminiviruses (Padidam *et al*., 1999), with unpredictable pathological consequences. In India, diseases caused by whitefly-transmitted begomoviruses are on increase, affecting the cultivation and economic yield of vegetables, including, tomato, pepper and chilli. Increased overlapping cultivation of closely related solanaceous and cucurbitaceous crops has resulted in an expanded host range of existing begomoviruses and has led to the emergence of new strains and recombinants. This is especially true for tomato, which is grown in diverse

agroclimatic zones throughout the year, thus perpetuating the virus inoculum and vector population (Varma *et al*., 2011, Ramappa *et al*., 1998). Considerable progress has been made in characterizing tomato begomoviruses in southern, western and northern India. At present, a total of 16 tomato infecting begomovirus species are known to be associated with ToLCD in India (Brown *et al*. 2012; Fauquet *et al*. 2008). Tomato leaf curl Bangalore virus (ToLcBav) (Muniyappa *et al*., 2000), Tomato leaf curl Karnataka virus (ToLCKaV) (Chatchawankanphanich and Maxwell 2002), and Tomato leaf curl Kerala virus (ToLCKeV) (Pasumarthy *et al*., 2010) from southern India (Hong and Harrison, 1995; Kirthi *et al.* 2002). Whereas Ageratum enation virus (AEV) (Swarnalatha *et al*., 2013), Croton yellow vein mosaic virus (Chowda Reddy *et al*., 2005; Pramesh *et al*., 2013) Papaya leaf curl virus (PaLCV), Tomato leaf curl New Delhi virus (ToLCNDV) (Srivastava *et al*. 1993), Tomato leaf curl Gujarat virus (ToLcGuV) (Chakraborty *et al*. 2003; Jyothsna *et al*., 2013), Tomato leaf curl Palampur virus (ToLCPaV) (Kumar *et al*., 2008), Tomato leaf curl Pune virus (ToLCPuV), Tomato leaf curl and Rajasthan virus (ToLCRaV) from northern India (Padidam *et al*, 1995, Chakraborty, 2008), Cotton leaf curl Burewala virus (CLCuBV) (Kumar *et al*., 2013), Tomato leaf curl Ranchi virus (ToLCRnV) (Kumari *et al*., 2011b), Tomato leaf curl Patna virus (ToLCPV) (Kumari *et al*., 2011a) and tomato leaf curl Joydebpur virus (ToLCJV) (Tiwari *et al*., 2012) have been reported from eastern India (Kumari *et al*., 2009, 2010, 2011b).

With an increasing number of samples analyzed from different locations, it is now evident that both monopartite and bipartite begomoviruses are distributed throughout India. Information regarding tomato begomoviruses from the eastern region of India – eastern U.P., Bihar, Jharkhand, West Bengal and the far north-eastern region comprising Assam, Manipur, Meghalaya and Mizoram is very limited. We surveyed the Pantnagar district and samples were collected from five poly houses and analyzed, results showed the presence of Tobacco curl shoot virus (TbCSV) causing tomato leaf curl disease in tharai region of Uttarakhand. All of the samples from Pantnagar that were tested showed the presence of AEV and TbCSV based on sequencing of genomic components of amplicons obtained with PAR1v722 and PAL1c1960 primers (Deng et al. 1994) and RCA. The Samples were also subjected to PCR amplifications with primers specific for ToLCNDV, ToLCBaV, ToLCGuV, ToLCKaV and betasatellites and were negative. Preliminary data generated in the present study indicate the predominant occurrence of TbCSV. Two tomato begomoviruses, ToLCPaV and ToLCRnV, have been reported from the neighboring state of Bihar. Interestingly sequence analysis of ToLCRnV has shown that this virus is a strain or isolate more closely related to TbLCSV (Kumari *et al*., 2011b).

Among begomoviruses, mixed infection and recombination serves as a key factor for evolution of a novel species. Indian tomato-infecting begomoviruses are also well documented for having one or more recombination events for their origin (Padidam *et al*., 1999; Kirthi *et al*., 2002; Chatchawankanphanich and Maxwell, 2002; Chakraborty *et al*., 2003). High probability of recombination, resulting into emergence of a new pathogenic population, has been reported in geminiviruses (Hou and Gilbertson, 1996; Padidam *et al*., 1999). In addition, recombination has also been reported to affect the host specificity leading to mobility to different unusual hosts (Varsani *et al*., 2008). Moreover, the recombination predictions, in which AEV (JX436472), ToLRnV (GQ994095) and TbCSV isolates (HG00365, HQ407395) were detected as probable parents for present TbCSV isolates, indicating that recombination can occur between virus species and isolates during mixed infection.

TbCSV reported first from china infecting oriental tobacco and tomato (Xie *et al*., 2002; Zhou *et al*., 2003; Li *et al*., 2004), occurs in different hosts throughout china such as ageratum (AJ971266, HG003650), *Mirabilis jalapa* (GU199584) and *Alternanthera philoxeroides* (GU199583), pepper (Qing *et al*., 2010), Its occurrence in Northern India was first observed on beans with high incidence at Varanasi, Uttar Pradesh (Venkataravanappa *et al*., 2012), subsequently it was also noticed on wild sunflower (Gen Bank acc.no. HQ407395). It is expected to spread easily throughout the north India and Indian subcontinent, as its land mass is continuous without any obstacles such as mountains or oceans. The peculiar feature of TbCSV is that it is a typical monopartite begomovirus and does not require a betasatellite to infect and produces disease symptoms in indicator hosts or the primary host, but intensifies symptoms in some hosts (Cui *et al*., 2004; Li *et al*., 2005).

Annual crops such as tomato are re-infected with geminiviruses every growing season from sources that must include other crops and weeds, as well as related hosts such as chilli, potato, and brinjal. These plants act as reservoirs of both the viruses and insect vectors in the off season. This annual cycle between crop and reservoir hosts is a stringent bottleneck that potentially

reduces the genetic diversity of the viruses in the crop. Each year only the best adapted viruses survive the bottleneck and spread within the crop. It is only recently that researchers have come to realize that weeds may harbor a far greater diversity of viruses and their satellites, that actually appears in the crop, and that it is possible that genetic changes (including for example component exchange and recombination) within weeds plays a major role in virus diversification. Information on the distribution and prevalence of the different begomovirus species and recombinant forms comprising the disease complex is crucial in guiding tomato breeding programs in the search for stable and durable sources of resistance. This type of study is also an important piece of information to understand natural genetic and population dynamic processes driving the emergence and continuing evolution of this unique virus complex. In addition, Brazil is considered to be one of the major diversity centers of the neotropical bipartite begomovirus cluster. However, few studies are available describing the evolutionary mechanisms associated with the upsurge of so many species and quasi-species in this group of viruses on Solanum hosts in India.

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SPECIAL ARTICLE

Invasive alien species : Problems and the way forward

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ABSTRACT: Increased global trade in agriculture has an unwanted impact in the form of increased chances of the introduction of exotic pests. Papaya mealybug, coconut mite, serpentine leaf miner are the recent most examples of the vulnerability of Indian agriculture to the alien species. This paper takes stock of the situation and draws road map to strengthen quarantine mechanism in the country to prevent the entry of exotic pests. Building public awareness and enhanced coordination among various implementing agencies are considered as important requisites to protect Indian agriculture from invasive pest species.

Keywords: Alien species, exotic pests, plant protection, quarantine

Movement of plants and plant materials to different places commenced with nomadic way of early man and the advancement in navigation and air transport paved way for the accelerated movement of alien plants and animal species to different continents from the place of origin. In a way it enriched species richness world over. However, some of the alien species were harmful replacing the indigenous population or adversely affecting the agricultural crops as pests. The alien species may not be harmful in their place of origin, as the invasiveness is curtailed by influence of many factors of the particular ecosystem. The alien species become invasive in introduced area due to absence of natural enemies and congenial environment parameters. Biological invasions produce severe, often irreversible impact on agriculture, recreation and natural resources. Invasive species are considered as the second greatest threat to native species, only behind habitat destruction. Accidental or intentional introduction of plant pests into newer areas is curtailed to some extent through legal mechanism implemented by the National Plant Protection Organizations (NPPO).

In India, the Directorate of Plant Protection, Quarantine and Storage under Ministry of Agriculture, is responsible for implementation of Destructive Insects and Pests Act, 1914 through Plant Quarantine (Regulation of Import into India) Order, 2003 to prevent entry, establishment and spread of exotic plant-pests into India to safeguard agriculture, horticulture and forest tree plants. Plant Quarantine Stations are established at various points of entry such as seaports, airports and land frontiers to implement the provisions of PQ Order, 2003. The international agricultural trade has increased multifold in the past two decades both in quantity as well as in number of different commodities. However, there is no commensurate increase in the human resource and infra structure facilities to handle the increased agricultural trade. Further, lack of required infrastructure and skilled human resources coupled with porous borders and less public awareness on quarantine pest, has resulted in the entry of many plant pests which are causing devastating damages.

Scientific evidence indicates that biological invasions are growing at an unprecedented rate, posing increasing threats to the diversity of life, and also disrupting native ecosystems. Global economies, as well as water availability, food security and human health are impacted adversely due to pest incursions. India, one among the 12 mega diversity countries in the world, is rich with 8% of world species. The richness of the varied agro-climatic zones and the ecological disturbances are congenial conditions for the invading alien species to thrive.

Recent Pest Incursions into India and the Economic Implications

- **Coffee berry borer, *Hypothenemus hampei*:** Since the time of its first report of occurrence in 1990 in Wayanad of Kerala, the coffee berry borer has spread in the major coffee growing areas in southern states which contribute to 88 Per cent (3,88,000 hectares) of coffee growing areas in India. Annually more than Rs.20 crores are spent towards control measures. European market is the major source for export of coffee from India, ever since

the introduction of coffee berry borer, the export is affected due to pesticide residue & mycotoxin in coffee berries.

- **The coconut eriophid mite, *Aceria gurrreronis* :** It was first noticed in 1997 in Ernakulam District of Kerala and has spread to major coconut growing areas of Tamil Nadu, Karnataka, Andhra Pradesh and Lakshadweep. In a survey conducted in 1999 at Kerala, nearly 42 per cent of plants (about 589 lakh) were affected and estimated yield loss was around 22 per cent. The percentage of reduction in nut weight due to mite infestation was estimated as 2.12 per cent (Sreejith, 2011). India is the third largest coconut producing country in the world and presently the crop is covered in an area of 1.9 million ha with an estimated production of 12.8 billion nuts per annum, which accounts for 22.36 per cent of world production. Annually more than Rs.100 crores are being spent towards management of this pest.

- **Spiralling white fly, *Aleurodicus disperses***: It was first reported in 1993 at Thiruvananthapuram, Kerala and later reported from Tamil Nadu, Andhra Pradesh, Karnataka and Maharashtra. Spiralling white fly is a threat to many crops as 280 plant species are hosts and the cause of 53 per cent yield loss of tapioca and heavy yield loss also observed in groundnut, banana, papaya, guava, chilli, coconut, rubber in India (Mani, 2010). Further, it was observed as a new pest of mulberry in 2010 and causing huge economic damage to silk worm rearing. The sooty mould formation is a secondary infestation due to the honey dew excretion by spiralling white fly, which adds to the owes of infestation and damage.

- **Papaya mealy bug**, ***Paracoccus marginatus***: The infestation was first noticed in 2007 on Papaya, at Coimbatore, Tamil Nadu. By 2009, the pest assumed the major pest status across the country and caused huge damage to mulberry, tapioca, Jatropha, cotton and several fruits, flowers and plantation crops in Tamil Nadu causing 90 per cent damage. There are 60 plant species as hosts for Papaya mealy bug, the pest has spread to almost all ecological niches in India, through transport of infested papaya fruits. Fortunately the pest could be successfully managed through the intervention of classical biological control wherein *Acerophagus papayae* was imported from USA.

- **Cotton mealy bug**, ***Phenococcus solenopsis***: The widespread damage it caused in the country in 2006 has been utmost concern to the entire continent. This species was not known to occur in India or Pakistan prior to the 2005, and it indicates that the pest must have been introduced through planting materials into India. India is a major producer of cotton in the world and cotton cultivation is the livelihood for over 60 million people in India. Cotton mealy bug is a major threat to cotton cultivation all over the country and causing significant economical damage. The pest has invaded all cotton growing areas in the country.

- **Erythrina gall wasp,** *Quadrastichus erythrinae*: is a major invasive pest on *Erythrina* spp. in black pepper plantations of Kerala and Karnataka. The Erythrina gall wasp was first noticed in 2005 and by 2006 spread to all districts of Kerala and Karnataka and also recorded from few districts in Maharashtra.

  Nearly 60 per cent damage of Erythrina plants was observed in Wayanad District of Kerala in 2006. Five species of Erythrina are hosts for this invasive alien species. The damage in Erythrina plants directly affects production of black pepper in these areas as Erythrina plants are used for trailing Black Pepper and Vanilla. The surveys conducted indicate that infestation of *Erythrina* spp. was one of the major constraints for growth and production of black pepper vines in recent years.

- **Eucalyptus gall wasp,** *Leptocybe invasa*: It was first reported in 2001 in Mandya District of Karnataka, and reported to be introduced from Australia through planting material. By 2007, the Eucalyptus gall wasp has spread to Tamil Nadu, Andhra Pradesh, Kerala, Pondicherry, Gujarat, Madhya Pradesh, Uttar Pradesh, Maharashtra, Goa and Delhi. Severe damage observed in 2007-08 and caused seedling mortality in Eucalyptus nurseries, infested fresh seedlings were destroyed in huge numbers to avoid spread. Eleven species of Eucalyptus are hosts for this pest. In India this pest poses threat to 8 million ha of Eucalyptus plantation. This pest is a threat to Paper Industry and forestry areas.

- **Sunflower downy mildew,** *Plasmopara halstedii*: was initially reported in Marathwada region of Maharashtra in 1992, where sunflower is extensively grown. A survey conducted in 1995-96 revealed

that 36.67 per cent of infection in the region (Shirshikar, 2005). Sunflower downy mildew is seed transmitted in nature and has spread to major sunflower growing States such as Karnataka, Andhra Pradesh apart from Maharashtra.

- **Sunflower necrosis virus:** It was first reported in Karnataka as a major problem in sunflower crop in 1997. Now the virus had spread to Maharashtra, Tamil Nadu, Andhra Pradesh, Karnataka infecting sunflower and pulses.

Most of these invasive alien species have got well established and become wide spread in India, due to lack of public awareness on exotic and invasive species and its consequences, absence of stakeholder (importer, exporters, food processing industry and farmers) involvement on risk analysis, pest risk management programme and the absence of official containment and eradication programmes. Bio-invasion by the alien species may lead to economic loss, ecological imbalance, threat to food security, decrease in availability of water and nutrition and disturbances in biodiversity.

Measures to prevent pest incursions: Current scenario

Through implementation of legal mechanism i.e. Plant Quarantine (Regulation of Import into India) Order, 2003, the imported plants and plant materials are regulated and the commodities are inspected, sampled and tested at the port of entry. The perishable live plants, bulbs, rhizomes, cuttings etc., imported for propagation, after completion of inspection at the port of entry are provisionally released to grow under Post-Entry Quarantine (PEQ) facilities established by the importers. There does not exist much stronger linkage from port of entry to PEQ facility and the procedures to be followed by notified Inspection Authority (who need to carry out regular monitoring of imported plants for presence of exotic pests in the PEQ area) during PEQ period. If quarantine pests are intercepted, the consignments are subjected to approved quarantine treatments and if treatment is not an option then the consignments are either deported or destroyed. The weak link between Plant Quarantine & Customs, unregulated import of seeds/plants/bulbs as accompanied baggage in small quantity go unnoticed at airports, has resulted in incursion of many economically important plant pests.

When a new or exotic pest is reported for the first time, no serious thought is given to contain and eradicate the pest through concerted efforts by the stakeholders. Whereas when the same pest assumes an epidemic form, measures are initiated to control the pest to minimize the damage/loss. The agricultural trade is increasing many folds and consequently the looming pest threats are also increasing. Coconut hispid beetle (*Brontispa longissima*) is one of the economically important pests of coconut and is polyphagous, which has already invaded into neighbouring countries such as Maldives and Myanmar, is a looming threat to India. The imported fruits may serve as major pathway of entry for exotic fruit flies, which might severely affect fruit production as fruit flies are polyphagous and can also harm vegetable. This calls for strengthening of plant quarantine system in India through policy, human resource, infra structure enhancement, public awareness and contingency plan to thwart the entry or establishment of the pest.

Contingency/emergency plan to combat pest incursion

There is a need to establish legislation to deal with exotic plant pests as regulatory system can only deal the management of pest incursion effectively. A nationally coordinated system of surveillance, inspection, testing, diagnosis and control using entry and post entry measures are required to prevent the establishment and spread of exotic plant pests that may have harmful effect on plants, human, animals and environment. These activities are responsibilities of Central Government, State/ Union Territory, Research Institutes, Agricultural Universities, Private/ Public sectors, Farmers and Public. At present the biosecurity of the nation is not addressed in a holistic manner with little coordination among various stakeholders. The harmonization and integration among the relevant sectors to deal with pest incursion management shall pave way for preventing the pest's entry, establishment and spread and save the nation from unwanted crop loss as well as expenditure towards control measures.

Need for invasive alien pest incursion management

Past experiences indicate lack of preparedness to combat the invasions of invasive alien pests on emergency basis, leading to entry and establishment of a number of invasive weeds and plant pests in recent years. There is a need to establish Emergency Plant Pest Incursion Management Protocols to combat further invasion of alien species into India that are likely to find their pathway through increased trade. There is a well organized preparedness mechanism in place to carryout monitoring, early warning and control on war-footing to

combat the invasion of locust into India in close cooperation with neighbouring countries. There is a need for such structured mechanism to be in place for managing the incursion of alien invasive plant pests. Further, it is pertinent to identify roles and responsibilities in the eventuality of plant pest incursion, besides enhancing the existing plant quarantine human resource to meet the increase in international trade of agriculture and strengthening the infra structure facilities required for quick detection and diagnosis. There is a need to enhance the capacity of all the stakeholders to ensure effective and timely interventions to prevent / manage pest incursions. The looming threat of invasive species needs to be addressed by having effective contingency / emergency plant pest incursion management plans. This calls for strengthening of plant quarantine system in India through policy, human resources, infra structure enhancement, public awareness and contingency plan to thwart the entry and / or establishment of pests.

Stages of emergency plan

There are many emergency plans in India for taking care in the event of natural calamities or man-made exigencies, however no such coordinated mechanism to address plant pest invasion are in place. In case of plant pest incursion, an emergency incursion management plan needs to be outlined and made available by Government of India (NPPO) for timely implementation to prevent further spread and eradication. This emergency pest incursion management plan is applied as an emergency measure to prevent establishment and/ or spread of an exotic pest following its recent entry. An emergency plan for Plant Pest Incursion needs to have the following phases as basic requirements.

Response Planning Phase: The NPPO is responsible for planning the course of action, identifying roles, responsibilities, funding, human resource, infra structure requirement, compensation (if needed), public awareness etc., in the event of any pest incursion. There is a need to establish a team of experts to give scientific inputs on biology, control measures and containment or eradication parameters, surveillance plan for identifying extent of spread and involve stakeholders for proper understanding of scientific nuances to carry out containment and/or eradication of pest incursion.

Investigation Phase: As soon as the first report of pest incursion is reported, it is the responsibility of the NPPO to take up eradication by involving concerned stakeholders. Surveillance of pest spread needs to be taken up as first step to identify potential distribution of the pest for delimiting / cordoning the area to initiate eradication measure. The appropriate control measures, human resource to handle eradication programme and other requirement to successfully carryout eradication can be planned based on surveillance report for immediate execution of the eradication plan.

Eradication Phase: The eradication process involves the establishment of a management team followed by the conduct of the eradication programme, following the established plan as prescribed by NPPO. The team of emergency management personnel will carry out eradication of the pest through treatment / destroying the plants as deemed fit. Further, it is necessary to prevent entry or exit of plant species which can harbor the pest. Vigilance is the key factor for successful eradication of plant pests.

Withdrawal Phase: The success of eradication of pest incursion can only be ascertained through surveillance for absence of the pest. The NPPO will need to verify the absence of the introduced pests through intensive surveillance in the eradicated area and then can order for withdrawal of control measures and emergency management team. Further, in international trade, the verification and the proof of absence of introduced pest serves as basis for evidence of absence of the specific pest. Otherwise, it will hamper export of agricultural commodities from India.

Review: Throughout the eradication process, the programme needs to be subjected to periodic review to analyze and assess information gathered, to check that objectives are being achieved, and/or to determine if changes are required.

Documentation: The entire emergency pest incursion management process needs to be documented as record, which will serve as model for tackling similar exigencies. The feasibility and economy involved, the resources utilized, the duration of eradication, the success or otherwise will help in formulating future strategies.

Protocol for emergency preparedness

There is a need for legal and institutional framework that clearly mandates basic aspects of prevention and management of invasive alien species. The basic requirements for dealing with alien species are prevention, early detection, eradication, and control.

- **Prevention** of introductions is the first and most cost-effective option. The lessons learnt from

several past experiences of highly destructive and invasive pests such as the spiralling whitefly, papaya mealybug, coconut eriophid mite, coffee berry borer, cotton mealy bug etc are to be taken as the guiding principles for establishing mechanisms for emergency actions in the event of new pest incursions. There are many economically important exotic pests which may likely enter and cause economic impact on many crops including forest ecosystem. Exclusion protocols provide the most efficient way to prevent pests that are likely to enter.

- **Early detection** of a potential invasive species is often crucial in determining whether eradication of the species is feasible. The possibility of early eradication or at least of effectively containing a new coloniser makes investment in early detection worthwhile. Surveillance is an essential component in preventing the establishment or spread of exotic pests.

- When prevention has failed, **eradication** is the preferred course of action. Eradication can be a successful and cost-effective solution in response to an early detection of a non-indigenous species. However, a careful analysis of the costs and likelihood of success must be made, and adequate resources mobilised, before eradication is attempted.

- The last step in the sequence of management options is the **control** of an invasive / exotic species when eradication is not feasible. The aim of control is to reduce the density and abundance of an invasive pest to keep it below economic threshold.

Successful eradication programmes in the past have been based on 1) mechanical control, e.g. hand-pulling of weeds or handpicking of snails or cutting down infested/ infected plants, 2) chemical control and 3) habitat management, (e.g. grazing and burning). However, most eradication programmes need to employ several different methods. Each programme must evaluate its situation to find the best methods in that area under the given circumstances. In comparison with other methods, classical biological control, when it is successful, is highly cost- effective, permanent, self-sustaining and ecologically safe because of the high specificity of the agents used. Biological control is particularly appropriate for use in nature reserves and other conservation areas because of its environmentally friendly nature and the increasing instances of prohibition of pesticide use in these areas. Integrated pest management, combining several methods, will often provide the most effective and acceptable control.

Way forward

Building on the guiding principles agreed to by the Conference of Parties under the Convention on Biological Diversity, the Global Strategy on Invasive Species (Jeffrey *et al.*, 2004) has identified ten strategic responses to guide policy maker to address the growing problem of Invasive Alien Species. The strategies are (i) building management capacity, (ii) build research capacity, (iii) promoting sharing information, (iv) developing economic policies and tools, (v) strengthening national, regional and international legal institutional frameworks, (vi) instituting a system of environmental risk analysis, (vii) building public awareness and engagement, (viii) preparing national strategies and plans, (ix) building invasive alien species issues into global change initiatives and (x) promoting international cooperation. There is a need to develop similar multipronged strategy to prevent the incursion of invasive alien species and if introduced then immediate measures to manage the invasive species. Some of the country specific strategies that can be employed to address the issue are:

Regulatory Framework : There is a need to formulate regulations both at National and State levels which support prevention, containment and eradication of invasive alien species and exotic plant pests. In India, the agriculture, horticulture and forestry are the State subjects. The production, protection and conservation of agriculture, horticulture and forestry resources in each State are managed by respective State Departments. There is a need to review the existing policies and regulations, empower the State Departments of Agriculture, Horticulture and Forestry in regulating the inter-state movement of plants and plant materials to prevent, contain and eradicate exotic and invasive species which may threaten the native plant eco-system. An effective mechanism for information exchange, surveillance, monitoring and early warning systems for prevention, containment and eradication needs to be developed. The NPPO, which is responsible for implementing the regulations at national level, has to secure necessary budgetary support to meet the cost for containment and eradication in the event of pest incursion, including compensation in the event of extreme step of destroying the host plants to contain / eradicate the pest.

Regional Cooperation : India has land contiguity with many neighbouring countries and close proximity to the Island nations of Sri Lanka and Maldives, as a result any new pest incursions in these neighbouring countries may eventually enter into India as pests do not recognize national boundaries. The cooperation with neighbouring countries and harmonization of phytosanitary measures in the South Asia will go a long way in protecting the biosecurity of the Region as well as the Nation. India being a lead country in the region, should take the initiative for identifying the potential pest threats to the region and engage in human resource development in the area of Pest Risk Analysis, Pest Surveillance, Pest Diagnostics and Pest Incursion Management for South Asian Region. Further, in the event of any new pest incursions in the neighbouring countries which may be of potential concern to India's biosecurity, the NPPO of India may have to take proactive role by providing necessary assistance to the neighbouring countries in containment and eradication.

Institutional Framework: The production, protection and conservation of Agriculture, Horticulture and Forestry resources in each State are managed by respective State Departments. Each department do carry out regular surveys and surveillance for various parameters of production, conservation but the component of surveillance for exotic or invasive plant pests need to be included in the regular activities of each department. The States which have land contiguity with neighbouring countries and close proximity to neighbouring island countries to establish 'State Biosecurity Authority / Council' with State regulatory support to safeguard the plant resources from exotic plant pests. There is a need to create District Biosecurity Committee under the chairmanship of District Collector and include the Joint Directors of Agriculture, Horticulture and District Forest Officer and representatives of Biosecurity Cells within the districts as members to review the incidence of any pest epidemics as well as new pest occurrence within their jurisdiction. The District Biosecurity Committee must meet and review the pest situation at least once in every month and submit periodical reports to the State Biosecurity Council. In the event of any new pest sightings the committee must immediately alert the neighbouring District Biosecurity Committee's as well as State Biosecurity Council and NPPO. The State Biosecurity Council must confirm the pest identity in coordination with NPPO, to initiate necessary emergency actions to contain and eradicate the new pest. A Block Biosecurity Committee may be formed involving block level officials of agriculture, horticulture, forestry including farmers, traders, NGOs and industries dependent on agriculture, horticulture and forestry as stakeholders for reporting any exotic pest / invasive species. The representative's of Block Biosecurity Committee should regularly interact and report to the District Biosecurity Committee.

Building Management Capacity: There is a need to establish "emergency preparedness teams" and "rapid response mechanisms" to tackle the incursion of invasive alien species and exotic plant pests. For successful containment, suppression and eradication of invasive alien species, regulatory support and interdepartmental coordination among various stakeholders are the key elements. There is a need for capacity building and networking of biodiversity specialists, environmentalists, plant quarantine specialists to address the issues posed by incursion of invasive alien species and exotic pests. To begin with capacity building efforts should be focused to build basic awareness on threats posed by potential invasive alien species and exotic plant pests among border control officials, quarantine officials, customs, food inspection authorities and stakeholders. The State Agricultural/Horticultural Units along the border areas should be made part of the emergency preparedness/rapid response network. The above officials should be trained to organize regular surveys and surveillance for detection of new pest.

Research Capacity: There is a need to strengthen research capacity to analyse the risks posed by the invasive alien species, risk assessment and risk management. There is a need to develop improved techniques to eradicate and contain invasive alien species through biological control and other environmentally sustainable strategies. Further, identifying the limiting factors which affect the spread of the invasive alien species can prevent its spread. There is a need to develop research in exclusion methods without affecting the trade of agricultural commodities. The "prediction model" and its wide publicity among the stakeholders as awareness tool need to be developed for better awareness and management.

Environmental Risk Analysis: The NPPO must identify the emerging quarantine and invasive pest threats to India through appropriate risk analysis and environmental assessment. It must provide the detailed biology, ecology, host range, morphology supported by photographs of the pest with key identification characteristics and host symptoms of looming pests to

the State Biosecurity Council, District Biosecurity Committee and Block Biosecurity Committees to organize awareness campaigns among the stakeholders to safeguard the Biosecurity through surveillance and early detection. The NPPO must develop guidelines on Do's and Dont's in the event of exotic / invasive pest sightings.

Awareness: The public awareness on invasive species is vital in preventing the entry, establishment and eradication. Creating awareness on harmful effects of invasive species, their economic impact and successful eradication programs carried out elsewhere in the curriculum of schools and colleges in go a long way in enhancing the awareness among the citizens. Regular workshops on looming threats of invasive species involving NPPO, State Biosecurity Council, District Biosecurity Committee and Block Biosecurity Committees will not only create awareness but also enable them in containment and eradication in the event of any new incursions.

CONCLUSION

India is still free from many economically important exotic plant pests and other invasive species. Emergency preparedness to prevent the entry, establishment and to initiate containment and eradication activity in case of pest incursion is needed. There is a well organized preparedness mechanism in place to prevent, control and eradicate locust invasion in the Country. However, this mechanism is confined only to the western borders of India and being implemented by NPPO for locust only. While drawing the lessons from locust control programme, urgent action is required for establishing a similar preparedness action plan for various looming threats of exotic pests and invasive alien species into India.

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SHORT NOTE

Effect of organic manures on the incidence of Asian citrus psyllid, *Diaphorina citri* Kuwayama

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Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is one of the major insect pests of citrus cultivars/groups *viz.*, Nagpur mandarin, *Citrus reticulata* Blanco, sweet orange, *Citrus sinensis* (L.) Osbeck and acid lime, *Citrus aurantifolia* Swingle (Shivankar and Rao, 2010). Both nymphs and adults suck the vital plant sap from young shoots and cause heavy de-blossoming, thereby seriously affecting the fruit set. The psyllid is also known to transmit the disease, huanglongbing (HLB), *Candidatus* Liberibactor asiaticus (Bove, 2006). It is active during spring and in dry spells during monsoon (Shivankar *et al.*, 2001). The ACP is important as a pest on Nagpur mandarin and as a vector of HLB on sweet orange (Das *et al*., 2002). The epidemics of *D. citri* on Nagpur mandarin were reported in central India during 1960-62, since then ACP has attained endemic pest status causing considerable loss (Shivankar and Rao, 2010). At present, the most common practice for management of ACP is through foliar application of insecticides particularly organo-phosphates, carbamates and neo-nicotinoids (Batra *et al*., 1990, Kalidas and Shivankar, 1994, Dahia *et al*., 1994, Singh *et al*., 1995, Patel *et al*., 1998, Chakravarthi *et al*., 1998, Rao and Shivankar,2010). However, safer and effective alternative methods are the need of the hour to contain ACP on sustainable basis. In this context, cultural methods like organic manuring play an important role in containing ACP in Nagpur mandarin orchards of central India. Therefore, the present study was conducted to assess the effect of organic manuring on ACP population which may play a vital role as one of the important components in the development of IPM module.

Effect of different organic manures *viz.*, farm yard manure (FYM) at the rate of 20 kg/tree, vermicompost at the rate of 10 kg/tree, poultry manure at the rate of 10 kg/tree, green manuring with cow pea, *Vigna unguiculata* (L.) and sun hemp *Crotalaria juncea* L. along with in-organic fertilizers (300g N, 100g P, 50g K/tree) in a 12 year old orchard of Nagpur mandarin on the incidence of *D. citri* conducted during 2010, 11 and 12 at Experimental Farm of National Research Centre for Citrus, Nagpur. Sun hemp and cowpea plants were sown in the basin of the Nagpur mandarin tree during rainy season and grown-up plants on reaching flowering stage were incorporated in to the soil. The experiment was laid out in completely randomized block design and each treatment replicated five times. Each replication consisted of two trees. Observations on psyllid population/5cm twig from two twigs on each side covering all the four directions of the tree, were recorded at fortnightly intervals during spring 2010, 2011 and 2012. The data were transformed to square root values and were subjected to analysis of variance.

Effect of organic manuring on ACP population showed that among the organic manuring treatments, ACP population was significantly low in vermicompost (15.91-18.55 population/5cm twig) than other treatments but was at par with FYM (16.38-19.46 population/5cm twig) during 2010 and 11. In all the three years, ACP population was significantly high in in-organic fertilizer treatment (24.0-36.36 population/5cm twig) than organic manure treatments (15.91-33.34 population/5cm twig) (Table 1). The results are in congruent with Ravi *et al.*(2006) who reported reduced incidence of leafhopper, *Amrasca biguttula biguttula* (Ishida) and whitefly, *Bemisia tabaci* Genn. in vermicompost treated Sunflower (*Helianthus annuus* L.). The low ACP populations in vermicompost treatment are probably due to the accumulation of more Potassium in soil as well as leaves of the Nagpur mandarin trees treated with vermicompost over the years (Anonymous, 2012). Further, increased levels of potassium fertilizer in Valencia orange (*Citrus sinensis* Blanco) plants grown under green house conditions resulted in decreased fitness (psyllid weight, egg production, development time) of psyllid population (Rogers, 2010). The organic vermicompost amendment

Table1. Effect of organic manures on the incidence of Asian citrus psyllid, *Diaphorina citri*

Treatment	No. psyllid nymphs/5 cm twig			
	2010	2011	2012	Pooled mean
FYM @ 20 kg/tree	16.38 (4.08)[a]	19.46 (4.40)[a]	23.30 (4.80)[b]	19.71 (4.42)
Vermicompost @ 10 kg/tree	15.91 (4.00)[a]	18.55 (4.27)[a]	18.10 (4.24)[a]	17.52 (4.17)
Poultry manure @ 10 g/tree	16.58 (4.10)[b]	27.18 (5.30)[b]	32.72 (5.71)[d]	25.49 (5.03)
Green manuring with cowpea	17.31 (4.19)[b]	27.67 (5.28)[b]	31.74 (5.63)[c]	25.47 (5.03)
Green manuring with sunhemp	17.73 (4.24)[b]	29.89 (5.30)[bc]	33.34 (5.77)[e]	26.65 (5.10)
Fertilizer 600g N+200g P+100g K	24.00 (4.92)[c]	32.22 (5.69)[c]	36.36 (6.02)[f]	31.86 (5.54)
SEd+	0.14	0.30	0.29	-
CD (p = 0.05))	0.30	0.64	0.32	-

Figures in parentheses are square root transformed values
Values followed by same letter in a column are not significantly different

probably increased the total phenol content and also the activity of enzymes *viz*., polyphenol oxidase and peroxidase in Nagpur mandarin trees which might be responsible for the reduced ACP incidence. The present study generated useful information on ACP population reduction in spring flushes of Nagpur mandarin through application of vermicompost at the rate of 10 kg/tree during rainy season which may well be taken in to consideration as an important component of IPM for the management of ACP in Nagpur mandarin orchards of Central India.

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SHORT NOTE

Population dynamics of sapota fruit mite, *Tuckerella kumaoensis* Gupta (Acari:Tuckerellidae) in Gujarat, India

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Sapota, *Manilkara achras* (Mills) Foseberg commonly also known as *Chiku* or Saposilla is a slow growing evergreen tree. India ranks first in the world in sapota production (14.24 lakh MT). In Gujarat it is mainly cultivated in South Gujarat in general and Navsari and Valsad districts in particular. Gujarat is one of the largest sapota producing states in India with an area of a 28,800 ha and an annual production of 3, 08,704 M.T., which accounts for 20 per cent of total sapota production of total sapota production in India (Patel *et al.*, 2013). Earlier, sapota was not much affected by insect pests and diseases but incidence of many insect pests has been reported during last two decade. The pest complex of sapota also includes a mite, *Tuckerella kumaoensis* Gupta and it affects the fruits of the sapota. The red coloured mite feeds on fruit surface from marble size fruit stage, resulted the fruit surface rough and thereafter suck the cell sap. The affected fruit surface becomes rough or black or dark coloured which results in qualitative loss of harvested products. For the effective management of any pest it is very essential to know its seasonal incidence. Considering the importance of the mite pest in sapota the present experiment was designed.

Population dynamics of the fruit mite in relation to weather parameters were carried out at Fruit Research Station, Navsari Agricultural University, Gandevi on sapota cv. Kalipatti during 2009-10 to 2011-12. For this purpose, ten trees were randomly selected from untreated orchard of sapota. Ten fruits from each tree were observed at weekly interval. The adult as well as immature stages has been counted from the basal region ($2cm^2$) of the fruit. The mite were observed under stereoscopic microscope. The mite population recorded was correlated with various abiotic factors *viz.*, maximum temperature, minimum temperature, average temperature, and morning, evening and average relative humidity for individual years as well as with three year pooled data. It is evident from the Table-1 that during 2009-10, the fruit mite incidence started from the 45th standard meteorological week (SMW) and observed through out the year. The population of fruit mite fluctuated at various time intervals. It showed two peaks during 2009-10, the first peak during 51st SMW (5.89 mites per fruit) and letter on it was again increased during 14th SMW i.e., April and reached to the maximum level i.e. 7.22 mites per fruit during 18th SMW. Then the population gradually decreased. During 2009-10 the mite population showed a significant positive correlation with maximum temperature (r=0.5519), while it was negatively correlated with minimum temperature (r=-0.4491), morning RH (r=-0.6935) and evening RH (r=-0.6988). During 2010-11, these data revealed that the *T. kumaoensis* population gradually increased and it has two peaks, the first is first during 1st SMW, first week of January where the mite population were 7.21 mites per fruit. Further, a second peak was also observed during 9th to 22nd SMW (February to June). The mite population during this period ranges 4.67 to 7.67 per fruit. The highest mite population per fruit was 7.67 in 20th SMW. The correlation studies revealed that the mite population showed a significant positive correlation (r=0.283) while negatively correlated with minimum temperature (r=-0.3412), morning RH (r=-0.7086) and evening RH (r=-0.6135). further, in the year 2011-12 the data showed that the mite were active round the year with various population levels. The mite population gradually increased from 6th SMW (5.00 mites per fruit) and reached maximum during 20th SMW. Then the population declined and it was nil during 31st to 36th SMW. Then again it increased. The correlation studies suggested that the mite population had a significant positive correlation (r=0.5066) with maximum temperature while it was negatively correlated with minimum temperature (r=-0.163), morning RH (r=-0.5619) and evening RH (r=-0.5083).

The pooled data of three years revealed that the fruit mite was active throughout the year and its population fluctuated at various time intervals. The mite population

Table 1. Population dynamics of sapota fruit mite, *Tuckerella kumaoensis* Gupta

Std. Week	No. fruit mites /2 cm²			
	2009-10	2010-11	2011-12	Pooled data
45	2.63	1.36	2.31	2.10
46	2.90	1.50	3.00	2.47
47	3.76	1.79	2.35	2.63
48	3.88	1.90	3.75	3.18
49	3.49	2.72	1.95	2.72
50	3.67	2.93	3.10	3.23
51	5.89	3.68	2.67	4.08
52	5.80	4.45	1.50	3.92
1	5.17	7.21	0.50	4.29
2	4.76	5.45	0.25	3.49
3	3.42	5.55	0.25	3.07
4	3.70	5.00	0.50	3.07
5	4.45	2.10	3.75	3.43
6	4.89	3.00	5.00	4.30
7	5.11	3.15	5.50	4.59
8	4.89	2.33	4.75	3.99
9	4.28	5.11	4.70	4.70
10	4.10	6.10	4.90	5.03
11	3.58	5.15	5.10	4.61
12	4.25	4.67	4.70	4.54
13	4.12	5.21	5.10	4.81
14	5.62	5.67	5.50	5.60
15	5.40	5.85	5.50	5.58
16	5.47	5.95	6.25	5.89
17	5.90	6.17	6.70	6.26
18	7.22	7.67	7.00	7.30
19	6.45	7.15	7.50	7.03
20	6.18	7.67	8.00	7.28
21	6.00	6.70	7.70	6.80
22	3.71	5.77	5.75	5.08
23	4.00	4.67	5.50	4.72
24	4.42	4.77	5.50	4.90
25	4.00	3.75	5.50	4.42
26	2.28	3.11	3.25	2.88
27	1.40	1.67	2.00	1.69
28	0.40	2.75	2.00	1.72
29	0.27	1.11	1.25	0.88
30	0.22	1.13	0.45	0.60
31	0.19	1.15	0.00	0.45

32	0.00	0.00	0.00	0.00
33	0.00	0.00	0.00	0.00
34	0.00	0.00	0.00	0.00
35	0.00	0.50	0.00	0.17
36	0.00	1.50	0.00	0.50
37	0.00	1.25	0.50	0.58
38	0.00	2.30	0.50	0.93
39	0.00	0.50	0.50	0.33
40	0.00	0.50	0.50	0.33
41	0.00	0.00	0.00	0.00
42	0.00	1.75	0.00	0.58
43	0.00	1.45	1.75	1.07
44	0.00	1.00	2.50	1.17

Table 2. Correlation of sapota fruit mite, *T. kumaoensis* Gupta with abiotic factors

Abiotic Factor	fruit mite density/2 cm^2			
	2009-10	2010-11	2011-12	Pooled data
Maximum Temperature	0.5519 **	0.2836 **	0.5066 **	0.4194**
Minimum Temperature	-0.4491 **	-0.3412 **	-0.1632 **	-0.3040 **
Average Temperature	0.1017 ^{NS}	-0.1095 ^{NS}	0.1238 ^{NS}	-0.0240 ^{NS}
Morning R.H.	-0.6935 **	-0.7086 **	-0.5619 **	-0.6256 **
Evening R. H.	-0.6988 **	-0.6135 **	-0.5083 **	-0.5924 **
Average R.H.	-0.7768 **	-0.7067 **	-0.575 **	-0.6640 **

Table 3. Multiple regression equation to predict population buildup of *T. kumaoensis* on the basis of weather parameters

Year	Regression Models	R^2	Multiple R
2009-10	Y= 17.8528 + 0.1193 (X_1) - 0.1461 (X_2) + 17.6748 (X_4) + 17.8267 (X_5) - 35.6884 (X_6)	63.38	81.84
2010-11	Y= 24.0075 + 0.0474 (X_1) - 0.0754 (X_2) + 24.4888 (X_4) + 23.6955 (X_5) – 47.4250 (X_6)	57.24	78.38
2011-12	Y= 6.257 + 0.2926 (X_1) - 0.1498 (X_2) + 20.6532 (X_4) + 20.7939 (X_5) – 41.5951 (X_6)	48.57	72.53
Pooled data	Y= 12.4468 + 0.1228 (X_1) - 0.0085 (X_2) + 22.9592 (X_4) + 23.0495 (X_5) – 46.1771 (X_6)	51.19	72.64

Y=a+bx, where, Y =mite population and X_1, X_2, X_4, X_5 and X_6 are maximum temperature, minimum temperature, morning RH, evening RH and mean RH, respectively.

gradually increased during 14th SMW (5.60 mites per fruit) and reached to its peak in 18th SMW i.e., 7.30 mites per fruit and then gradually shows a declining trends. In mid August (32nd to 34th SMW) no mite was recorded. The correlation studies of the three years pooled data suggest that the fruit mite population showed a significant positive correlation (r= 0.4194) with maximum temperature, whereas it has a negative correlation with minimum temperature (r= -0.3040), morning RH (r=-0.6256) and evening RH (r=-0.5924). The multiple regression equation developed from all three years data as well as from pooled data is also presented in Table 3. During 2009-10, the regression analysis revealed that total impact of weather parameters on population buildup of *T. kumaonensis* was 63.38 per cent, whereas for 2010-11 it was 57.24 per cent and for 2011-12 the R^2 value is 48.57 per cent. The R^2 value for the pooled data was 51.19 per cent. Patel (1997) also reported that *T. kumaonensis* showed highly significant positive correlation with maximum temperature, non-significant negative correlation with minimum temperature, average temperature and morning relative humidity and a highly significant negative correlation with evening relative humidity and average relative humidity at Navsari. Under the present study the *T. kumaonensis* also showed a highly significant positive correlation with maximum temperature, while it showed a significantly negative correlation with minimum temperature, morning evening and mean relative humidity. Further, he also reported 61.09 per cent variation in the buildup of mite population due to weather parameters at Navsari. The present findings also showed similar trends and based on these findings it is possible to predict mite infestation and device suitable management practices.

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Pest Management in Horticultural Ecosystems,
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SHORT NOTE

Occurrence of rhinoceros beetle, *Oryctes rhinoceros* (L.), on banana cultivars in Kerala

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The coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera:Scarabaeidae) is one of the most damaging pests of coconut and African oil palm in south and south-east Asia and the Western Pacific islands. Adults of *O. rhinoceros* are 30-50 mm long and 14-21mm breadth, black or reddish black in colour, stout and possesses a characteristic cephalic horn which is larger in males. The pygedium is densely clothed with reddish brown hairs on the ventral surface in the female (Nirula *et al.,* 1952), a feature which helps in distinguishing it from the male. The adults are the destructive stage, they bore into the crown of the palm resulting in wedge shaped or "V" cuts in the fronds that unfurl. The beetle feeds on tissue juices. Some of the crushed fibre is pushed outside the entrance hole, where it indicates the insect's presence. Orian (1959) reported one third of seedling palms destruction by rhinoceros beetle in Chagos Islands. Ramachandran *et al.* (1963) has reported a loss in yield of 5.5 to 9.1 per cent in coconut due to beetle attack. In India, damage of inflorescence is also reported in severely infested areas which cause reduction in yield up to 5.7% (Nair *et al*., 2002). Attack by adults reduce yield and destroy seedlings. They provide entry points for lethal secondary attacks by the palm weevil, *Rhyncophorus* or by other pathogens (Bedford, 1980). Apart from coconut and African oil palm, other host plants of *O. rhinoceros* include the date palm, arecanut palm (Nair, 2002) and a variety of palms grown for ornamental purpose, including *Roystonea regia*, *Livistona chinensis*, *Corypha umbraculifera* and *Raphia ruffia* (Gressitt, 1953; Bedford, 1980) *Arenga*, *Borassus*, *Corypha*, *Elaeis, Metroxylon*, *Nypa*, *Oncosperma* and *Phoenix* (Lever, 1969). Fletcher (1914) listed out aloes and sugarcane apart from palms as host and Sadakathulla and Ramachandran (1990) recorded sugarcane as host of the pest. The damage caused by this pest on non palm hosts was not causing serious concern so far.

Regular pest surveillance was being carried out to identify any new pest outbreak, pest status change etc. by a team of subject experts in farmers' plots throughout the year by Krishi Vigyan Kendra - Alleppey, Kerala. Special field surveys were undertaken during 2007-2009 in various locations, in Southern State, Kerala, following frequent field reports from farmers on occurrence of 'big beetles' in banana (*Musa* sp.). Subsequent field observations on banana planted as an intercrop as well as monocrop in coconut gardens revealed infestation by rhinoceros beetle, *O. rhinoceros* which was not noticed in any of the field surveys conducted during the last ten years.

The adult beetles of *O. rhinoceros* were found attacking banana plants of various ages from four to six months after planting. It was first noticed in Nooranadu village of Alleppey District, Kerala, India and later at different locations of the district *viz*., Chenganoor, Kayamkulam and Ambalappuzha during 2007-2009. Subsequent observations during 2010-2012 revealed *O. rhinoceros* damage on banana from different parts of South India.

The infestation was first noticed in south part of Kerala, India. More than ten thousand plants were observed at random from different locations from June 2008 to January 2012. External symptoms of beetle attack appeared as a cut/chewed wound on the banana pseudostem (Fig.1) very similar to symptoms caused by *Scapanes australis*. On close examination, the damaged pseudostem revealed tunnels made by the pest resembling the damage by the pest on coconut spindle leaf, and the beetles were found feeding inside the tunnel (Fig.2). In coconut, beetle cut and chews the spindle leaf and occasionally inflorescence. Tunnels had an average depth of 2.0cm with a horizontal diameter of 1.7cm and vertical diameter of 4.3 cm (Fig. 3). The tunnelling and chewing symptoms were noticed on the pseudostem at different heights i.e., 15, 83, 90, 95,110,134,155cms, above the ground level. Percentage infestation in different plots varied from 2.5 to 4.7%. Repeated attack on the same

Fig.1. Initial damage symptoms of *O. Rhinoceros* on banana

Fig. 2. Rhinoceros beetle damage on banana pseudostem

Fig. 3. Entry hole on pseudostem

plant was not observed, but recurrence of infestation was recorded in the same plot. Infestation was noticed only in 'Nendran' (genome group AAB) and 'Njalipoovan' (Genome group AB) varieties of banana indicating the varietal preference by the pest (Table 1). Other varieties such as 'Palayankodan (AAB)', 'Robusta (AAA)' and 'Red banana (AAA)' cultivated near to the infested plots were free from infestation. The major banana pest of the region, pseudostem weevil, *Odoiporus longicollis* (Coleoptera: Curculionidae) also showed maximum preference to 'Nendran' variety as reported by Visalakshi *et al.* (1989).

Severely damaged plants toppled down and found more prone to wind damage. In infested banana plants where entry of the pest was near the leaf axil, leaves were found drooping down. Symptoms of infestation and damage by *O. rhinoceros* to the tune of 4-20% were noticed in coconut palms adjacent to the infested banana plots. The pest attack on banana was noticed both in monocropping as well as intercropping under coconut. This is the first field report of *O. rhinoceros* infestation on banana plants as a pest. The sole report of rhinoceros beetle's occurrence on banana dates back to fifties (Nirula *et al* ., 1952). Since then there have been no records on banana being attacked by *O. rhinoceros*. Padmanabhan and Sudararaj (1999) and Padmanabhan *et al*. (2001) reported several coleopteran pests on banana but there was no mention of rhinoceros beetle. Melanesian rhinoceros beetle, *Scapanes australis* was recorded as a main pest of juvenile coconut palms in Solomon Islands (Bedford, 1976). The adult beetles were found boring into the stems of banana plants in different parts of Papua New Guinea (Szent-Ivany and Barrett, 1956; Mararuai, 2010). But *Scapanes* was not reported from India. This is the first detailed report on the occurrence, extent of damage and varietal preference of *O. rhinoceros* on banana in India. It warrants a constant vigil on the movement of rhinoceros beetle to banana plantations wherever they are cultivated near coconut plantations.

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Table 1. Infestation of *O. rhinoceros* on banana cultivars at different locations

Cultivar	Infestation (%) of *O.rhinoceros* on banana plants			
	Nooranadu	Chenganoor	Kayamkulam	Ambalappuzha
'Nendran' (AAB)	3.5	4.7	2.6	2.5
'Njalipoovan' (AB)	3.0	3.8	2.5	3.2
'Red banana' (AAA)	0.0	0.0	0.0	0.0
'Palayankodan' (AAB)	0.0	0.0	0.0	0.0
'Robusta' (AAA)	0.0	0.0	0.0	0.0

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SHORT NOTE

Management of spiraling whitefly, *Aleurodicus dispersus* (Russel) in guava, *Psidium guajava* L.

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The spiraling whitefly (SWF), *Aleurodicus dispersus* (Russel) is a polyphagous pest with a characteristic spiraling pattern of oviposition on the underside of leaves. The pest is native to Central America, the Caribbean region and the Pacific islands. It was first observed in Florida in 1957 (Russel, 1965) and was reported in Hawaii on the Oahu island in 1978 (Paulson and Kumashiro, 1985). Since then it started to spread rapidly to most tropical regions of the world. It was reported from India in 1993 from Kerala (Palaniswami *et al.,* 1995) and later from other parts of peninsular India (Reddy and Chandurkar, 1999). Spiraling whitefly has extensive host range covering 481 plants belonging to 295 genera from 90 families of vegetables, fruits and ornamentals trees (Srinivasa, 2000). Important hosts reported so far are citrus, avocado, guava, plantain, banana, coconut, soybeans, cassava and stone fruit (John *et al.,* 2007). It damages plants by sucking the sap of leaves and excreting a sticky honeydew and white waxy flocculent substance which affects the photosynthesis and overall appearance of the plants (Mani and Krishnamoorthy, 1999; Chien *et al.,* 2002). A loss of 80 per cent in fruit yield has been recorded in guava attacked by the pest in four continuous months in Taiwan (Wen *et al.*, 1995).The management options for *A. dispersus* include cultural, biological and chemical methods. Of these, the chemical control is recommended to reduce the whitefly populations during sensitive crop growth stages to minimize the yield losses. Some chemicals such as tobacco extract (4%), neem oil (2%), fish oil rosin soap (4%) and detergent soap solution (5%), malathion (0.1%) and carbaryl (0.1%), dichlorvos (0.08%), triazophos (0.08%) were found to be effective in suppressing the nymphal and adult whitefly population (Ragumoorthy and Kempraj, 1996; Ranjith *et al.,* 1996; Asia Mariam, 1999; Muralikrishna, 1999; Geetha, 2000; Mallapanavar, 2000). Earlier, λ-cyhalothrin has been found effective against pupae and adults of *A. dispersus* (Yao *et al*., 2008). However, guava being frequently harvested crop, application of chemical insecticides may warrant sufficient waiting periods that are otherwise not observed by many growers. The present investigation was carried out to find out the best options to bring down the populations of *A. dispersus* with eco-friendly botanical formulations.

The study was conducted at the experimental farm of the Indian Institute of Horticultural Research, Hessaraghatta (12° 58' N; 77° 35' E), Bangalore on 25 years old trees of guava (cv. Allahabad Safeda) during December 2012. Treatments *viz*., triazophos at 1.5 mL/ L, neem soap at 10 g/L, neem guard at 5g/L, λ - cyhalothrin at 0.5 mL/ L, dichlorvos at 1 mL/ L, pongamia oil (PO) at 10g/L, dichlorvos + PO, pongamia soap at 10 g/L and water spray were evaluated against spiraling whitefly *A. dispersus* along with untreated check. Guava trees infested with whiteflies were selected for administering the treatments. Ten leaves were randomly collected from each treated tree and each leaf is served as replication. The detached leaves were carefully kept in polythene covers and tied with rubber band to prevent any escape of whiteflies. The polythene covers were brought to laboratory and observations on number of eggs, nymphs, pupae and adults per leaf were recorded under stereo microscope. Post- treatment observations were recorded at 24 h, 48 h, 96 h, 7 days, 14 days and 21 days after spraying (DAS). Data were subjected to ANOVA using '*F*' test criteria at p=0.05 level.

The results pertaining to the management of spiraling whiteflies on guava using botanicals and insecticides are given in the Fig 1. At 24 hr after the spray neem guard, neem soap, Dichlorovas with Pongamia oil was found to be superior in reducing the pupal population significantly ($F= 3.05$; $df=9$; $p<0.003$) compare to the other treatments. Whereas, after 48 hr of post treatment reduction in nymphal ($F = 3.42$; $df=9$; $p<0.001$), pupal

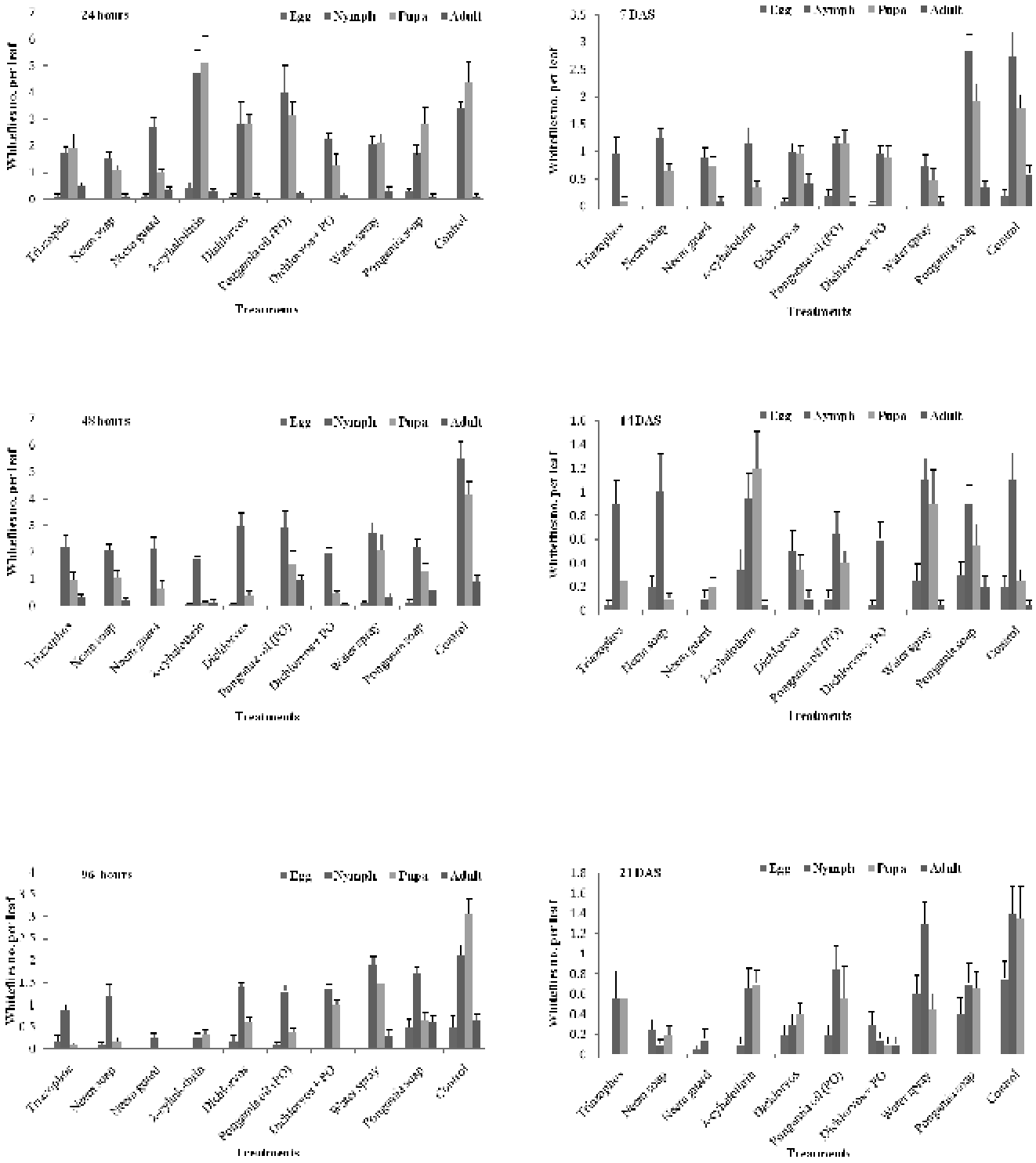

Fig. 1. Effect of different treatments on various life stages of *A. dispersus*

(F= 5.67; df=9; p<0.00) and adult (F= 4.63; df=9; p<0.00) populations are significantly different. Among all the treatments λ-cyhalothrin found to be superior in reducing nymphal and pupal population of whiteflies, whereas, neem guard found to be superior in reducing adult population. At 96 hr after the spray reduction in nymphal (F= 6.91; df=9; p<0.001), pupal (F= 16.65; df=9; p<0.00) and adult (F= 4.85; df=9; p<0.00) populations are found to be significant. Among all the treatments neem guard was found to be superior in reducing the nymphal and pupal populations, adult populations were found to be low in all the treatments except in water spray and pongamia soap when compared to control. At 7 days after spray (DAS) there was significant difference in the reduction of nymphal (F= 4.69; df=9; p<0.00), pupal (F= 4.33; df=9; p<0.00) and adult (F= 2.49; df=9; p<0.01) populations among the treatments. Triazophos, neem guard and water spray found be superior in reducing whiteflies population when compared to the control. At 14 DAS only pupal (F= 2.62; df=9; p<0.01) population was found to be significant among the treatments. Dichlorvos with pongamia oil and neem soap was found to be effective in reducing the pupal population compare to the other treatments. At 21 DAS only nymphal (F= 3.009; df=9; p<0.00) population was found to be significantly different among the treatments. Neem soap, neem guard and Dichlorvos with pongamia oil was found to be effective in reducing the nymphal population.

Whiteflies develop rapidly in warm weather, and populations can build up quickly in situations where natural enemies are destroyed and weather is favorable. Thus, whiteflies are difficult to manage once their populations have reached high levels and further repeated applications of a product can lead to development of high resistance levels. For sustainable management of whiteflies, the spray interventions should take place at low levels of whitefly population. The present study was carried out to understand which treatments will have immediate effect on different developmental stages of whitefly, when its populations are at their lowest.

With respect to egg stage, there was no significant difference between the treatments in causing egg mortality implicating, at low population level even a water spray can bring down the egg number. Incase of nymphal population, the botanicals *viz.*, neem soap, neem guard, pongamia oil and pongamia soap along with water spray found to be either superior or on par with other chemical treatments in bringing down the *A. dispersus* nymphal population at different intervals after spray. Similar results were also reported in case of pupal number also. In case of adult population of *A. dispersus*, there was no significant difference among the treatments at different intervals indicating the superiority of botanicals in bringing down the *A. dispersus* incidence at low population levels. Earlier studies also reported that spraying of neem oil (2%), neem seed kernel extract (3%), fish oil rosin soap (4%) and detergent soap solution (5%) were found to be effective in suppressing the nymphal and adult whitefly population (Ranjith, 1996; Asia Mariam, 1999, Kavitha Kirubavathy *et al*., 1999 and Geetha, 2000). In addition, in the present investigation the pongamia products *viz*., oil and soap were also found effective in reducing the whitefly population. These findings clearly indicate that in context of integrated management of *A. dispersus*, botanical based formulations *viz*., neem, pongamia will serve as effective management interventions at low population levels.

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SHORT NOTE

Host range of invasive Jack Beardsley mealybug, *Pseudococcus jackbeardsleyi* Gimpel and Miller in Karnataka

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The movement of invasive mealybugs (Hemiptera: Pseudococcidae) which are major pests of a wide range of agricultural, horticultural and ornamental plants worldwide has been documented by Muniappan (2011). As envisaged in the paper after the invasion of papaya mealybug, and *Phenacoccus madeirensis* (Madeira mealybug), the Jack Beardsley mealybug was recorded in India (Shylesha and Joshi, 2011, Mani *et al.*, 2012). Certain of attributes of the Pseudococcidae, *viz*., wide host range, short generation time, cosmopolitan nature, ability to transmit some important plant viruses, etc., have contributed to their enormous damage potential (Meyer *et al.*,2008). In this regard, *Pseudococcus jackbeardsleyi* Gimpel and Miller (Hemiptera: Pseudococcidae), known as the Jack Beardsley mealybug, a polyphagous species of neotropical origin commonly occurring in Caribbean and Central and South America, that is known to attack 93 plant species including several vegetable and fruit and ornamental crop species (CAB Intl., 2001) has entered India infesting several of crop plants. The invasive mealybug is greyish in colour; thin filaments around the body, caudal pair about one half of the length of the body, and ovisac covering hind part of the body (Williams 2004a). The presence of ovisac differentiates it from *Pseudococcus longispinus* (Targioni Tozzetti). Morphological details of the *P. jackbeardsleyi* occurring in India are given by Mani *et al.*, (2012).

Survey for invasive insects in southern parts of India revealed the occurrence of *P. jackbeardsleyi* in Tamil Nadu, and Karnataka. It was found associated with papaya mealybug on papaya (Fig.1) at Ravindranath Tagore Nagar in Bangalore. Other plants in the area like *Cordyline terminalis* (Agavaceae), an ornamental plant native to Southeast Asia, Australia, New Zealand was found to harbour *P. jackbeardsleyi*. The nymphs were found scattered on the leaves singly, similarly it was found on flowers of custard apple (*Anonna squamosa),* Purple martin (*Streptocarpus* sp.) Jasmine (*Jasminum multiform*) in pure form (Fig. 3 and 4). Along with papaya mealybug, *Paracoccus marginatus*, it was found in papaya, tapioca, chrysanthemum and Indian spinach (*Basella alba*) (Fig. 3 and 4). It is associated with *P. solenopsis* on parthenium and chrysanthemum. In some crops it was associated with aphids and spiralling whiteflies as in case of basil, chrysanthemum (Fig. 2) and jasmine. The Jack Beardsley mealybug is distributed throughout the Neotropical region and a few countries in southern Asia (Williams and Watson, 1988).

Jack Beardsley mealybug is a polyphagous species known to attack 93 plant species including several vegetable and fruit and ornamental crop species (CAB Intl., 2001). United States quarantine department recorded on a wide diversity of hosts from annuals such as peppers, eggplant, and tomatoes to many tropical fruit trees, and tropical shrubs, and ornamentals. It has been recorded on more than 35 host plant families at quarantine

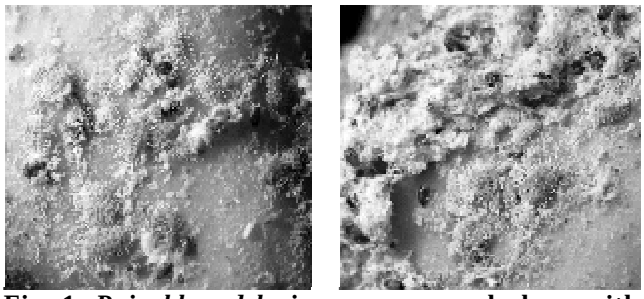

Fig. 1. *P. jackbeardsleyi* on papaya and along with *P. marginatus*

Fig. 2. *P. jackbeardsleyi* on *chrysanthemum* along with *P. solenopsis* and aphids

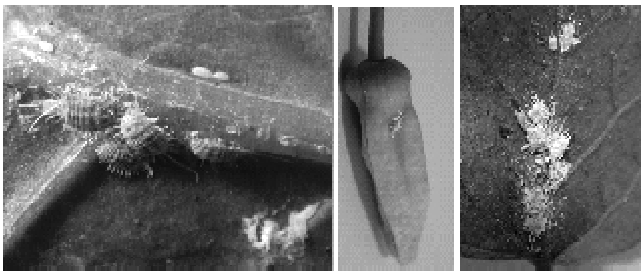

Fig. 3. *P. jackbeardsleyi* on *Basella alba*, Anonna flower and jasmine

Fig. 4. *P. jackbeardsleyi* on *Streptocarpus* sp. (purple martin), tapioca and *Cordyline* sp. plants

interceptions at US ports. Importation consignments of fresh potatoes from Mexico were found to contain *P. jackbeardsleyi* (USDAAPHIS, 2003). As many as 22 plant species have been reported as hosts of *P. jackbeardsleyi* in Asian countries (Williams, 2004a, b) including banana, *Aglaonema, Dieffenbachia*, tomato, potato, pepper, *Hibiscus*, *Anthurium*, orchids, floral ginger, *Annona, Dracaena*, and ivy gourd. It was originally identified as the banana mealybug in Hawaii (Beardsley, 1986).

Ever since the first report of this invasive mealybug, the host range is expanding day by day in India. Several of the host plants of *P. jackbeardsleyi* are economically important. As in case of the other invasive species observed *viz.*, *P. solenopsis* and *P. marginatus*, in the beginning the establishment was on weeds and ornamental crops was fast and co existence with several other sucking pests was observed. This invasive mealybug is a very slow establishing species and is expanding slowly. Some of the local natural enemies like *Cryptolaemus montrouzieri*, *Spalgis epius* and some species of gnats are keeping the spread under check.

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SHORT NOTE

Oviposition preference of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) to date palm cultivars

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Date palm (*Phoenix dactylifera* L.) is one of the oldest fruit trees of the world and is closely associated with the life of the people in the Middle East since ancient times. The Kingdom of Saudi Arabia produces over a million tones of dates annually from an estimated 25 million palms (FAO, 2010). The crop has a high socioeconomic importance not only due to its food value, but also its capacity to provide shelter, fiber, aesthetic beauty and furniture (Mousavi *et al*, 2009). Worldwide, 2000 or more date cultivars are known to exist (Ali-Mohamed and Khamis, 2004). More than 400 different date palm cultivars are reported from Saudi Arabia (Anonymous, 2006). With an estimated three million palms, the Al-Hassa oasis (25^{O}19' 60"N latitude and 49^{O} 37' 60" E longitude) in the Eastern Province is the largest date palm grown area in the Kingdom. The crop is attacked by several insect pests of which the red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier), (Coleptera: Curculiondae) is the most serious one. It has been identified as a category-1 pest of date palm in the Middle-East by the Food and Agriculture Organization of the UN and is reported from over 50 per cent of the date growing countries worldwide (Faleiro, 2006). RPW was first recorded in the Middle East from Rass-El-Khaima in the United Arab Emirates during 1985 from where it spread to all the Gulf countries infesting 5 to 6 per cent palms in the region (Zaid *et al.,* 2002). The RPW has a wide geographical and host range and is reported to infest 40 palm species worldwide (Anonymous, 2013). Studies carried out in China (Ju *et al.*, 2011), showed that *Phoenix canariensis* and *Washingtonia filifera* were more suitable hosts for RPW while *P. sylvestris* was the least preferred. Barranco *et al*. (2000) and Dembilio *et al*. (2009) reported antibiotic and antixenotic mechanisms in some palm species. *Washingtonia filifera* and *Chamaerops humilis* could not be naturally infested by RPW adult females and antibiosis was found to be the main mechanism operating in *W. filifera* (Dembilio *et al.*, 2009). RPW is known to mostly attack young date palm of less than 20 years old (Abraham *et al*., 1998). Farazmand (2002) and Al-Ayedh (2008) found that date palm cultivars with high sugar content enhanced growth of RPW. Infestation begins with gravid female weevils getting attracted to palm volatiles for laying eggs which hatch into damage inflicting larvae. Infested date palms exhibit several symptoms depending on the stage of attack *viz*. oozing of brownish fluid mixed with palm tissue excreted by feeding larvae that has a typical fermented odor, tunneling of palm tissue by larvae, presence of adults and pupae at the base of fronds, drying of infested offshoots, fallen pupae around an infested palm, drying of outer leaves and fruit bunches and toppling of the trunk in case of very severe and extensive tissue damage (Abraham *et al*, 1998). Early detection of infested palms is very difficult but essential in order to take appropriate measures at the earliest (Faleiro, 2006; Dembilio and Jacas, 2011).The pest is currently managed mainly through a pheromone based Integrated Pest Management (IPM). As the oviposition is the first event in the infestation cycle, exploiting host plant resistance to oviposition would be of immense help in developing resistant varieties. With this objective, we assessed the extent of egg laying by RPW in 25 important Saudi Arabian cultivars.

The Date Palm Research Centre (DPRC), Ministry of Agriculture, Al-Hassa, Saudi Arabia has a a diverse genetic pool of over 100 date palm cultivars of national, regional and international date collections planted in the gene bank. Twenty five date palm cultivars (Table 1) from the Kingdom of Saudi Arabia were assessed for

Table 1. Oviposition preference of red palm weevil to date palm cultivars

S. No.	Cultivar	Mean number of eggs laid / week		
		Trial -I	Trial-II	Cumulative Mean
1	Murheim	4.80 hi	4.80 efg	4.80 jkl
2	Hilali	6.80 fghi	6.60 efg	6.90 ijkl
3	Shahal	3.60 i	0.00 g	1.80 l
4	Barhei	1.20 i	4.60 efg	2.80 kl
5	Sheshi	1.20 i	2.60 fg	1.90 l
6	Shabibi	2.00 i	5.80 efg	3.90 jkl
7	Hatmi	2.40 i	7.60 efg	5.00 jkl
8	Reziz	10.80 fghi	1.00 fg	6.90 ijkl
9	Gaar	6.00 ghi	0.00 g	3.00 kl
10	Kheneizi	45.20 ab	13.80 def	31.30 b
11	Voseili	33.20 bc	0.00 g	16.60 defgh
12	Khalas	26.40 cde	11.00 efg	18.70 defg
13	Tanajib	17.60 defgh	11.80 efg	14.70 efghi
14	Taiar	9.60 fghi	5.60 efg	7.60 hijkl
15	Khasab	30.40 cd	12.20 efg	21.30 cdef
16	Sukari Ahmer	7.60 fghi	15.80 cde	11.70 ghijk
17	Rotana	18.80 defg	5.20 efg	12.20 fghijk
18	Sugai	12.40 fghi	14.00 def	13.20 fghij
19	Ajwa	48.80 a	56.60 a	48.97 a
20	Dinari	19.60 def	28.40 bc	24.00 bcde
21	Anbara	16.80 efgh	26.40 bcd	21.60 cdef
22	Nabat Sultana	5.20 hi	46.00 a	25.60 bcd
23	Zamli	6.40 jhi	4.00 efg	5.20 jkl
24	Majnaz	3.20 i	3.20 efg	3.20 kl
25	Turi	27.60 cde	31.20 b	29.40 bc
	CD (p=0.05)	13.15	13.07	9.42

Values followed by different letters within the same column are significantly different at 5% significance level.

their preference for egg laying by the red palm weevil. Adult RPW male and females were obtained from the 'whole palm' insect rearing facility at DPRC. Newly emerged adult weevils (1:1) selected for the trial were reared as a primary culture in perforated plastic boxes (40x25x30cm) with sugarcane pieces to feed on. This ensured mating and fertility of the adult female weevils. Subsequently, three to four week old fertile female weevils were selected for the egg laying trial. Ten gravid females and six mature males were released in a round perforated plastic box (diameter: 24 cm; height: 8cm) containing petiole bits (1x1x1 inch each) of the selected date cultivars. Freshly pruned petiole surface releases palm volatiles that attracts adult female weevils for oviposition (Abraham *et al.*, 1998) and was therefore offered for egg laying to female weevils in this trial. Male

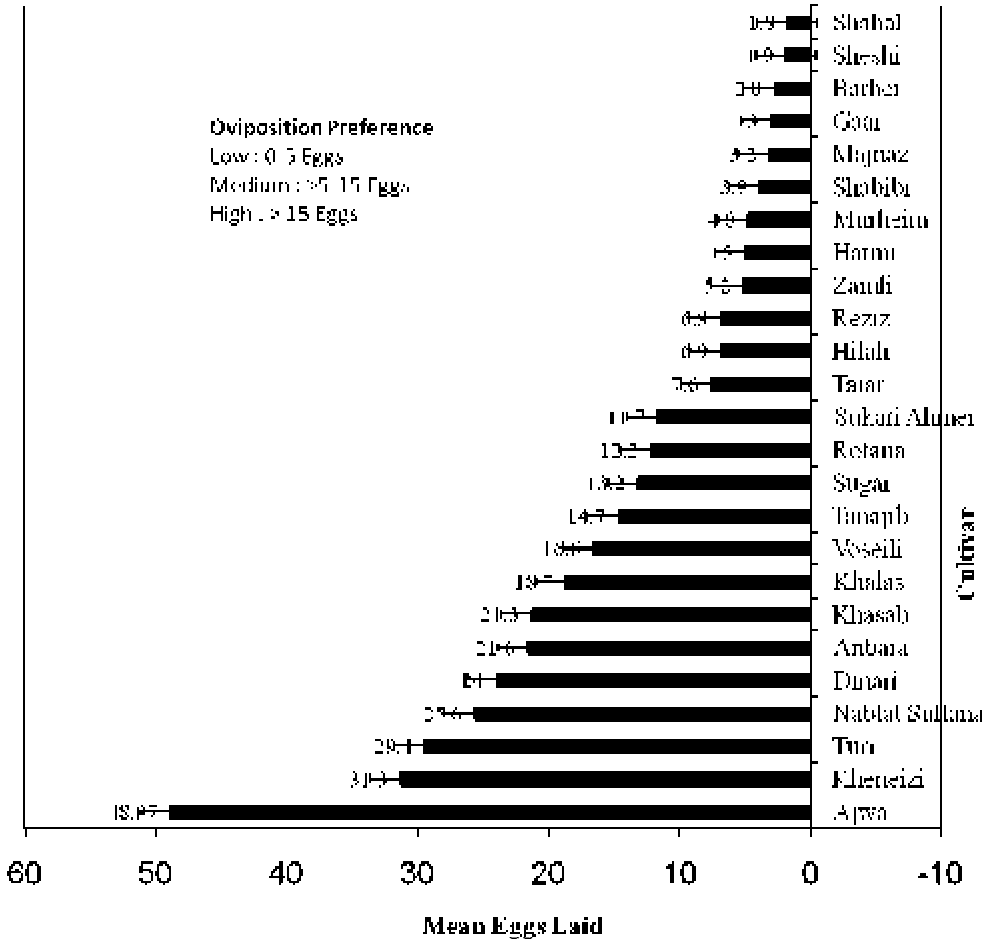

Fig. 1. Oviposition preference in date palm cultivars by *R. ferrugineus*

weevils were released with the female weevils to ensure mating and sustain fertility of female weevils (Faleiro *et al.*, 2003). Three petiole bits of each cultivar were offered for egg laying to the above mentioned gravid adult females in the box. One box contained five cultivars. The boxes containing the adult weevils and date petiole bits were covered with a perforated lid and kept in a dark room (25^0C; 75 % RH) for a week to facilitate oviposition. Number of eggs laid/ larvae hatched in each petiole bit was recorded on the 7th day after start of trial by carefully peeling the petiole fibre. The treatments were replicated five times and the trial was repeated twice. The experiment was laid out in the Completely Randomized Design. Data on mean egg laying was subjected to ANOVA. Means were separated by LSD at $t_{5\%.}$

Significant differences were recorded in the number of eggs laid by RPW in the date palm cultivars tested (Table 1 and Figure 1). Least mean number of eggs laid was in the cultivar 'Shahal', while the highest oviposition was recorded in the cultivar 'Ajwa'. Al-Baqshi *et al.* (2008) recorded high degree of antixenosis (non-preference) for egg laying in the cultivars 'Shahal', 'Murheim', 'Gaar' and 'Sheshi' with the cultivar 'Khalas' exhibiting a low degree of antixenosis for egg laying by RPW. This is in agreement with our findings. Al-Ayedh (2008) recorded significantly greater numbers of eggs of RPW in the cultivar 'Sukkary' as compared to the cultivar 'Khalas' which was attributed to the higher sugar content in 'Sukkary'. Based on cumulative analysis (Fig.1), eight cultivars (Shahal>Sheshi>Barhei >Gaar >Majnaz> Shabibi> Murheim>Hatmi) with a mean egg laying of 0-5 were considered to exhibit high degree of antixenosis or non-preference for oviposition by RPW. Further, eight cultivars (Zamli>Reziz>Hilali>Taiar>Sukari Ahmer>Rotana>Sugai>Tanajib) recording a mean egg lay of more than 5 to 15 eggs showed moderate antixenosis while nine cultivars (Voseili>Khalas>Khasab> Anbara>Dinari>Nabat Sultana>Turi>Kheneizi>Ajwa) with more than 15 eggs were most preferred and exhibited a low degree of antixenosis for egg laying by RPW.

Studies carried out in Spain revealed that the palm species *Chamaerops humilis* and *Phoenix theophrasti* showed antixenotic and antibiotic mechanisms of resistance, respectively against RPW (Dembilio *et al.*, 2009). The coconut cultivar, 'Malayan Yellow Dwarf' was least preferred for egg laying by RPW while, maximum number of eggs were laid in 'Chowghat Green Dwarf' (Faleiro and Rangnekar, 2001). Reports from Iran suggest that sugar in date palm varieties enhanced growth, daily oviposition and reduced mortality of RPW while, calcium was found to inhibit the growth of RPW (Farazmand, 2002). 'Khalas' is a premier date palm cultivar of Saudi Arabia (Al-Abdoulhadi *et al.*, 2011) predominant in the Eastern Province of the Kingdom (Al-Abbad *et al.*, 2011) and as indicated above, is among the most preferred for egg laying by RPW. The study provides a valuable insight into the inherent risk level the farmers of the region encounter based on the date palm cultivar grown and should adopt the necessary control measures (Abraham *et al.*, 1998) especially in young plantations where RPW preferred cultivars like Khalas that belong to the high risk category are grown. The findings of this study also provides a base line for breeding date palm cultivars resistant to RPW for incorporating host plant resistance as a component of the RPW-IPM strategy in the future, as proposed previously by Abraham *et al*. (2002).

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SHORT NOTE

Efficacy of different biopesticides and insecticides against mealy bugs on custard apple

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In India, custard apple is grown over a vast area in the states of Andhra Pradesh, Maharashtra, Tamil Nadu, Assam and Orissa. The custard apple is infested by 20 species of insect pests (Butani, 1979), of which the mealy bug is the most important one. Three species of mealy bugs *viz*., *Maconellicocus hirsutus*, *Planococcus citri* and *Ferrisia virgata* are serious pests of this crop. Mealy bugs live in protective areas such as cracks and crevices of the bark, at the base of petioles, on the under side of leaves and between the fruit eyes. Eggs of mealy bugs protected by waxy filamentous secretion of ovisac are almost impossible to reach with insecticide. Hence, it is very difficult to manage mealy bug. Control of mealy bugs with chemical pesticide has certain limitations of which one is the problem of hazardous levels of residue. Recently the incidence of mealy bugs becomes a serious concern in many custard apple orchards in Maharashtra State. Once the pest incidence increased, it is very difficult to control it with conventional insecticide. The present investigation was undertaken to know the efficacy of different group of insecticides against mealy bugs on custard apple.

A field experiment was conducted at the Department of Horticulture, MPKV, Rahuri, Maharashtra during the *Mrig bahar* of 2009-10. The experiment was conducted in a randomized block design with three replications and each replication has two plants. There were eight treatments *viz*.,T_1: *Verticilium lecanii* (1.15%) WP (Phule Bugicide) at the rate of 6 x10^5 spores/g (3 g/litre), T_2: *Verticilium lecanii* 3% S (Phule Bugicide) at the rate of 6 x10^5 spores/g (3 g/litre), T_3: *Beauveria bassiana* 1.15% WP (Phule *Beauveria*) at the rate of 10x10^5 spores/g (5 g/litre), T_4: NSE at the rate of (5%) (50 g/litre), T_5: buprofezin 25 SC at the rate of (0.02%) (0.8 ml/litre), T_6: imidacloprid 17.8 SL at the rate of (0.005%) (0.3 ml/litre), T_7: diafenthiuron 50 WP at the rate of (0.02%) (0.4 ml/litre) and T_8: dichlorvos 76 WSC (0.05%) (0.66 ml/litre) T_9: control (water spray). Each treatment was consisted of two sprays applied at an interval of 15 days by initiating first spray with the appearance of mealy bug incidence. Effectiveness of insecticides was judged on the basis of level of mealy bug incidence on randomly selected fruits. The pre-count of nymphs and adults of mealy bugs was recorded on a day prior to application and post-counts at 3, 7 and 10 days after each spray. The per cent mortality was worked for 3, 7 and 10 days after application of insecticides. The generated data on survival of mealy bug was transformed into d n+1 values and subjected for statistical analysis. The yield per plant was recorded and economics of spray treatment was also worked out.

The data on the effect of insecticides on mealy bugs are presented in Table1. At 1st spray, mealy bug population was in the range of 19.42 to 23.67/fruit on a day before spray treatment and it was statistically non-significant. All insecticide treatments were significantly superior over untreated control in minimizing the incidence of mealy bug on 3rd, 7th and 10th day after spray treatment (DAS). The infestation of mealy bugs was reduced on 3rd, 7th and 10th DAT. On 10th DAS, the population of mealy bug recorded in chemical spray treatments was ranged 3.5 to 7.50 nymphs and adults/fruit. Irrespective of chemical insecticides, the mealy bug count recorded was between 3.75 to 6.00 nymphs and adults/fruit in the treatments of entomopathogenic fungi. Non chemical treatment i.e. NSE 5 per cent was relatively less effective recording 10.08 nymphs and adults/fruit. Buprofezin (0.02%) was found to be most effective in minimizing the mealy bug population and recorded 3.50 nymphs and adults/fruit on 10 DAS followed by *V. lecani* (1.15% WP) and *V. lecanii* (3% S) which recorded 3.70 and 4.00 nymphs and adults /fruit, respectively.

After 2nd spray, mealy bug population was in the range of 7.25 to 13.08/fruit before initiation of treatment and it was also statistically non-significant. All the insecticide treatments were significantly superior over untreated control in minimizing the incidence on mealy bug on fruits. On 10 DAS, the chemical spray, non chemical spray and entompathogenic fungi treatments

Table 1. Efficacy of different insecticides against mealy bugs on custard apple

Treatment	No. of nymphs and adults/ fruit													
	1st spray				2nd spray				Mean of two sprays					
	Pre count	3DAS	7 DAS	10 DAS	Pre count	3DAS	7 DAS	10 DAS	Pre count	3DAS	7 DAS	10 DAS	Fruit yield (t/ha)	Incremental benefit cost ratio
V.lecanii 1.15% WP	23.58 (4.96)*	16.33 (4.16)	7.58 (2.93)	3.75 (2.18)	7.25 (2.86)	4.92 (2.43)	2.25 (1.80)	1:13.4	15.42 (3.91)	10.63 (3.30)	4.92 (2.37)	2.50 (1.84)	11.28	13.4
V.lecanii 3% WP	23.67 (4.96)	17.92 (4.35)	7.75 (2.96)	4.00 (2.23)	7.33 (2.87)	5.33 (2.51)	2.25 (1.80)	1:7.60	15.50 (3.92)	11.63 (3.43)	5.00 (2.38)	2.63 (1.87)	11.08	7.60
B. bassiana 1.15% WP	22.00 (4.78)	18.83 (4.45)	9.50 (3.23)	6.00 (2.64)	8.92 (3.14)	7.25 (2.87)	3.75 (2.18)	1:4.55	15.46 (3.91)	13.04 (3.66)	6.63 (2.71)	4.13 (2.22)	9.28	4.55
NSE 5%	23.00 (4.90)	14.83 (3.98)	11.58 (3.54)	10.08 (3.32)	8.58 (3.09)	5.42 (2.52)	4.58 (2.36)	1:1.38	15.79 (3.99)	10.13 (3.26)	8.08 (2.95)	6.71 (2.70)	7.71	1.38
Buprofezin 0.02%	19.83 (4.54)	5.17 (2.48)	4.17 (2.26)	3.50 (2.12)	8.08 (2.97)	2.00 (1.73)	1.75 (1.66)	1:6.81	13.96 (3.76)	3.59 (2.11)	2.96 (1.96)	2.29 (1.78)	12.14	6.81
Imidacloprid 0.005%	22.58 (4.86)	7.25 (2.87)	6.00 (2.65)	7.08 (2.84)	8.67 (3.09)	2.75 (1.94)	2.33 (1.82)	1:5.28	15.63 (3.98)	5.00 (2.41)	4.13 (2.25)	4.79 (2.36)	9.21	5.28
Diafenthiuron 0.02%	22.83 (4.88)	7.50 (2.91)	6.25 (2.69)	7.17 (2.85)	8.17 (3.01)	2.75 (1.94)	2.25 (1.80)	1:1.17	15.50 (3.95)	5.13 (2.42)	4.29 (2.26)	4.88 (2.37)	8.18	1.17
Dichlorvos 0.05%	22.08 (4.79)	4.50 (2.34)	6.33 (2.70)	7.50 (2.91)	8.92 (3.14)	1.92 (1.71)	2.25 (1.80)	1:3.27	15.50 (3.95)	3.21 (2.03)	4.29 (2.25)	5.13 (2.42)	8.73	3.27
Untreated control	19.42 (4.51)	21.75 (4.76)	23.58 (4.96)	24.83 (5.08)	13.08 (3.74)	12.08 (3.62)	10.92 (3.45)	10.92 (3.45)	16.25 (4.13)	16.32 (4.19)	17.25 (4.21)	17.88 (4.27)	6.95	0.0
S.E.±	0.18	0.10	0.12	0.10	0.17	0.08	0.06	0.07	0.17	0.09	0.09	0.08	0.74	
C.D. at 5%	N.S.	0.32	0.37	0.30	N.S.	0.24	0.20	0.21	N.S.	0.28	0.28	0.24	2.22	

* Figures in the parentheses indicate √n+1 transformed values.

DAS = days after spraying

reducing of 1.08 to 2.75 , 3.33 to 5.42 and 1.25 to 2.25 the nymphs and adults population, respectively. Buprofezin (0.02%) was found to be most effective in minimizing the mealy bug population and recorded 1.08 nymphs and adults /fruit followed by *V.lecanii* (1.15% WP) and *V. lecanii* (3% S) which recorded 1.25 nymphs and adults /fruit. The pooled means of two sprays showed increasing trend of incidence from 16.32 to 17.88 nymphs and adults/fruit in untreated control on 3rd, 7th and 10th DAS. The chemical, non-chemical and entomopathogenic fungi treatments observed 2.29 to 5.13, 6.71 to 10.13 and 2.50 to 4.13 nymphs and adults/ fruit on 10 DAS. Buprofezin (0.02%) was found to be most effective in minimizing the mealy bug population and recorded 2.29 nymphs and adults /fruit followed by *V. lecanii* (1.15% WP) and *V. lecanii* (3% S) which recorded 2.50 and 2.63 nymphs and adults /fruit, respectively. The decreasing order of effectiveness of different treatment was buprofezin > *V. lecanii* (1.15%) WP > *V. lecanii* (3%) WP > *B. Bassiana* > imidacloprid > diafenthiuron > dichlorvos > NSE when compared with control. The results of the present investigation confirmed with Bedford *et al*. (1996) who reported that buprofezin significantly reduced mealy bug (*Planococcus citri*) population. Shrivastava (1997) also reported that buprofezin (0.0625%) was very effective in controlling the second instar nymphs of *Dochica mangiferae* and mortality was 100 per cent after 16 days of spray under laboratory condition. The satisfactory control of the pest by *V. lecani* in the present study is in agreement with Kulkarni *et al.* (2007) and Jayachakravarthy (2001) on *F. virgata* and *M.hirsutus*, respectively. The result in respect of effectiveness of *B. bassiana* was in conformity with Suresh *et al.* (2010) who reported that *B. bassiana* was moderately effective against mealy bugs. Balikai (1999), Shelke (2001) and Bhosale *et al.* (2009) reported that dichlorvos 76 WSC was effective in management of mealy bugs. Deshmukh (1999) found that dichlorvos (0.095%) was highly effective against *P.citri.* These results support to the present finding.

All the insecticide treatments significantly increased fruit yield over control except NSE 5 per cent (Table 1). The highest yield (12.4 t/ha) was recorded in buprofezin (0.02%) treated trees followed by *V. lecanii* 1.15 % WP (11.28 t/ha) and *V. lecanii* (3%) S (11.08 t/ha) and it was significantly at par with buprofezin. The next effective treatment was *B. bassiana* (1.15%) WP (9.28 t/ha) and it was at par with imidacloprid (0.005%) (9.21 t/ha), dichlorvos (0.05%) (8.73 t/ha), diafenthiuron (0.02%) (8.18 t/ha) and NSE (5%) (7.71 t/ha). The incremental benefit cost ratio (IBCR) ranged from 1.17 to 13.40 in different treatments. The most economical treatment was *V.lecanii* (1.15%) WP and recorded (13.40) followed by *V.lecanii* (3%) WP (7.60) and buprofezin (0.02%) (6.81). The IBCR was 1:5.28 and 1:4.55 in imidacloprid (0.005%) and *B. bassiana* (1.15%) WP treatments, respectively as against control (1:3.27). Whereas, NSE (5%) and dichlorvos (0.05%) recorded less IBCR as compared to control. Our study clearly indicates that *V. lecanii* is effective and economical thus proving to be a potential alternative to insecticides.

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SHORT NOTE

Effect of vegetable oils on pulse beetle, C*allosobruchus chinensis* L. (Coleoptera: Bruchidae) infestation and germination of pea seeds (*Pisum sativum*)

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The pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae), causes substantial losses to the pulses in the storage (Alam, 1971; Singh *et al.*, 2001 and Righi-Assia *et al.*, 2010), though the initial infestation occurs in the field itself. It causes weight loss, decreased germination potential and reduction in commercial value of the seed (Okunola, 2003). It is a serious pest of peas, mung bean, cowpea and lentil and has also been reported attacking cotton seed, sorghum and maize (Ahmed *et al*., 2003). The use of synthetic organic pesticides for the control of insect-pests of stored seeds has led to the development of resistance and toxic residues in food grains. The use of plant products as grain protectants is an age old practice (Weaveret *et al*., 1992) and reported to be quite safe and promising (Jilani *et al*.,1988 and Ahmed *et al*., 2003). The use of vegetable oils has been known to protect seeds from bruchids infestation in many pulse crops (Okunola, 2003; Khalequzzaman *et al*., 2007). Therefore, there is a need to evaluate various botanicals to identify the ones which are not only effective but also safe to the quality and vigour of seeds treated. Keeping this in view the present study was conducted. The culture of *C. chinensis* was raised on pea seeds and maintained under controlled conditions at 27 ±1 ^{0}C and 70% R.H. The freshly harvested pea seeds were sterilized in oven at 55^{0}C for 4 hours as per method of Mookherjee *et al*. (1968). The sterilized seeds were put in half kg capacity glass jars into which five pairs of freshly emerged *C. chinensis* adults were released. The jars were tightly covered with muslin cloth and were kept in BOD incubator for raising the culture. Seven vegetable oils *viz.*, mustard, neem, *karanj*, Cedar wood oil, apricot and olive were used to coat the seeds at 1, 3 and 5 per cent concentrations. The oils were thoroughly mixed by vigorously shaking in different plastic containers filled with seed. Five pairs of the newly emerged adults of *C. chinensis* were released in each plastic container which was covered with muslin cloth and was tightened with rubber band. The treatments, including control, were maintained in three replications. All the adults were allowed to remain in the container until their natural mortality under room temperature. After storage period of two months, the data on seed damage, seed weight loss and seed germination was recorded. The per cent seed damage was calculated by adopting the procedure given by Adams and Schulten (1978). Loss in seed weight was calculated by taking initial weight and final weight of the seed and per cent weight loss was calculated. The per cent seed germination was calculated by taking 100 seeds from each container. The seeds were sandwiched between towel paper as proposed by Sen and Ghosh (1999). The paper towel was then kept in seed germinator chamber at 25 ± 1^{0}C. The germination percentage was calculated as per the ISTA procedure (Anonymous, 1985).

It is evident from Table 1 that all the tested oils provided significant protection to pea seeds from the damage by *C. chinensis.* Neem oil was the most effective treatment allowing only 0.11 per cent damage followed by *karanj* (0.18%), cedar (0.29%), mustard (0.47%) olive (0.87%) and apricot (1.43%). Seed damage in untreated control was 9.72 per cent. No seed damage was recorded in neem oil at 5 per cent concentration and it was at par with its lower concentrations (3 and 1 %), *karanj* (5, 3 and 1%), cedar and mustard at 5 and 3 per cent, olive and apricot at 5 per cent. The present results are in conformity with the results of Tripathi *et al*. (2006) who reported that neem oil was effective in reducing the damage to grains of pigeon pea by *C. chinensis.* It is clear from Table 1 that neem, *karanj* and cedar oil minimized the weight loss in treated seeds to 0.11, 0.22 and 0.30 per cent, respectively as against 11.02 per cent in the control. Among all these treatments,

Table 1. Effect of vegetable oils on quality characters of treated pea seeds caused by *C. chinensis*

Treatment	*Mean Seed Damage (%)[1]				*Mean weight loss (%)[2]				*Mean seed germination (%)[3]			
	Concentrations (%)											
	5	3	1	Mean	5	3	1	Mean	5	3	1	Mean
Mustard	0.07 (0.75)	0.33 (0.91)	1.01 (1.21)	0.47 (0.96)	0.07 (0.75)	0.40 (0.95)	1.00 (1.21)	0.49 (0.97)	82.00 (64.98)	80.67 (63.96)	70.67 (57.39)	77.78 (62.11)
Neem	0.00 (0.71)	0.07 (0.75)	0.27 (0.88)	0.11 (0.78)	0.00 (0.71)	0.07 (0.75)	0.27 (0.79)	0.11 (0.78)	93.33 (75.28)	91.33 (72.99)	89.33 (71.06)	91.33 (73.11)
Karanj	0.00 (0.71)	0.13 (0.79)	0.39 (0.92)	0.18 (0.82)	0.00 (0.71)	0.27 (0.88)	0.40 (0.95)	0.22 (0.84)	92.00 (73.65)	89.33 (71.06)	84.67 (67.06)	88.67 (70.59)
Cedar	0.07 (0.75)	0.27 (0.88)	0.53 (1.00)	0.29 (0.88)	0.07 (0.75)	0.30 (0.89)	0.53 (1.00)	0.30 (0.88)	84.67 (67.21)	82.00 (65.04)	76.67 (61.14)	81.11 (64.47)
Apricot	0.19 (0.83)	1.71 (1.47)	2.37 (1.69)	1.43 (1.33)	0.23 (0.85)	1.73 (1.47)	2.40 (1.69)	1.46 (1.34)	72.00 (58.12)	70.67 (57.26)	62.00 (51.95)	68.22 (55.78)
Olive	0.13 (0.79)	1.01 (1.22)	1.47 (1.39)	0.87 (1.13)	0.17 (0.81)	1.00 (1.22)	1.47 (1.39)	0.88 (1.14)	80.67 (64.09)	78.67 (62.75)	69.33 (56.37)	76.22 (61.07)
Control	9.34 (3.13)	9.80 (3.21)	10.00 (3.24)	9.72 (3.19)	9.7 (3.19)	11.57 (3.46)	11.80 (3.49)	11.02 (3.38)	42.00 (40.39)	46.00 (42.70)	44.00 (41.54)	44.00 (41.54)

*Mean of three replications,

Figures in parentheses are $\sqrt{x+0.5}$ transformed values[1, 2]

Figures in parentheses are arc sine transformed values[3]

CD (p=0.05) Treatment: (0.13), Concentration: (0.08), Treatment x Concentration: (0.22)[1], (0.23)[2]

Treatment: (3.06), Concentration: (2.00), Treatment x Concentration: (5.29)

mixing of oils of neem and *karanj* at 1 per cent was effective to minimize weight loss due to *C. chinensis* infestation. The present findings corroborate the findings of Tripathi *et al*. (2006) who observed that neem oil at 2ml/kg of pigeon pea seeds against *C.chinensis* reduced the weight loss. Singh *et al*. (2006) recorded no weight loss on pea seeds treated with mustard oil at 2ml/ kg. Data also revealed that seed germination in various treatments ranged from 68.22 to 91.33 per cent, as against 44 per cent in control. Oils of neem and *karanj* were the best and equally effective even at 1 per cent concentration (89.33 and 84.67% germination, respectively). Thus seed germination of stored pea was improved due to the protection from damage by *C. chinensis* provided by these oils. None of the treatments apparently reduced the seed germination indicating that these vegetable oils can be used safely for the control of *C. chinensis*. Present study finds support from the work of Sreeramaiah and Bommegouda (1992) who reported higher germination of chickpea seeds treated with neem oil at 5 ml/kg. Khaire *et al*. (1992) reported that neem, *karanj* and mustard oils when used against *C. chinensis* were safe from seed germination point of view. Singh and Yadav (2003) reported no effect on germination of green gram seeds treated with neem, karanj, mustard and olive oil recorded after 6 to 8 hour, 90, 150 and 210 days after treatment. Our results indicate that vegetable oils are not only effective against pulse beetle but also do not affect seed germination.

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SHORT NOTE

Evaluation of sticky colour traps against coffee berry borer, *Hypothenemus hampei* (Ferrari)

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The coffee berry borer, *Hypothenemus hampei* (Ferrari), (Coleoptera: Scolytidae) is the most serious pest of coffee in many of the world's coffee producing countries (Le Pelley, 1968). In India, this pest was first noticed in a few plantations of Gudalur district of Tamil Nadu, during 1990 (Kumar *et al*., 1990). The adult female beetle enters into the coffee beery through the naval region, make galleries and lays eggs in the hardened endosperm. The hatched out larvae feed and destroy the coffee berry (Wrigley, 1988; Water house and Norris, 1989). Various chemical and bio pesticides have been tested to control the pest in India (Anonymous, 1995). An integrated approach involving all strategies of control is presently advised and practiced in India. The aim of the present study is to compare the efficacy of sticky colour traps for the management of coffee berry borer as an eco-friendly approach.

Field trials were conducted during the years 2008 and 2012 (October to January) to study the effect of sticky colour traps against coffee berry borer. For this purpose, five estates viz., RCRS Chundale Edaguni Estate, Edaguni Viyyanad Estate, Meppadi, Nabiraj Estate, Madakkimala Abyala Estate, Abyala were selected from Wayanad district of Kerala and Kodagu district of Karnataka. The variety of coffee grown in these estates was Sln.274 (*Coffea canephora*) of 30 to 50 years old. The spacing maintained between the plants was 3m x 3m with medium shade pattern. The trial was laid out in randomized design blocks, keeping four replications and each replication consisted of four sticky colour traps. The sticky traps are made up of metallic cylinder with the dimension of 15 cm height and 10 cm diameter. There were six treatments, red, green, white, yellow, blue and black. The colour cylinders were applied with a thin film of poly butane glue. The traps were installed in the field on a wooden stick just above the plant canopy at a distance of 20 feet apart and 5.6 feet height. The weekly observations were recorded during the cropping period (October to January). The trapped beetles were collected by using pluckers and identified in the laboratory. The results of this study clearly indicate that white colour sticky traps were most attractive followed by yellow and red. The blue and green sticky traps were less attractive and black colour sticky trap was least attractive (Fig. 1). Mu mu thein *et al*. (2011) reported that a higher number of the putative vectors, *Matsumurattix hiroglyphicus* and *Xamatotettix flavovittatus* were trapped on blue and yellow as compared to white, orange, green and colourless sticky traps. It was reported that the yellow and orange colour traps were significantly attractive for leaf hopper, *Emposca decipiens* in cotton (Demirel and Yilirim, 2008). The red coloured sticky traps caught more *Scaphodeus titanus*, grapevine "Flavescence doree" phyto plasma vector than white, yellow and blue (Lessio an Alma, 2004). Sascha Buhholz *et al.* (2010) reported that white and yellow pitfall traps caught the highest numbers of individuals of Apidae, Araneae, Carabidae, Diptera and Formicidae. The catches of winged arthropods such as Diptera an Apidae were great in white and yellow traps, which are common flower colours (Kirk, 1984; Muhlenberg, 1993). The flower-inhabiting species *Thrips obscuratus* (Craword) and *Certothrips frici* (Vzel), were

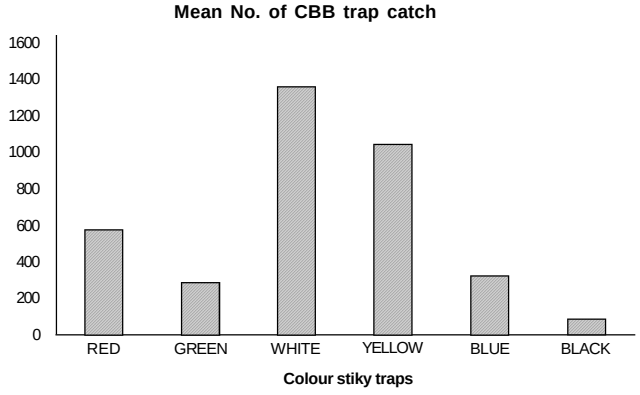

Fig. 1. Mean No. of coffee berry borer trap catch in different sticky colour traps

more attracted to white and yellow traps respectively, colours associated with the flowers of their host plants (Anonymous,1992). The polyphagous flower and foliage inhabiting *Thrips tabaci* L. was most attracted to yellow traps grass inhabiting *Limothrips cerealium* (Haliday), was equally attracted to all colour traps (Anonymous 1992). Shadwick and Harens (2007) reported that yellow and white cylinder sticky traps were equally attracted to flying clover root weevil adults. It was reported that yellow sticky cylinder traps were highly attracted to Argentine stem weevil and Lucerne weevils (Pottinger, 1966; Goldson *et al*. 1984; Baeker *et al* 1989). Prokopy and Owens (1983) suggested that attraction to yellow is characteristic of all herbivorous insect. In conclusion, white and yellow colour sticky traps are suitable for monitoring and managing the coffee berry borer.

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SHORT NOTE

Natural enemy complex of *Thrips tabaci* Lindeman in onion and garlic

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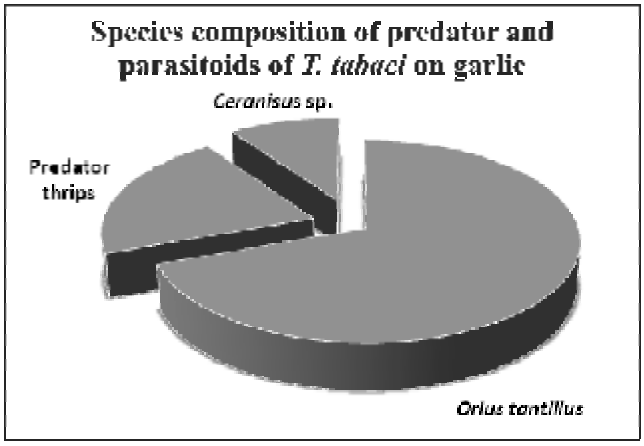
Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) is a major pest of onion and garlic crops. Yield losses in onion and garlic due to thrips were estimated to be upto 50 per cent during *rabi* season (Anonymous, 2001, Juan Anciso 2002, Diaz-Montano *et al.*, 2011). Several management practices are in vogue worldwide to control thrips and the major strategy is by chemical means followed by cultural, mechanical and biological methods. Biological methods include utilising natural enemies like micropathogens, insect predators and insect parasitoids. Although many species of predators and parasitoids of thrips, are known, a little is known about the specific natural enemies group against *T. tabaci.* Further, their adaptability in onion and garlic ecosystem is also not well studied. To address these issues with respect to onion and garlic ecosystem we made a study during 2012 *rabi* season at Directorate of Onion and Garlic Research (DOGR), Rajgurunagar. Predator and parasitoid complex associated with *T. tabaci* occurring in onion and garlic was significantly varying. In onion ecosystem, the major group found was parasitoids (>80%) followed by predators (16%). But in the garlic, predator (90%) group was at the higher end followed by parasitoid (10%) group. Predicted reason behind non-preferance of predators in onion is the odour of the onion foliage or leaf structure may not provide any shelter for the predators for multiplication. But in case of garlic, higher number of predators were observed than parasitoids.



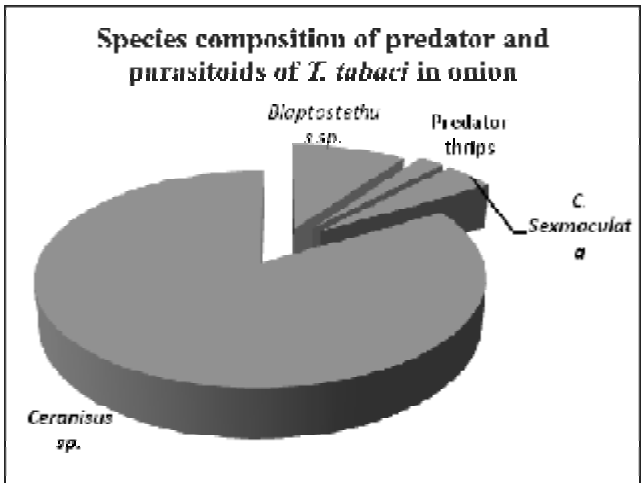


Table 1. List of natural enemies recorded on *Thrips tabaci* in onion and garlic

Species	Family	Order	Group
Ceranisus menes	Eulophidae	Hymenoptera	Parasitoid
Blaptosthethus sp.	Anthocoridae	Hemiptera	Predator
Cheilomenes sexmaculata	Coccinellidae	Coleoptera	Predator
Aelothrips sp.	Aelothripidae	Thysanoptera	Predator
Orius tantillus	Anthocoridae	Hemiptera	Predator

In onion, the distribution of natural enemies of *T. tabaci* comprised of 80 per cent parasitoides of *Ceranisus menes* (Hymenoptera: Eulophidae) followed by 10 per cent of predators *Blaptosthethus* sp. (Hemiptera: Anthocoridae), 4 per cent coccinellid predator *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae), and 2 per cent Predatory thrips of *Aelothrips* sp. (Thysanoptera: Aelothripidae). Jayanthi Mala, (2012) reported the occurrence of *C. menes* as a new parasitoid of *T. tabaci* in onion. In garlic, natural enemies of

T. tabaci distribution revealed that 70 per cent was predator *viz*., *Orius tantillus* (Hemiptera: Anthocoridae) followed by 20 per cent of predatory thrips, *Aelothrips* sp. (Thysanoptera: Aelothripidae) and 10 per cent of Hymenopteran parasitoides *viz., C. menes*. The higher preference of *O. tantillus* to garlic over onion might be due to congeniality of microclimate. We also observed the varietal preference by *O. tantillus* within garlic entries. Garlic line with broad leaves were highly preferred by predators. It is evident that these predators and parasitoids occur naturally and contribute to suppression of thrips population. Augmentation of natural enemies should be taken care by minimising the insecticidal sprays or by using botanicals or less harmful insecticides during the onset of thrips infestation. If habitat is created to enhance the survival of natural enemies, the natural enemies population will involve in natural control of pest complex. The information generated can be used to develop a bio-intensive IPM technology in onion and garlic ecosystem.

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SHORT NOTE

Newer insecticides for the management of aphid, *Aphis gossypii* Glov. in gherkins (*Cucumis anguria* L.) and their effect on the predator, *Coccinella septempunctata* L.

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Gherkins (*Cucumis anguria* L.), popularly known as 'pickling cucumber' or small cucumber was introduced to India in 1990 and became popular among small and marginal farmers in the states of Karnataka, Tamil Nadu and Andhra Pradesh (Chakravarthy *et al*., 2008). The fruits of gherkins are directly consumed and mainly exported. It is attacked by several insect pests and cotton aphid, *Aphis gossypii* Glover is one of them (Virakthmath *et al.,* 2004). Apart from sucking large amount of fluid from the growing plants and debilitating the plants aphids transmit the dreaded cucumber mosaic virus disease as a vector in a non persistent manner (Srinivasulu *et al*., 2010). The infected plants exhibit mosaic, vein banding and blistering on malformed leaves and fruits thereby reducing marketability. Presently, the neonicotinoid insecticide imidacloprid is recommended (Mhaske *et al*., 2007) over the earlier recommended insecticide dimethoate (Balikai, 2004) for its control. However, there are reports of resistance development by the pest to pyrethroid and organophosphate insecticides in the past (Kerns and Gayler, 1992). Keeping these in view, the present investigation was undertaken to find out novel insecticides effective against the pest which are in pipeline.

A field experiment was conducted in a randomized block design during the winter seasons of 2009-10 and 2010-11 to evaluate the bio-efficacy of a new anthranillic diamide insecticide, cyantraniliprole (Cyazypyr) (HGW 86) at the Central Research Station farm, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, using a gherkin variety 'Ruturaj' in plots of 5m x 4.5m size with a spacing of 1.5m x1.5m. The crop was grown with package of practices recommended for the state except plant protection. There were eight treatments replicated three times. The insecicide treatments included cyantraniliprole (HGW 86) 10per centOD at the rate of 45, 60, 75, 90 and 105 g a.i./ha and two checks *viz*., spinosad 45 SC at the rate 56 g a.i./ha and imidacloprid 17.8 SL at the rate 25 g a. i./ha and an untreated control (UTC). Chemical treatments were applied on appearance of the pest attack on the leaf through spraying with high volume knapsack sprayer fitted with hollow cone nozzle and using 500 litres of spray fluid per hectare. Altogether three applications were done at 10 days intervals during both the seasons of field experimentation. Weekly observations on the pest population were taken on five tagged plants per plot. The data were recorded on three terminal leaves per plant at pre-treatment and at 3 and 7 days after each application. Besides, the total number of plants showing cucumber mosaic virus (CMV) symptoms per plot was recorded on completion of sprays and the per cent CMV infested plants was calculated. The population of predatory coccinellid lady beetle, *Coccinella septempunctata* L. was recorded on the same five tagged plants per plot from all treatment plots of bio-efficacy evaluation trial prior to every application and at 0, 3 and 7 days after each application. Treatment-wise marketable gherkin fruit yield was recorded at each picking and a total of five economic pickings were done. The data were subjected to transformation before statistical analysis following Gomez and Gomez (1984) to compare the treatment effects.

The aphid population (both nymphs and adults) (Table1) did not vary significantly at one day before spraying (DBS) during 2009-10 (67.48-78.80) and 2010-11 (116.36-120.48) implying uniform distribution of the pest throughout the experimental field. The population of aphid remained significantly low in all the insecticidal treatments except spinosad at 3 DAS during both the seasons showing ineffectiveness of spinosad in controlling the aphids. But, at 7 DAS, except in spinosad treatment, in which the aphid population remained at par

Table 1. Effect of insecticides on population of *Aphis gossypii* on gherkin at Bhubaneswar

Treatment	Dose g a.i./ha	No. of aphids/3 terminal leaves								Reduction in CMV affected plants (%)	Yield t/ha	
		2009-10				2010-11						
		1 DBS	3 DAS	7 DAS	Reduction over control (%)	1 DBS	3 DAS	7 DAS	Reduction over control (%)		2009-10	2010-11
Cyantraniliprole HGW 86 10% OD	45	78.80 (1.90)	12.20 (1.12)^{b}	29.46 (1.48)^{c}	59.30	120.48 (2.08)	57.68 (1.77)^{d}	86.30 (1.94)^{d}	28.25	25.54	3.51^{e}	3.82^{e}
Cyantraniliprole HGW 86 10% OD	60	67.48 (1.83)	8.35 (0.97)^{b}	18.80 (1.30)^{c}	74.03	118.15 (2.07)	20.54 (1.33)^{c}	35.90 (1.57)^{c}	70.15	55.67	3.86^{d}	4.04^{d}
Cyantraniliprole HGW 86 10% OD	75	72.56 (1.87)	6.40 (0.86)^{b}	5.58 (0.82)^{b}	92.29	116.36 (2.07)	12.80 (1.14)^{c}	18.42 (1.28)^{c}	84.68	62.83	4.32^{c}	4.67^{c}
Cyantraniliprole HGW 86 10% OD	90	75.82 (1.88)	4.80 (0.76)^{a}	3.62 (0.66)^{a}	95.00	119.52 (2.08)	8.70 (0.99)^{b}	7.40 (0.92)^{b}	93.85	85.32	4.94^{a}	5.02^{a}
Cyantraniliprole HGW 86 10% OD	105	69.87 (1.85)	3.20 (0.62)^{a}	2.40 (0.53)^{a}	96.68	118.74 (2.07)	6.54 (0.88)^{b}	4.87 (0.77)^{b}	95.95	86.65	5.08^{a}	5.14^{a}
Spinosad 45% SC	56	71.24 (1.86)	68.39 (1.84)^{c}	65.72 (1.82)^{d}	9.23	117.65 (2.07)	106.62 (2.03)^{e}	110.18 (2.05)^{d}	8.39	58.36	4.88^{b}	4.96^{b}
Imidacloprid 17.8% SL	25	74.35 (1.87)	1.80 (0.45)^{a}	1.41 (0.38)^{a}	98.05	119.84 (2.08)	1.24 (0.35)^{a}	1.46 (0.39)^{a}	98.79	77.98	4.27^{c}	4.58^{c}
Untreated Control	-	68.12 (1.84)	69.70 (1.85)^{c}	72.40 (1.86)^{d}	-	116.88 (2.07)	118.20 (2.08)^{e}	120.28 (2.08)^{d}	-	-	2.48^{f}	2.60^{f}
SE (m) ±	-	(0.04)	(0.11)	(0.09)	-	(0.03)	(0.08)	(0.10)	-	-	0.06	0.05
CD (P=0.05)	-	(NS)	(0.32)	(0.28)	-	(NS)	(0.24)	(0.29)	-	-	0.18	0.16

Figures in the parentheses are log (x+1) transformed values
Means followed by a common letter in a column are not significantly different from each other
DBS= Day before spraying, DAS= Days after spraying, NS= Non Significant

Table 2. Effect of insecticides on population of *Coccinella septumpunctata* on gherkin at Bhubaneswar

Treatment	Dose g a.i./ha	No. of predators / 5 plants							
		Season-I (2009-10)				Season-II (2010-11)			
		1 DBS	0 DAS	3 DAS	7 DAS	1 DBS	0 DAS	3 DAS	7 DAS
Cyantraniliprole HGW 86 10% OD	45	4.67 (2.27)	3.84 (2.07)	3.80 (2.07)	4.20 (2.17)	5.33 (2.41)	4.87 (2.32)	5.02 (2.35)	5.97 (2.54)
Cyantraniliprole HGW 86 10% OD	60	5.33 (2.41)	3.82 (2.07)	3.91 (2.10)	4.22 (2.17)	5.33 (2.41)	4.34 (2.20)	5.21 (2.39)	6.04 (2.56)
Cyantraniliprole HGW 86 10% OD	75	4.33 (2.20)	4.23 (2.17)	3.90 (2.10)	4.12 (2.14)	5.00 (2.34)	4.52 (2.24)	5.17 (2.38)	5.92 (2.53)
Cyantraniliprole HGW 86 10% OD	90	4.67 (2.27)	4.08 (2.12)	4.01 (2.12)	3.81 (2.07)	5.67 (2.48)	4.91 (2.32)	5.42 (2.43)	5.74 (2.50)
Cyantraniliprole HGW 86 10% OD	105	5.00 (2.34)	3.92 (2.10)	4.10 (2.14)	4.01 (2.12)	5.33 (2.41)	4.07 (2.14)	5.38 (2.42)	5.85 (2.52)
Spinosad 45% SC	56	4.67 (2.27)	3.74 (2.05)	3.90 (2.10)	4.11 (2.14)	5.67 (2.48)	4.56 (2.25)	5.27 (2.40)	5.96 (2.54)
Imidacloprid 17.8% SL	25	5.00 (2.34)	4.10 (2.12)	3.93 (2.10)	3.92 (2.10)	5.67 (2.48)	4.48 (2.23)	5.14 (2.37)	6.10 (2.57)
Untreated Control	-	4.67 (2.27)	3.93 (2.10)	4.10 (2.14)	4.33 (2.20)	5.33 (2.41)	4.34 (2.20)	4.56 (2.25)	5.27 (2.40)
SE (m) ±	-	(0.10)	(0.06)	(0.04)	(0.06)	(0.07)	(0.08)	(0.07)	(0.06)
CD (P=0.05)	-	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)

Figures in the parentheses are x+0.5 square root transformed values
Means followed by a common letter in a column are not significantly different from each other
DBS= Day before spraying, DAS= Days After Spraying, NS= Non Significant

with UTC, all other treatments recorded significantly lower aphid population during both the seasons. Imidacloprid 17.8 SL at the rate of 25 g a.i/ha recorded significantly lowest aphid population/3 terminal leaves (1.46) during 2010-11 followed by cyantraniliprole (HGW86) 10 per cent OD at the rate of 90 and 105 g a.i./ha (4.87 and 7.40) with 98.79, 93.85 and 95.95 per cent reduction in aphid population over untreated control (UTC), respectively, whereas during 2009-10 all the above three treatments recorded aphid population at par (1.41-3.62) with 95.00-98.05per cent reduction over control, compared to other treatments at 7 DAS. Highest per cent reduction in cucumber mosaic virus transmitted plants was observed in the insecticide treatments comprising of imidacloprid 17.8 SL at the rate of 25 g a.i./ha, cyantraniliprole (HGW 86) 10per cent OD at the rate of 90 and 105 g a.i./ha (85.32 – 87.98) over UTC. Efficacy of imidacloprid against aphids has earlier been documented by Elbert *et al.* (1998). There is no report in the literature regarding bio-efficacy of cyantraniliprole (HGW86) against nymphs and adults of *A. gossypii* implying the present finding a first report. The anthranillic diamide insecticide group possesses anti-feedant properties that differ between chemicals of this group and insects (Gonzales-Coloma *et al*., 1999) which might be the reason of record of low population of aphids vis-à-vis record of lower incidence of cucumber mosaic virus in cyantraniliprole treated gherkin plots in the present field evaluation.

The population of predator, *C. septumpunctata* did not vary at 1 DBS during 2009-10 (4.33-4.67) and 2010-11 (5.00-5.67) indicating uniform distribution in the experimental sub-plots (Table 2). At zero hour after insecticide sprays, although slight reduction in their number was observed in treated plots but, it was on par with UTC during both the seasons. On 3rd and 7th DAS the predatory coccinellid population also did not differ significantly in insecticidal treated plots from that of UTC indicating safety of tested chemicals to the predators. Misra (2011) reported safety of related anthranillic diamide group of insecticide chlorantraniliprole to coccinellid predators which corroborates with the present findings. Ghosh *et al.*, (2010) reported safety of spinosad to important predators recorded in tomato field including coccinellid *Menochilus sexmaculatus*. Safety of imidacloprid to the natural enemies including *C. septempunctata* has already been documented (Bozsik, 2006) which is in agreement with the present findings. The marketable fruit yield (tonnes/ha) was significantly more in all the insecticidal treatments during 2009-10 (3.51-5.08 t/ha) and 2010-11 (3.82-5.14 t/ha) compared to UTC (2.48 and 2.60 t/ha, respectively). Among the treatments, significantly highest fruit yield was recorded in the treatments cyantraniliprole (HGW86) 10per cent OD at the rate of 90 and 105 g a.i./ha during both 2009-10 (4.94-5.08 t/ha) and 2010-11 (5.02-5.14 t/ha) registering 99.19, 104.84 and 93.08, 97.69per cent increase in fruit yield over UTC, respectively followed by spinosad 45 SC at the rate of 56 g a.i./ha (4.88, 4.96 t/ha, respectively) with 96.77 and 90.77per cent increase in marketable fruit yield over UTC, respectively. In other treatments the recorded fruit yield increase over control was lower than these three treatments. Thus, it may be concluded from the present field evaluation study that imidacloprid 17.8 SL at the rate of 25 g a.i./ha and cyantraniliprole (HGW 86) 10 per cent OD at the rate of 90 g a.i./ha are not only effective against *A. gossypii* in gherkins crop but also lead to reduction in the cucumber mosaic virus infected plants.

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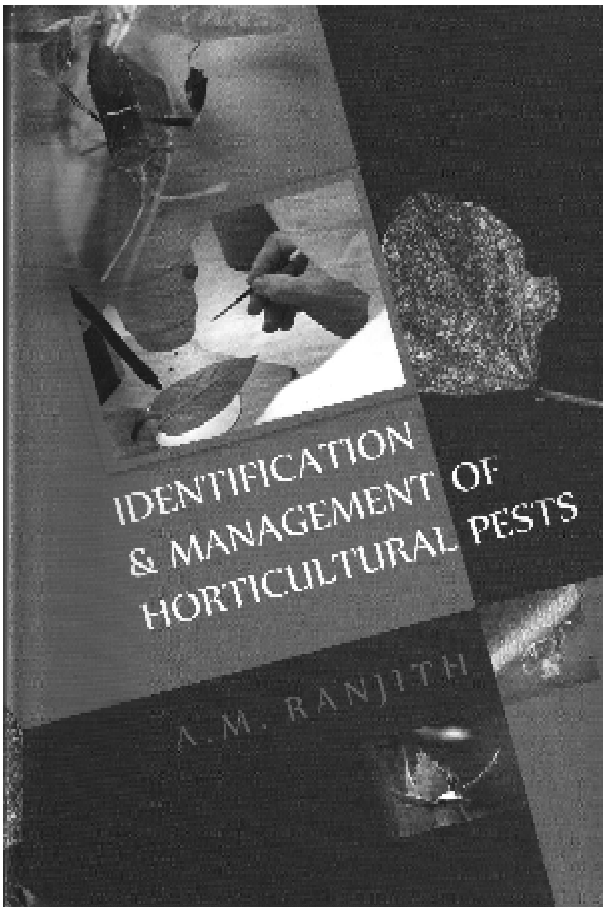
BOOK – REVIEW



The effort of compiling all major pests, possibly of the complete horticultural sector has been clearly achieved in a scientific and easy to use manner in the form of a reader friendly book titled ***"Identification and management of horticultural pests."*** An important requirement of students, especially of those appearing for higher exams and information required by plant protection officers has been met by this book authored by Dr. A.M Ranjith, Professor, Department of Entomology, Kerala Agricultural University, Thrissur, Kerala, India. This book is divided into seven sections that make the reader's task of information search easier. Fruits, medicinal and aromatic plants, ornamental plants, plantation crops, spices, tubers, vegetables (arranged in the same order) are the seven sections which are further divide into chapters where 78 crops, their important pests and management are covered. A comprehensive database of more than 400 pests occurring on all major horticultural crops is found in this book. Special care has been taken to mention the common name and the scientific name (both old and revised) of the insects. Clear photographs included in this book make it coupled with the review, also make it a source for pest management courses. Thus the book also serves as a text book.

The book contains around 350 pages of authentic information gathered from highly reliable sources and the author's 32 years of teaching and research experience. All the pages of this book are made of photo glossy paper and are neatly bound in a hard wrap bearing an attractive bright, easy to identify cover, apt for student use. Costing 1250 rupees, this book would serve as an asset to the entomology and applied zoology section of any library.

The author, Dr. Ranjith being a topper himself during his Ph. D and M. Sc. days, has kept in mind the key necessities of students and farmers while compiling this book. Published by New India Publishing Agency, New Delhi, the book will be a useful field guide to all those involved in pest management.

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AAPMHE launches Plant Protection Science Club-IIHR

The Association for Advancement of Pest Management in Horticultural Ecosystems (AAPMHE) launched the Plant Protection Sciences Club (PPSC) at Indian Institute of Horticultural Research (IIHR), Bengaluru on the 3rd August, 2013. The club was inaugurated by Dr. A. S. Sidhu, Director, IIHR, Bengaluru, who appreciated the concept of the club and wished all the best. Dr. P. Chowdappa, President, AAPMHE in his address emphasised the importance of such interactive platforms for students and researchers to get exposure to the latest advances in plant protection research. Dr. A. Krishnamoorthy, Head, Division of Entomology and Nematology and Dr. M. Krishna Reddy, Head, Division of Plant Pathology, IIHR also complimented the AAPMHE for the initiative and assured their active cooperation in carrying forward the club activities.

The main objective of the PPSC is to create a platform for interaction on the current developments in innovative and improved management tactics of various pests and diseases in horticultural crops. The club will meet on the first Saturday of every month with one or two presentations by interested members on the topics of current relevance in the field of horticultural plant protection. The membership is open to all those interested in plant protection research.

Dr. V. Sridhar, Principal Scientist, Division of Entomology, IIHR presented the inaugural seminar on the topic 'Allele mining – Prospects in Plant Protection'. The major emphasis of the lecture was about the basics of alleles and how the understanding of different alleles of a gene helps in the development of pest resistant/tolerant cultivars and pesticide resistance management. The inaugural function, coordinated by Dr. V. Sridhar, Secretary, AAPMHE was attended by more than 50 members including the scientists, research fellows and students.

PPSC TEAM, IIHR

GUIDELINES FOR AUTHORS

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

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

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

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

SHORT NOTES

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