# BIOTIC AGENTS FOR THE MANAGEMENT OF AMERICAN SERPENTINE LEAF MINER, *Liriomyza trifolii* (Burgess) (DIPTERA: AGROMYZIDAE)

By

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**(2009-21-103)**

**THESIS**

*Submitted in partial fulfilment of the requirements*

*for the degree of*

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# DECLARATION

I, hereby declare that this thesis entitled **"Biotic agents for the management of American serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)"** is a *bonafide* record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
30.10.2014

**Jyothi Sara Jacob**

# CERTIFICATE

Certified that this thesis entitled **"Biotic agents for the management of American serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)"** is a *bonafide* record of research work done independently by **Mrs. Jyothi Sara Jacob** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the Advisory committee of Mrs. Jyothi Sara Jacob (2009-21-103), a candidate for the degree of Doctor of Philosophy in Agriculture with major in Agricultural Entomology, agree that the thesis entitled **"Biotic agents for the management of American serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)"** may be submitted by Mrs. Jyothi Sara Jacob, in partial fulfillment of the requirement for the degree.

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**Jyothi Sara Jacob**

*Dedicated to*

*Eldho, Miriam*
*&*
*our loving parents*

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# Introduction

# 1. INTRODUCTION

India is the second largest producer of vegetables all over the world with 160 million metric tonnes during 2010 - 2011. About 40 per cent yield loss is caused by insect pests in vegetable production (Krishnamoorthy and Gangavisalakshy, 2014).

The leaf miners, *Liriomyza* spp. are economically important phytophagous pests of several vegetable crops coming under the family Agromyzidae (Diptera). Six species of *Liriomyza* are reported as polyphagous pests (Morgan *et al.*, 2000; Linden, 2004).

*Liriomyza trifolii* (Burgess) commonly known as the American serpentine leaf miner, is one of the predominant and economically important species. The native place of *L. trifolii* was reported to be Florida in United States of America and Caribbean Islands (Spencer, 1973). During 1990s, it was introduced to India through infested plant materials. The first report of the occurrence of *L. trifolii* in India was in the Proceedings of the Annual Castor Research Workers' Group Meeting held at Hyderabad (DOR, 1991). The pest surveillance conducted in Kerala by KHDP (1998) and Smitha (2003) revealed severe incidence of *L. trifolii* on cowpea, ash gourd, bitter gourd and tomato and higher incidence of this pest was reported during the months of January to March. The damage is caused by the maggots which are leaf miners, feeding on the mesophyll tissues leaving the epidermis intact, resulting in serpentine mines on the upper leaf surface. Heavy infestation causes desiccation and drying of leaves (Chandler and Thomas, 1983). Outbreak of *L. trifolii* adversely affected the yield in cowpea (Singh and Meroett, 1980) and the infestation of the pest caused 70 per cent loss of tomato yield (Zoebisch *et al.*, 1984). The wide host range, short life cycle and faster development of resistance to insecticides make the management of *L. trifolii* very difficult.

The natural parasitism of *L. trifolii* by the parasitoids was found to be high in the crop fields which are not sprayed with chemical insecticides. Several parasitoids which attack the larval and pupal stages of *L. trifolii* were reported and about 22 species of parasitoids were reported from India parasitizing *L. trifolii.* The mass multiplication and release of the major indigenous parasitoid species to *L. trifolii* infested crops especially in glass house conditions are being practiced outside India.

Biological control and use of ecofriendly insecticides offer great promise in the management of *L. trifolii.* But before arriving at recommendations, it is essential to investigate the diversity and role of indigenous enemies of *L. trifolii.*

In addition to parasitoids, the entomopathogenic nematodes (EPNs) were also reported from other countries to suppress *L. trifolii* in polyhouses. The utilization of indigenous isolates of EPNs enhances the efficacy of controlling native insect pests (Bedding, 1990). The potential of indigenous isolates of EPNs in controlling soil inhabiting insects is well known. Detailed studies on the effective management of foliar insects, including leaf miners with EPNs are lacking. Hence the isolation and bioefficacy studies of native EPNs against the leaf miner, *L. trifolii* will be appreciated. The compatibility of native isolates of EPNs with commonly used insecticides is also required.

Hence, the present study was conducted with following objectives:

- Collection and identification of indigenous natural enemies of American serpentine leaf miner, *L. trifolii* and also to assess the pathogenicity of the entomopathogens and thereby to explore the feasibility of utilizing them along with insecticides component for the management of *L. trifolii*

# Review of literature

# 2. REVIEW OF LITERATURE

The American serpentine leaf miner, *Liriomyza trifolii* (Burgess) was described by Burgess in 1880 as *Oscinis trifolii* as it was collected from white clover (*Trifolium repens* L.). It is known in several common names like broad bean leaf miner, celery leaf miner and chrysanthemum leaf miner. This leaf miner has wide distribution and host range. Several natural enemies, particularly parasitoids, play an important role in regulating the population of *L. trifolii.* The literature pertaining to the natural enemies and management of the American serpentine leaf miner are reviewed hereunder.

## 2.1 Natural enemies of *Liriomyza trifolii*

### 2.1.1 Parasitoids

#### 2.1.1.1 Outside India

Forty five species of parasitoids belonging to Chalcidoidea and Braconidae were reported from different parts of the World to parasitize the larval and pupal stages of *L. trifolii* to a tune of 51 to 98 per cent was reported by Neuenschwander *et al.* (1987). A list of parasitoids of *L. trifolii* are given in Table 1.

#### 2.1.1.2 In India

About 22 species of parasitoids were reported to parasitize *L. trifolii* from India and is presented in Table 2. Most of the parasitoids belong to the hymenopteran families of Eulophidae and Braconidae.

**Table 1. Parasitoids of *Liriomyza trifolii* reported from countries outside India**

| Sl. No. | Parasitoids | Place | References |
|---|---|---|---|
| 1 | Braconidae - *Opius dimidiatus* (Ashm.), Pteromalidae - *Halticoptera circulus* (Wlk.), Eulophidae - *Derostenus variipes* Crawford, *Diglyphus intermedius* (Girault) | Florida | Genung and Janes (1975) |
| 2 | Eulophidae - *Closterocerus cinctipennis* Ashm. | USA | Chandler (1982) |
| 3 | Eulophidae - *Diglyphus begini* (Ashmead), *D. intermedius*, *Chrysocharis parksi* (Crawford) | Europe | Woets *et al.* (1985) |
| 4 | Eulophidae - *Chrysonotomyia formosa* (Westwood) | Italy | Bene and Rumine (1985) |
| 5 | Eulophidae - *Chrysonotomyia punctiventris* (Crawford) | Guam | Schreiner *et al.* (1986) |
| | | USA | Lynch and Johnson (1987) |
| 6 | Eulophidae - *Hemiptarsenus semialbiclavus* (Girault), *Chrysonotomyia* sp. | Senegal | Neuenschwander *et al.* (1987) |
| 7 | Eulophidae - *C. formosa, D. isaea* (Walker), *Cirrospilus vittatus* (Walker), *Hemiptarsenus dropion* (Walker), *Pnigalio* sp. | Tuscany | Bene (1989) |
| 8 | Braconidae - *O. dimidiatus*, *O. dissitus* Muesebeck *H. circulus* (Pteromalidae) | Southern Florida | Parkman *et al.* (1989) |

| Sl. No. | Parasitoids | Place | References |
|---|---|---|---|
| 9 | Eulophidae - *Chrysocharis pentheus* (Walker), *Chrysonotomyia okazakii* (Kamijo), *Chrysonotomyia* sp., *Halticoptera* sp. 1, *Halticoptera* sp. 2, *H. varicornis*<br>Braconidae - *Opius* sp. | Central Taiwan | Lin and Wang (1992) |
| 10 | Eulophidae - *D. intermedius*, *D. begini*, *Neochrysocharis punctiventris* (Crawford)<br>Braconidae - *O. dissitus* | Florida | Schuster and Wharton (1993) |
| 11 | Eulophidae - *Chrysonotomyia rexia* Narendran, *C. vittatus*, *D. chabria*, *D. isaea*, *H. varicornis*, *Hemiptarsenus zilahisebessi* Erdos<br>Braconidae - *Opius* sp. | Spain | Cabello *et al.* (1994) |
| 12 | Eulophidae - *C. parksi*, *Diglyphus* sp.<br>Braconidae - *Opius* sp. | Arizona, USA | Palumbo *et al.* (1994) |
| 13 | Eulophidae - *N. formosa*, *H. varicornis*, *C. okazakii* (Kamijo), *C. pentheus*, *N. okazakii* | Japan | Arakaki and Kinjo (1998) |
| 14 | Eulophidae - *D. isaea*, *D. poppoea* Walker, *Diglyphus crassinervis* Erdos, *Dacnusa sibirica* Telenga | Portugal | Godinho and Mexia (2000) |

| Sl. No. | Parasitoids | Place | References |
|---|---|---|---|
| 15 | Eulophidae - *Chrysocharis orbicularis* (Nees), *C. pubicornis* (Zetterstedt), *C. vittatus*, *Diaulinopsis arenaria* (Erdos), *D. crassinervis*, *D. isaea*, *H. circulus*, *Halticoptera* sp., *H. dropion*, *H. zilahisebessi*, *N. formosa*, *Pediobius acanthi* (Walker), *Hemiptarsenus* sp., *Ratzeburgiola incomplete* Boucek, *Gronotoma* sp., *N. formosa*, Braconidae - *Opius* sp. | Jordan | Al-Ghabeish and Allawi (2001) |
| 16 | Eulophidae - *Chrysonotomyia okazakii* Kamijo | Taiwan | Chin and Chih (1998); Chin and Chih (2001) |
| 17 | Eulophidae - *Asecodes* sp. nr. *notandus* (Sivestri) (), *Cirrospilus ambiguous* Hanssan and LaSalle, *H. varicornis*, *N. formosa*, *Quadrastichus* sp. nr. *liriomyzae* Hanssan and LaSalle | Southern Thailand | Petcharat *et al.* (2002) |
| 18 | Eulophidae - *D. sibirica*, *D. isaea* Braconidae - *Opius pallipes* Wesmael | Russia | Ushchekov (2002) |
| 19 | Eulophidae - *H. varicornis*, *Quadrastichus* sp., *Neochrysocharis* sp., *Granotoma* sp., one unknown species Braconidae - *Opius* sp. | Florida | Herlinda (2003) |
| 20 | Eulophidae - *Diglyphus albiscapus* Erdos, *D. albiscapus* , *N. formosa*, *D. pusztensis* (Erdos and Novicky) | Nara Prefecture | Matsumura *et al.* (2003) |

| Sl. No. | Parasitoids | Place | References |
|---|---|---|---|
| 21 | Eulophidae - *Quadrastichus plaquoi* Reina & Salle | Italy | Reina and Salle (2004) |
| 22 | Eulophidae - *Neochrysocharis beasleyi* Fisher & La Salle, *N. okazakii*, *N. formosa*, *Asecodes delucchii* (Boucek), *Chromatomyia horticola* (Goureau) | Central and Southern Vietnam | Hoa *et al.* (2005) |
| 23 | Eulophidae - *C. vittatus.*, *H. zilahisebessi*, *Closterocerus formosus* Westwood [*Neochrysocharis formosa* (Westwood)], *D. isaea*, *D. crassinervis*, *Pnigalio pectinicornis* | Iran | Talebi *et al.* (2005) |
| 24 | Eulophidae - *C. vittatus*, *H. zilahisebessi*, *C. formosus*, *D. isaea*, *D. crassinervis*, *Pnigalio* sp. | Iran | Asadi *et al.* (2006) |
| 25 | Braconidae - *Bracon kirgisorum* Telenga, *Opius basalis* Fischer, *O. monilicornis* Fischer, *O. quasipulvis* Fisher, *O. exiguous* Wesmael<br>Eulophidae - *Chrysocharis liriomyzae* Delucchi, *C. vittatus*, *D. crassinervis*, *D. isaea*, *D. minoeus* Walker, *H. zilahisebessi*, *N. formosa*, *Pediobius metallicus* Nees, *Pnigalio soemius* Walker<br>Pteromalidae - *Cyrtogaster vulgaris* Walker, *Sphegigaster brevicornis* Walker | Turkey | Cikman *et al.* (2006) |
| 26 | Eulophidae - *C. pentheus*, *N. formosa*<br>Braconidae - *Opius* sp. | Japan | Tokumaru and Abe (2006) |
| 27 | Eulophidae - *N. formosa*<br>Pteromalidae - *H. circulus* | Japan | Tokumaru *et al.* (2007) |

| Sl. No. | Parasitoids | Place | References |
|---|---|---|---|
| 28 | Eulophidae - *N. formosa, C. pentheus* | Japan | Saito *et al.* (2008) |
| 29 | Eulophidae - *Chrysocharis* sp., *Cirrospilus* sp., *D. crassinervis, D. isaea, Neochrysocharis* sp. | Egypt | Fadl and El-Khawas (2009) |
| 30 | Eulophidae - *N. okazakii, C. pentheus, A. delucchii, N. formosa, H. varicornis* | Vietnam | Tran (2009) |
| 31 | Eulophidae - *H. varicornis* | Indonesia | Baliadi and Tengkano (2010) |
| 32 | Braconidae - *O. dissitus, Opius* sp., *C. cinctipennis*<br>Eulophidae - *Neochrysocharis* sp.<br>A species under Figitidae | Mexico | Escoboza *et al.* (2010) |
| 33 | Eulophidae - *N. formosa* | South Texas | Hernandez *et al.* (2010) |
| 34 | Eulophidae - *D. isaea, C. pentheus* (Walker), *N. formosa, Neochrysocharis* sp.<br>Braconidae - *Dacnusa sasakawi* Takada | Japan | DuChing and Saito (2011) |

**Table 2. Parasitoids of *Liriomyza trifolii* reported from India**

| Sl. No. | Parasitoids | Places | References |
|---|---|---|---|
| 1 | Eulophidae - *H. varicornis*, *Gronotoma* sp. | Karnataka | Virakthamath *et al.* (1993) |
| 2 | Eulophidae - *Tetrastichus* sp. | Bengaluru | Jagannatha (1994) |
| 3 | Eulophidae - *Cirrospilus variegatus* (Masi), *C. ambiguous*, *Chrysonotomyia* sp. | Gujarat | Kapadia (1995) |
| 4 | Eulophidae - *Chrysonotomyia* sp., *H. varicornis, Quadrastichus* sp. | India | Men *et al.* (1998) |
| 5 | Eulophidae - *C. rexia*, *Asecodes* sp., *Closterocerus agromyzae* Narayan, Subba Rao and Ramachandra Rao, *Hemiptarsenus brevipedicellus* Shafee and Rizvi<br>Pteromalidae - *Herbertia indica* Burks<br>Diapriidae - *Entomacis* sp.<br>Braconidae - *Agathidini* sp. | Kerala | Regi *et al.* (2003) |
| 6 | Eulophidae - *C. rexia*<br>Scelionidae - *Gryon* sp.<br>Braconidae - *Bracon* sp. | Kerala | Smitha (2003) |
| 7 | Eulophidae - *C. rexia*, *Oomyzus liriomyzae* Narendran | Maharashtra | Galande and Ghorpade (2007) |

| Sl. No. | Parasitoids | Places | References |
|---|---|---|---|
| 8 | Eulophidae - *Asecodes* sp., *Chrysonotomyia* sp., *Closterocerus indica* Khan *et al.*, *Diglyphus* sp., *H. varicornis*, *Quadrastichus* sp. Braconidae - *Dacnusa* sp., *Opius* sp. Pteromalidae - *H. indica* | Jammu and Kashmir | Bhat *et al.* (2009) |
| 9 | Eulophidae - *N. formosa*, *Diglyphus* sp., *Asecodes* sp., *Chrysocharis* sp. | Himachal Pradesh | Sharma *et al.* (2011) |

The parasitoids, *Aprostocetus* spp., *Tetrastichus* spp. and *Toxares* sp. were recorded from several host insect other than *Liriomyza* spp. Hence the research works on these three parasitoid groups are reviewed here.

### 2.1.1.3. *Aprostocetus* spp.

*Aprostocetus krishnieri* Mani was the first species reported under this group. It is an important internal parasitoid of the amaranthus stem boring weevil, *Hypolixus truncatulus* (Boh.) (Iyer, 1942). *Aprostocetus (Tetrastichus) sokolowskii* (Kurd.) was reported from *Plutella xylostella* (L.) from India (Patel and Patel, 1968). *Aprostocetus* sp. was recorded from sorghum midge, *Contarinia sorghicola* (Coq.) (Kishore *et al.*, 1977). *A. purpureus* (Cameron) was described as natural enemies of *Planococcus* spp., *Coccus viridis* (Green) and *Ferrisia virgata* (Cockerell) (Reddy *et al.*, 1990). *Aprostocetus neglectus* (Domenichini) parasitized San Jose scale, *Quadraspidiotus perniciosus* (Comstock) (Rawat and Pawar, 1992) and *Aprostocetus niger* (Girault) on gall insect, *Trioza fletcheri minor* Crawford (Singh *et al.*, 1995). Oriental mealybug, *Planococcus lilacinus* (Ckll.) (Homoptera: Pseudococcidae) was also reported as a host of *A. purpureus* (Mani, 1995) and *A. purpureus* was also recorded as hyperparasitoid on the exotic parasitoid *Leptomastix*

*dactylopii* How. parasitizing citrus mealybug *Planococcus citri* (Risso) (Krishnamoorthy and Mani, 1996). Kausalya *et al.* (1997) reported the parasitization of *A. gala* (Walker) and *A. coimbatorensis* (Rohwer) on sorghum midge, *Stenodiplosis sorghicola* Coquilett also from India. *A. bangaloricus* Narendran and *A. santalinus* Narendran was mentioned as parasitoids from Coccoidea (Homoptera) attacking sandalwood, *Santalum album* L. from India (Hayat *et al.*, 2003).

The parasitization of *Aprostocetus obtusae* Narendran & David was reported on *Melanagromyza obtusa* (Malloch) (Diptera: Agromyzidae) from India (Narendran *et al.*, 2005). *Aprostocetus* sp. was also reported from *Rastrococcus iceryoides* Green (Das and Sahoo, 2005), eucalyptus gall wasp, *Leptocybe invasa* Fisher & Salle (Hymenoptera: Eulophidae) (Vastrad *et al.*, 2009; Kavithakumari *et al.*, 2010).

### 2.1.1.4 *Tetrastichus* spp.

Several species of *Tetrastichus* were recorded as polyphagous parasitoids attacking the oothecae of cockroach, lac insect, scales, leaf hoppers and midges. Oothecae of two Indian species of cockroach were attacked by *T. hagenowii* (Ratz.) and *T. asthenogmus* (Wtstn.) (Boucek *et al.*, 1979; Narashimham, 1984). *Tetrastichus purpureus* (Cameron) [*Aprostocetus purpureus* (Cameron)] parasitize lac insect, *Kerria lacca* (Kerr) (Srivastava and Mehra, 1980; Subbarayudu and Maheswar, 1998) and scale insect (*Melanaspis glomerata* (Green)) from India (Jadhav and Varma, 2001). *Tetrastichus diplosidis* (Crawford) occur as a larval ecto-parasite of the sorghum ear head midge, *Contarinia sorghicola* (Coquillett) in India (Thontadarya *et al.*, 1985). Sugarcane leaf hopper, *Pyrilla perpusilla* Walker was found to be parasitized by *Tetrastichus pyrillae* Crawford and *T. gala* Crawford in India (Gholap and Chandele, 1985).

### 2.1.1.5 *Toxares* spp.

*Toxares* sp. was commonly reported as aphid parasitoids. In India, *Toxares shigai* Takada was recorded as a parasitoid from *Brachycaudus helichrysi*

(Kaltenbach) from *Prunus* sp. and *M. persicae* (Sulzer) (Stary and Ghosh, 1975). *Toxares deltiger* (Hal.) occurs on *Aphis citricola* van der Goot, *Brachycaudus helichrysi* (Kaltenbach), *Capitophorus hippophaes* (Wlk.), *Myzus ornatus* Laing, *M. persicae, Metopolophium euryae* (Tak.) and *Schizaphis rotundiventris* (Signoret) in India (Stary and Ghosh, 1978). *Toxares shigai* Takada was observed on *Aphis farinosa* (Bohzko) (Takada and Rishi, 1980) and *Brachycaudus helichrysi* (Kaltenbach) from *Prunus* sp. (Stary and Ghosh, 1975), *M. persicae* (Stary and Ghosh, 1975) and *Chaitophorus leucomelas* Koch (Takada and Rishi 1980).

In Japan, *A. gossypii* was found parasitized by *Toxares macrosiphophagum* (Takada, 1992).

Since *Toxares* spp. were commonly recorded as aphid parasitoids, it cannot be considered as an efficient parasitoid of *L. trifolii*.

### 2.1.2. Predators of *Liriomyza trifolii*

Mirids were recorded as the common predators of *L. trifolii*. It included *Cyrtopeltis modestus* (Dist.), *Dicyphus cerastii* Wagner, *Dicyphus tamaninii* Wagner and *Macrolophus caliginosus* Wagner (Parrella *et al.*, 1982; Nedstam and Kron, 1999; Carvalho and Mexia, 2000; Lucas and Alomar, 2002; Cantane *et al.*, 2004). The adults and nymphs were preyed upon leaf miner larvae or pupae.

A ponerine ant (Formicidae: Ponerinae) was recorded to attack the larvae of *L. trifolii* in Colombia (Prieto and Ullola, 1982).

A lynx spider in the family Oxyopidae (Arachnida) was recorded as a predator the adults of *L. trifolii* (Prieto and Ullola, 1982).

A number of predaceous flies were observed to prey on *Liriomyza*, including empidids (Diptera: Empididae) and muscid flies (Diptera: Muscidae) in Israel (Friedberg and Gijscoijt, 1984).

The larvae and adults of the predatory thrips, *Franklinothrips vespiformis* Crawford were reported to attack the larvae of *L. trifolii* (Arakaki and Okajima, 1998)

Predatory dipterans of the families Dolichopodidae, Empididae and Muscidae were reported to capture and kill agromyzid adults in Alahan Panjang (West Sumatra) (Rauf *et al.*, 2000).

## 2.2 Entomopathogens against *Liriomyza trifolii*

### 2.2.1 Isolation of entomopathogens from *Liriomyza trifolii*

Insect pathogens have not been recorded from *L. trifolii*. Hence the literature on the effectiveness of entomopathogenic bacteria and fungi against *L. trifolii* are reviewed here.

### 2.2.2 Entomopathogenic fungi

Studies were carried out on the susceptibility of pupae of *L. trifolii* and *L. sativae* to 11 strains of entomogenous fungi, namely, *Beauveria bassiana* (Bals.) Vuill. (4 strains), *Metarhizium anisopliae* (Metchn.) (3 strains), *Paecilomyces farinosus* (Holmsk.) (1 strain) and *P. fumosoroseus* (Holmsk.) (3 strains). Puparia were placed in peat inoculated with suspensions of *B. bassiana*, *M. anisopliae*, *P. farinosus* and *P. fumosoroseus* infected at a rate of about $10^8$ conidiospores/ g. *L. trifolii* was observed to be susceptible to *P. farinosus* (23.5% adult emergence) and to two strains of *P. fumosoroseus* (2.5 and 4% adult emergence) at 25°C. *Metarhizium anisopliae* 78 and *P. farinosus* 46 were found highly effective as the emergence of adults was only 23.5 per cent and 27.5 per cent of pupae (Bordat *et al.*, 1988).

The efficacy of *Isaria fumosorosea* Wize strain Apopka-97 *(PFR97)*, alone and in combination with four fungicides (triflumizole, pyraclostrobin, chlorothalonil and azoxystrobin), four insecticides (acephate, abamectin, thiamethoxam and pymetrozine), two insect growth regulators (novaluron and pyriproxyfen), one bioinsecticide *i.e.* Dipel (*Bacillus thuringiensis* serovar. *kurstaki*) and one fertilizer/repellent (sincocin), in controlling *L. trifolii* was investigated on gerbera

daisy (*Gerbera jamesonii* Bolus) and sunflower in Hobe Sound (Florida, USA) during the autumn of 2008. The number of adult flies emerged significantly decreased with time for the pesticides, *PFR97* alone and *PFR97* + pesticides in gerbera (Wekesa *et al.*, 2011).

The efficacy of formulations of *B. bassiana*, *V. lecanii*, *M. anisopliae* and *P. fumosoroseus*, in comparison with botanical insecticide, Nimbecidine against *L. trifolii* was studied. Among all entomopathogenic fungi, *M. anisopliae* was found to be the most efficient. The application of Nimbecidine and Bio-Magic caused 69.9 per cent and 68.9 per cent reduction respectively in larval population after two applications (El-Salam *et al.*, 2013).

### 2.2.3 Entomopathogenic bacteria

The effects of *Bacillus thuringiensis* Berliner on *L. trifolii* were investigated in Turkey in bean. The application was done at a rate of $60\times10^{6}$ $mg^{-1}$ *B. thuringiensis* spore (recommended rate - 75 g/100 l) once in 15 days for nine weeks. Observations were made on the emergence of leaf miner adults and parasitoids from *B. thuringiensis*-treated and non-treated plots. The density of leaf miner was found to be reduced in treated plots than in untreated plots. The effective control of *L. trifolii* was reported with the application of *B. thuringiensis* once in every two to three weeks (Cikman and Comlekcioglu, 2006).

A *Wolbachia*-infected strain of *L. trifolii* and a naturally occurring *Wolbachia*-free strain was observed. An antibiotic-treated *Wolbachia*-free strain was also developed. From the studies it was observed that only the eggs resulting from the mating of infected male and *Wolbachia*-free female failed to hatch almost completely. Hence, *Wolbachia* strain that showed strong Cytoplasmic Incompatibility and perfect vertical transmission in *L. trifolii* could be used in insect pest control (Tagami *et al.*, 2006).

## 2.3 Compatibility of parasitoids with botanical insecticides

The toxicity of several insecticides including botanicals to the eggs, larvae and pupae of *D. isaea* was studied. Azadirachtin was observed to cause only 5.7 per cent mortality to the larvae of *D. isaea* (Dong *et al.*, 2003).

Laboratory studies were conducted for determining the effect of the extract of mature and immature fruits of chinaberry tree, *Melia azedarach* L. on the survival of *D. isaea* in Florida. They observed that extracts of *M. azedarach* was compatible with the parasitoid *D. isaea* that contributed to lower leaf miner populations (Hammad and McAuslane, 2010).

The effects of Neemazal T/S at 0.5 and 0.6 per cent on the important parasitoids of *L. trifolii* larvae, namely, *O. quasipulvis*, *Chorebus* sp. (Braconidae) and *D. isaea* (Eulophidae) were studied under laboratory and green house conditions on tomato in Turkey. Neem had less impact on the parasitoids of *L. trifolii* (Yildirim and Baspinar, 2012).

## 2.4 Compatibility of parasitoids with chemical insecticides

The effects of methamidophos, pyrazophos, acephate, abamectin, naled and cyromazine on parasitoids of *L. trifolii* were studied in field trials conducted in beans (*P. vulgaris* cv. Iluro) in Spain. Abamectin produced an index value of 26.67 which was not significantly different from the control. Rest of the pesticides had significantly lower parasitism indices than the control (2.07 - 20.15) (Ferrer *et al.*, 1987).

In Belgium, the applications of buprofezin at 0.75 g a.i./ 10 l (two times) at an interval of eight days against *L. trifolii* on Gerbera and tomato crop were shown to have no adverse effect on *D. sibirica* and *D. isaea* (Veire and Vacante, 1988).

The tolerance of several species of parasitoids of *L. trifolii* to permethrin and fenvalerate was determined in Hawai. The eulophid, *D. begini* was significantly more

tolerant to both compounds than *C. punctiventris* and *Ganaspidium utilis* (Beardsley). Low tolerance to permethrin and fenvalerate were observed for the pteromalid, *H. circulus* and *G. utilis*. *D. begini* was reported to have higher $LC_{50}$ to both pyrethroids (Manson and Johnson, 1988).

The effect of abamectin on *O. dissitus* and *H. semialbiclava* was assessed. Laboratory studies carried out at 25±1°C, 75±5% RH and LD 12:12 proved that the larval and adult stage of *H. semialbiclava* was highly sensitive to abamectin. The braconid, *O. dissitus* was more resilient and was able to eradicate a population of *L. trifolii* in its presence (Nielsen and Bordat, 1989).

The toxicity of methomyl, permethrin, methamidophos, thiodicarb, endosulfan, fenvalerate, abamectin, cyromazine and *B. thuringiensis* subsp. *kurstaki* was evaluated against the adults, larvae and pupae of *D. intermedius* and *N. punctiventris* in the laboratory. Permethrin and methomyl were toxic to all life stages of the parasitoids. Methamidophos was toxic to adult parasitoids but was less toxic to larvae and pupae of parasitoids. Endosulfan was highly toxic to *N. punctiventris* but less toxic to *D. intermedius*. Thiodicarb, fenvalerate and abamectin were less detrimental to atleast some life stages of both parasitoids when compared to methomyl, permethrin, methamidophos or endosulfan. The entomopathogenic bacteria, *B. thuringiensis* and cyromazine were found to be least toxic to all life stages of parasitoids especially to that of *D. intermedius* (Schuster, 1994).

Studies conducted on the resistance of *D. begini* against fenvalerate in USA showed that decline in $LC_{50}$ of fenvalerate was observed in females when they were kept unexposed to insecticides upto 10 months during laboratory rearing. Less susceptibility of male and female adults were observed to those from resistant colonies than from susceptible colonies (Spollen *et al.*, 1995).

The application of selective insecticides like buprofezin, pyridaben, dicofol, fenpyroximate, fenbutatin oxide and flufenoxuron was harmless to the parasitoid

complex and was effective against *L. trifolii*. Application of non selective pesticides, namely, permethrin, methomyl, ethofenprox and prothiofos resulted in destruction of parasitoid complex and outbreak of leaf miner was followed. More than one month was needed for recovery of high parasitism after application of non selective pesticides (Saito *et al.*, 1996).

The effects of 28 insecticides, eight acaricides and 18 fungicides were studied on the adults and larvae of *D. isaea* and *D. sibirica*. IGRs (buprofezin, flufenoxuron, pyriproxyfen and teflubenzuron), *B. thuringiensis*, sodium oleate, pymetrozine and some acaricides and fungicides were observed to be harmless to *D. isaea* and *D. sibirica* (Ozawa *et al.*, 1998).

Laboratory studies were conducted for the effect of spinosad against the larval and adult emergence of ectoparasitoid, *D. isaea*. Spinosad was found to be toxic to *D. isaea*, causing 45.73 per cent larval mortality. It inhibited adult emergence also and only 5.47 per cent adults were emerged in the treatment against 79.14 per cent in control (Gahbiche, 2001).

The larval and pupal mortality and sublethal effects of abamectin and cyromazine against *H. varicornis* and *D. isaea* were evaluated. Significant mortality was observed on larvae and pupae of both parasitoid species with the application of abamectin but cyromazine was not harmful. Cyromazine was harmless on the progeny production and longevity of the parasitoid when *H. varicornis* was exposed to it. The number of progeny was unaffected with the application of cyromazine (Bjorksten and Robinson, 2005).

The effect of abamectin on the larvae and adults of *D. isaea* in laboratory and greenhouse was studied. Direct application and uptake of abamectin had negative effect on the survival of *D. isaea* adults. Abamectin residue present on chrysanthemum leaves had significant negative effect on the longevity of adult females of *D. isaea* upto five days after application. Abamectin was lethal to *D. isaea*

larvae when applied directly to larvae or when contaminated leaf miner larvae were consumed by parasitoid larvae (Kaspi and Parrella, 2005).

The toxicity of imidacloprid, pymetrozine and lufenuron against *N. formosa* was studied in the laboratory. The median lethal concentrations (24 h.) for these three insecticides were estimated as 0.033 µg/ 0.5 ml for imidacloprid, 75.57 µg/ 0.5 ml for pymetrozine and 0.417 µg/ 0.5 ml for lufenuron. The survival of the parasitoid was found to decrease rapidly with time even in low concentrations than $LC_{50}$ showing the harmful effects of these insecticides to *N. formosa*. The reduction in female longevity was observed (Tran *et al.*, 2005).

The toxic effects of six insecticides, namely, cartap hydrocloride, thiocyclam, triazophos, pyrazophos, abamectin, and cyromazine on the different life stages of parasitoids *H. varicornis* and *N. formosa* were studied in Taiwan. Cartap hydrocloride, thiocyclam and triazophos were the most toxic insecticides to parasitoids followed by pyrazophos, abamectin and cyromazine. The adult stage was more vulnerable to the tested insecticides than the immature stages. *N. formosa* was more tolerant to five of the insecticides tested than *H. varicornis*, but the susceptibility of both parasitoids to triazophos was similar. Incorporation of cyromazine into *L. trifolii* control programs was recommended as cyromazine could conserve parasitoids (Chin *et al.*, 2007).

The effect of novaluron, abamectin, lambda cyhalothrin and spinetoram against the adults of two important parasitoids of *L. trifolii*, namely, *N. formosa* and *G. nigrimanus* (Kieffer) was studied. Spinetoram was found to be the most harmful. Novaluron exhibited the least lethal effects to adult parasitoid. Abamectin caused significant mortality to both parasitoid species in direct application and insecticide intake bioassays. Mortality was high for *G. nigrimanus* in residue assay. Variation was observed on the effects of lambda cyhalothrin between the two parasitoids. It was found harmful to *G. nigrimanus* in direct application while it had no effect on *N. formosa*. In insecticide intake bioassay, lambda cyhalothrin had no effect in survival

of either species but in insecticide residue bioassay, the survival was reduced in both species (Hernandez *et al.*, 2011).

## 2.5 Entomopathogenic nematodes (EPNs) against *Liriomyza trifolii*

### 2.5.1 Evaluation of EPNs against *Liriomyza trifolii*

The activity of six strains of *Steinernema feltiae* (Filipjev), two strains of *Heterorhabditis* sp. (Rhabditida: Heterorhabditidae) and a strain of *H. heliothidis* (Khan, Brooks and Hirschmann) was evaluated against *L. trifolii* in the laboratory in Italy. The entomopathogenic nematode, *S. feltiae* appeared more active than any of the *Heterorhabditis* species. Strain 0 of *S. feltiae* caused 76 per cent mortality to *L. trifolii* (Colombo and Locatelli, 1985).

Variation in effectiveness was reported between EPN species belonging to same genus. The symbiotic bacteria found in *S. feltiae*, *Xenorhabdus bovienii* Akhurst and Boemare was more pathogenic to *Liriomyza* spp. than *Xenorhabdus nematophilus* (Poinar and Thomas) associated with *S. carpocapsae* leading to greater efficacy of *S. feltiae* than *S. carpocapsae* (Akhurst and Boemare, 1990).

The foliar application of *S. carpocapsae* ($5 \times 10^8$ IJs/ha) on chrysanthemums infested with *L. trifolii* caused 64.2 per cent mortality in the laboratory in USA (Harris *et al.*, 1990).

Laboratory evaluation of *H. bacteriophora* Poinar on leaf miners resulted in 76 and 90 per cent mortalities (Olthof and Broadbent, 1991).

*Steinernema carpocapsae* caused 53 to more than 83 per cent mortality to the larvae of leaf miners in green house trials (Olthof and Broadbent, 1992).

Forty eight to 98 per cent mortalities to the larvae of *L. trifolii* was reported when 20 strains and/or species of steinernematid and heterorhabditid nematodes were evaluated under laboratory conditions in USA. The commercially available *S. carpocapsae* All Strain and the Hawaiian isolate of *S. feltiae* MG-14 strain caused 69

and 67 per cent mean mortality respectively in the fog house under high RH (81 to 91%). Mortality of *L. trifolii* infected with *S. carpocapsae* All Strain was observed to be more than 65 per cent at an average RH of more than 92 per cent (Hara *et al.*, 1993).

All larval stages, the prepuparium and early puparium (< 1 h after pupation initiation) were reported as susceptible stages to *S. carpocapsae* (LeBeck *et al.*, 1993).

Laboratory studies were conducted for the effectiveness of *S. carpocapsae* against *L. trifolii*. *S. carpocapsae* All Strain (10000 infective juveniles (IJs)/ml) was applied on chrysanthemums (cv. Manatee Iceberg) as foliar sprays on the second instar larvae of *L. trifolii* under conditions of high humidity in plastic cages. More than 85 per cent mortality was observed and glycerine was observed as the most effective adjuvant (Broadbent and Olthof, 1995).

## 2.6 Compatibility of EPNs with chemical insecticides

The toxic effects of 14 organophosphorus (OP) insecticides, seven carbamate insecticides, four synthetic pyrethroid insecticides, cartap hydrochloride and imidacloprid to the IJs of *S. carpocapsae* was studied. Cartap hydrochloride and two OPs (profenofos and pyraclofos) were reported as most toxic causing 83.4, 57.1 and 47.8 per cent mortality, respectively, at 100 µg/ml after 48 h. Diazinon, diclorvos, fenthion, malathion, trichlorfon, propetamphos and prothiofos showed weak toxicity at 100 µg/ml. OPs (with the exception of acephate, malathion and temephos), one carbamate (methomyl), two pyrethroids (permethrin and ethofenprox) and cartap hydrochloride apparently inhibited the pathogenicity of IJs of *S. carpocapsae* (Zang *et al.*, 1994).

The compatibility of insecticides, namely, endosulfan, phosphamidon, cypermethrin, malathion, monocrotophos and phorate with *S. carpocapsae* was evaluated in Uttar Pradesh. The IJs were found compatible with endosulfan,

phosphamidon, cypermethrin, malathion and monocrotophos and incompatible with phorate (Gupta and Siddiqui, 1999).

The compatibility of IJs of *S. feltiae* with chemical insecticides to control larval stages of the South American leaf miner, *L. huidobrensis* was investigated. IJs were directly exposed to five insecticides, namely, abamectin, deltamethrin, dimethoate, heptenophos and trichlorfon for 24 hours and the effects were studied against *Galleria mellonella* L. in a standard sand tube bioassay. Treatments with trichlorfon and dimethoate were not observed to reduce the ability of nematodes to locate and infect *G. mellonella* larvae to an unacceptable level. But nematode infectivity was observed to be reduced significantly when exposed to abamectin, deltamethrin and heptenophos (Head *et al.*, 2000).

The tolerance of fenvalerate, quinalphos and endosulfan with *Steinernema* and *Heterorhabditis indica* isolates was investigated. The per cent survival of IJs treated with neem ranged from 88.8 to 99.2 per cent followed by endosulfan (68 - 97.6%) and fenvalerate (66.4 - 98.4%). Quinalphos was reported to be deleterious to some isolates as survival and infectivity were impaired (Hussaini *et al.*, 2001a).

The compatibility of *S. glaseri* with phorate, chlorpyriphos and quinalphos was assessed against white grub (*Holotrichia consanguinea* Blanch.) on groundnut. Phorate (Phorate 10 G) was applied in furrows (recommended rate of 25 kg/ha), whereas chlorpyriphos (Durmet 20 EC) and quinalphos (Ekalux 25 EC) were applied as seed treatment (25 ml/kg seed) and as a post-sowing soil treatment (4 l/ha) with irrigation water. Survival of IJs in soil samples collected from 0 to 7 days post-treatment showed that *S. glaseri* was compatible with phorate, chlorpyriphos, and quinalphos even at their recommended rates (Bharat *et al.*, 2001).

Studies were conducted in USA to assess the interaction of EPNs, *H. bacteriophora* and *S. glaseri* (Steiner) with neonicotinoids, primarily thiamethoxam. In laboratory, greenhouse and field experiments, imidacloprid provided stronger and

more consistent synergism with nematodes than thiamethoxam (Koppenhofer *et al.*, 2002).

In USA, the compatibility of *H. bacteriophora* HP88 strain and *S. carpocapsae* All Strain with selected pesticide formulations, namely, thiamethoxam 25% a.i. (Meridian), chlorpyriphos 23.5% a.i. (Dursban), trichlorfon 80% a.i. (Dylox 80), halofenozide 1.5% a.i. (Mach 2), imidacloprid 75% a.i (Merit 75 WP), carbaryl 43% a.i. (Sevens SL), aluminium tris with 80% a.i. and mefenoxam with 45% a.i. was evaluated against turfgrass insects in tank-mixes under laboratory conditions. The pathogenicity of IJs were tested against *G. mellonella* larvae at 22°C to 26°C for 96 h. Viability of *S. carpocapsae* was unaffected by any of the pesticides, while aluminium tris and trichlorfon significantly reduced the pathogenicity of *S. carpocapsae* at all concentrations. Viability of *H. bacteriophora* was significantly reduced when treated with thiamethoxam and trichlorfon. The exposure to halofenozide, aluminium tris, trichlorfon and carbaryl significantly reduced pathogenicity of *H. bacteriophora*. Imidacloprid, at the recommended rate significantly increased the pathogenicity of *H. bacteriophora* (Alumai and Grewal, 2004).

The survival and infectivity of *S. carpocapsae*, *Steinernema arenarium* (Artyukhovsky) and *H. bacteriophora* were tested after exposing the IJs to different concentrations (250, 500, 1000 and 2000 ppm) of fipronil. The entomopathogenic nematode, *H. bacteriophora* was observed to be very tolerant to all concentrations of fipronil, with the highest mortality of 17 per cent after 72 h of exposure to 2000 ppm of fipronil. Similar response was observed in the case of *S. carpocapsae*, where the highest mortality of 11.25 per cent was observed to IJs after 72 h of exposure to 2000 ppm of fipronil. *S. arenarium* was found to be more sensitive to fipronil and at 2000 ppm mortality rates of 94.60 per cent and 100.00 per cent were observed after 24 and 72 h, respectively. Fipronil had negligible effects on the infectivity of the three nematode species tested. The IJs which survived after the

exposure to different concentrations of fipronil tested, infected and reproduced in *G. mellonella* larvae (Pino and Jove, 2005).

The effect of combinations of chlorantraniliprole and *H. bacteriophora* for control of third - instar white grubs in turf grass was assessed. The greenhouse experiments showed that the combinations had a synergistic or additive effect on the mortality of the oriental beetle, *Anomala* (=*Exomala*) *orientalis* (Waterhouse). A synergistic effect was observed by *H. bacteriophora*-chlorantraniliprole combinations on mortality of japanese beetle, *Popillia japonica* Newman, and northern masked chafer, *Cyclocephala borealis* Arrow, larvae in greenhouse experiments. Synergistic and additive effects on larval mortality were also observed in field experiments with *A. orientalis* and *P. japonica*. The progeny production of *H. bacteriophora* per dead larva showed no difference between *H. bacteriophora* alone and in the combination treatments. Chlorantraniliprole and *H. bacteriophora* were found compatible in tank mixes. The survival, infectivity and reproduction of *H. bacteriophora* were not affected by agitation in solution with 900 ppm chlorantraniliprole (Koppenhofer and Fuzy, 2008).

The compatibility of *H. indica, S. carpocapsae* and *S. glaseri* with 18 insecticides was evaluated in Brazil. Among all insecticides tested, Lorsban (chlorpyriphos), Decis (deltamethrin), Match (lufenuron), Deltaphos (deltamethrin+triazophos), Dimilin (diflubenzuron), Stallion (gama cyhalothrin), Karate Zeon (lambda cyhalothrin), Tracer (spinosad), Vexter (chlorpyriphos), Galgotrin (cypermethrin), Certero (triflumuron), and Talcord (permethrin) were compatible with the three nematode species tested under laboratory conditions (Negrisoli *et al.*, 2010).

Laboratory studies were conducted to find out the efficacy of three strains of steinernematid and two strains of heterorhabditid nematodes alone or in combination with eight conventional insecticides for the control of black cutworms, *Agrotis ipsilon* (Hufnagel). Among insecticides, tebufenozide, chlorpyriphos, isazophos, and

diazinon were more effective in controlling *A. ipsilon* larvae than RH - 0345, neem oil, azadirachtin and carbofuran and all insecticides were compatible with the EPNs (Seal *et al.*, 2010).

Studies were conducted in Czech Republic on the survival and infectivity of *S. feltiae* after the exposure to eight insecticides (a.i. kinoprene, lufenuron, methomyl, metoxyfenozide, oxamyl, piperonyl-butoxide, pyriproxyfen, tebufenozide), under laboratory conditions. In the study, *S. feltiae* was reported to be tolerant to all tested insecticides causing mortality which varied from 2.26 per cent to 18.68 per cent after 72 h (Radova, 2010).

The compatibility of profenophos, quinalphos, phenthoate, lambda cyhalothrin, chlorpyriphos, monocrotophos, triazophos and imidacloprid with a strain of *H. indica,* ICRI-18 was tested. Profenophos, triazophos and quinalphos reduced nematode survival from five to 20 per cent. Phenthoate, lambda cyhalothrin, chlorpyriphos and monocrotophos caused 70 to 90 per cent mortality to the IJs. Imidacloprid was observed as compatible with more than 90 per cent survival of IJs (Prakash *et al.*, 2011).

Effect of endosulfan and monocrotophos was assessed for the activity of IJs of *S. masoodi* (Ali *et al.*), *S. seemae* (Ali *et al.*), *S. carpocapsae* and *S. mushtaqi* (Perves). IJs pre-exposed to insecticides were tested for its infectivity against larvae of *Corcyra cephalonica* Stainton under laboratory conditions. Results showed that *S. mushtaqi* was more compatible with tested insecticides followed by *S. masoodi* and *S. seemae*. But *S. carpocapsae* was recorded as least compatible with tested insecticides. The activity of IJs was more affected with endosulfan followed by monocrotophos. The infectivity of IJs against larva of *C. cephalonica* was not much affected after 24 h of exposure to insecticides as compared to control (Rashid and Ali, 2012).

Azadiractin, chlorpyriphos, cypermethrin, fipronil, imidacloprid, malathion, thiamethoxam and chlorantraniliprole were reported as compatible with osmotically

treated and untreated IJs of *S. carpocapsae* Strain All. But bensultap, emamectin benzoate, phoxim and rotenone were harmful to the IJs of *S. carpocapsae* Strain All as reduction in infectivity of IJs to *G. mellonella* was observed (Xun *et al.*, 2012).

The compatibility of *S. carpocapsae* (POBe strain) to imidacloprid, thiamethoxam and spinosad at concentrations of 0.05 per cent and 2 per cent (for 72 h) was evaluated. IJs of *S. carpocapsae* were compatible with imidacloprid and spinosad (Kulkarni *et al.*, 2013).

The efficacy of soil treatments of three native EPNs against *Tuta absoluta* (Meyrick) larvae, pupae and adults under laboratory conditions was estimated. The compatibility of EPNs was also studied. The nematodes used for the experiments were *S. carpocapsae* (B14), *S. feltiae* (D114) and *H. bacteriophora* (DG46). The insecticides used for the study included flubendiamide (Fenos), chlorantraniliprole (Altacor) and metaflumizone (Alverde). *H. bacteriophora* was recorded to be more sensitive than *S. feltiae* and *S. carpocapsae* during the first 48 h of exposure to the three insecticides tested. The survival of the IJs of *S. carpocapsae* exposed to chlorantraniliprole and metaflumizone was not significantly different from that in the control (Pino *et al.*, 2013).

The compatibility of EPNs depend upon the strain specificity also in addition to species specificity (Laznik and Trdan, 2014).

## 2.7 Compatibility of EPNs with botanical insecticides

The effect of neem oil on the activity, penetration rate and infectivity of *Steinernema bicornutum Tallosi, Peters & Ehlers* was determined by Hussaini *et al.*, (2001b). The progeny production of two isolates of *S. bicornutum* and two *H. indica* isolates estimated by using the larvae of *G. mellonella* showed that the penetration rate of *S. bicornutum* isolates (30 - 40%) was impaired by the exposure to neem oil for 72 h. No additive or synergistic response was observed in progeny production of pesticide exposed IJs.

The effects of different formulations of neem in greenhouses on *S. feltiae* was studied. Neem as pure oil at the field recommended concentrations (5 - 10 ml/l) had no effect on the viability and virulence of *S. feltiae* up to 120 h incubation. The neem formulations, namely, nimbecidine and neem oil when mixed with a bactericidal soap (commonly used as a surfactant with neem oil) caused 13 to 25 per cent mortality of *S. feltiae*. Virulence of the nematodes was not affected by neem oil, nimbecidine or soap (Krishnayya and Grewal, 2002).

The pathogenic effect of *H. indica* and two neem based biopesticides (Neem and Nimor) on *G. mellonella* larva was estimated under laboratory conditions. The effectiveness was tested individually or in combination with *H. indica*. IJs of *H. indica* combined with Neem or Nimor resulted in 100 per cent mortality to larvae of *G. mellonella* but after 48 h of interaction (Sankara *et al.*, 2009).

Effect of aqueous suspension of botanical (Nemmarin) was studied on the activity of IJs of *S. masoodi*, *S. seemae*, *S. carpocapsae* and *S. mushtaqi*. IJs, pre exposed to insecticides were tested for its infectivity of IJs against larvae of *C. cephalonica* under laboratory condition. Nemmarin had little effect on the activity of IJs (Rashid and Ali, 2012).

## 2.8 Evaluation of botanical insecticides against *Liriomyza trifolii*

Application of 0.4 per cent crude neem extract caused significant mortality to the last instar larvae and pupae of *L. trifolii* when applied as soil drenches to chrysanthemums in greenhouses in USA. The insecticidal effects were observed for three weeks. The mortality of prepupae of the agromyzid that were reared on untreated plants and placed on neem-drenched soil was 89 per cent (Larew *et al.*, 1985).

The effectiveness of Margosan-O, a commercial formulation of neem seed extract, was tested against *L. trifolii* on greenhouse chrysanthemums. The treatments were given as systemic soil drench and foliar spray. The results suggested that the

systemic uptake of a 0.33 per cent formulation caused significant reduction in the number of pupae and adults reared from treated plants, but no reduction was observed in the number of mines. The application of Margosan-O as foliar spray (0.41, 0.84 and 1.25 %), significantly reduced the number of adults reared from treated plants and did not inhibit plant growth (Knodel *et al.*, 1986).

Various extracts of neem seed kernels were tested against preimaginal stages of *L. trifolii* on *P. vulgaris* seedlings. The aqueous, methanolic and ethanolic extracts were active in pre-infestation spraying, whereas only one per cent methanolic extract showed good activity on post infestation application (Meisner *et al.*, 1986).

Trials were conducted to show the effectiveness of in-transit treatment of chrysanthemum cuttings with neem extracts (as Margosan-O) for the control of *L. trifolii*. According to them, treatment lasted long enough to disrupt the life-cycle and was found to reduce the chances of re-infestation from subsequent generations of the agromyzid (Sanderson *et al.*, 1989).

The fecundity and longevity of adults of *L. trifolii* were studied after treating with neem seed extract on immatures. Results showed that soil drenching of azadirachtin (1 and 2 ppm) applied to infested chrysanthemums significantly reduced the fecundity of females. Longevity of males was found significantly reduced by drenching of 2 ppm azadirachtin. The fecundity of females treated with 1 ppm azadirachtin was observed to peak later, but decreased more rapidly than that of untreated females (Parkman and Peinkowski, 1990).

Azam (1991) tested the toxicity of neem oil (0.5, 0.75, 1.0, 1.25 and 1.5%) against larvae of *L. trifolii* in cucumber leaves in the laboratory. More than 80 per cent mortality was obtained to larvae and pupae of *L. trifolii* at concentrations of 1 to 1.25 per cent.

Laboratory evaluation was carried out with Neem Azal-S and Margosan-O against the adults and larvae of *L. trifolii*. At high concentration, the feeding

deterrence of both compounds was observed against the adults, and lasted for five days after treatment. Ovipositional deterrence was also observed for both formulations and the per cent ovipositional deterrent index (ODI) reached 80.7 and 52.6 for Neem Azal-S and Margosan-O (2%), respectively (Dimetry *et al.*, 1995).

The efficacy of some botanical insecticides along with chemical insecticides in laboratory against *L. trifolii* on cotton was evaluated. Neem oil 50 EC (0.3%) (TNAU formulation) was observed to cause high larval mortality at 24 hours after treatment. Significantly higher larval mortality was obtained after the application of neem oil (3%), neem seed kernel extract (5%) and *iluppai* (*Madhuca longifolia* (Koenig)) oil (3%). But neem oil (2%), neem seed kernel extract (2.5%) and pungam (*Pongamia pinnata* Linn.) oil (3%) were ineffective in causing larval mortality of *L. trifolii* (Jeyakumar and Uthamasamy, 1997).

The efficacy of six formulations of neem against *L. trifolii* on tomato was evaluated in Orissa, India. In the study, Multineem was observed as the most effective and the leaf infestation was reduced by 82.2 per cent. The least effectiveness was observed in the case of Neemazal with a reduction in infestation of 73.1 per cent, in tomato (Patanik, 1997).

Of the four concentrations (1, 2, 3 and 4%) of extracts of leaves and seeds of eight sub-tropical plants namely, *Acacia nilotica* Linn., *Annona squamosa* Linn., *A. indica*, *Boswellia sacra* Flueck, *Crotalaria juncea* L., *Jatropha dhofarica* Radcl-Sm, *Myrtus communis* L. and *Sueda aegyptiaca* (Hasselq.) against *L. trifolii* attacking cucumber (*Cucumis sativus* L.), the neem extract produced the highest mortality (above 94%) (Azam *et al.*, 2003).

The effectiveness of aqueous extracts from *Urginea maritima* L. (Liliaceae) and *Euphorbia myrsinites* L. (Euphorbiaceae), against *L. trifolii* on infested tomato was assessed in the laboratory and field. All dilutions (1:100, 1:50, and 1:25) of both plant extracts were observed to cause significant control of the leaf miner larvae and

maintained populations below those of the untreated control plants in all trials. The aqueous extracts from these two plants exhibited both translaminar and systemic activity (Civelek and Weintraub, 2004).

The study conducted at Uttar Pradesh showed that the botanical insecticide, Achook was the most effective followed by Kuchala (*Strychnos nux-vomica* L.) against *L. trifolii* (Nath and Singh, 2006).

A new cucurbitaceous glucoside, 23-O-β-D- glucopyranosyl-7 hydroxy-3-O malonyl cucurbita - 5, 24 dien- 19- al, named as momordicine V was isolated from bitter gourd (*Momordica charantia* L.) leaves in Japan. The oviposition of *L. trifolii* was significantly deterred when treated at 26.16 $\mu g/cm^2$ (Kashiwagi *et al.*, 2007).

NSKE (5%) and neem oil (3%) were recorded as the best treatments next to abamectin (0.0025%) and cypermethrin (0.01%) in reducing the infestation of *L. trifolii* with lower number of mines per leaf (Ramesh and Ukey, 2007).

The botanical formulations like Neem Azal T/S, Neem Azal T/S + TS/fort and petroleum ether extract of *Curcuma longa* L. were evaluated in the field for the management of different pests attacking broad bean including *L. trifolii* in Egypt. Reduction in the number of living *L. trifolii* larvae was observed compared to non-treated control. Neem Azal T/S+ additive (TS/fort) was observed to be the most effective in controlling leaf miners attacking broad bean in the field either by killing, deterrent or antifeedant effect (Dimetry *et al.*, 2010).

The efficacy of botanicals, neem oil (3%), karanj (*P. pinnata*) oil (3%), NSKE (5%), azadiractin 0.03 EC (3%) and neem cake at 250.00 Kg/ ha was evaluated against *L. trifolii*. NSKE was the most effective against the larval instars resulting in a mean larval mortality of 53.4 per cent and low damage level (25.50 %) (Ganapathy *et al.*, 2010).

The effectiveness of four botanical formulations, Gronim1% EC at 0.05% (Azadiractin @ 0.0005%), Achook 0.15 EC at 0.4% (Azadiractin @ 0.0006%),

aqueous extract of neem (*A. indica*). seed kernels and leaves of Naffatiya (*Ipomea carnea* Jace.) tested for their bio efficacy against *L. trifolii* revealed that Gronim was the most effective (Patel and Jhala, 2010).

The relative efficacy of different integrated pest management modules (alternate spray of chemical pesticides, biopesticides and botanicals) against *L. trifolii* was assessed. It was found that the IPM module composed of alternate spray of lambda cyhalothrin 5 EC (0.005%), *B. bassiana* at 1.25 kg/ha, abamectin 1.9 EC (0.0009%) and azadirachtin 1500 ppm at 2 ml/ l was at par with insecticidal module (Wagh and Patil, 2012).

The effect of two different doses of neem (Neem Azal T/S) *i.e.* 0.5 per cent and 0.6 per cent was verified on *L. trifolii* maggots in Turkey and confirmed that neem could control the maggots effectively (Yildirim and Baspinar, 2012).

### 2.8 Evaluation of chemical insecticides against *Liriomyza trifolii*

The management of *L. trifolii* commonly relay on the application of chemical insecticides. Studies were conducted at different places to evaluate the efficacy of different insecticides for reducing the population of *L. trifolii* in different crops. A list of chemical insecticides evaluated against *L. trifolii* and the effective ones reported by various research workers is presented in Table 3.

**Table 3. Chemical insecticides tested against *Liriomyza trifolii***

| Sl. No. | Crop | Insecticides tested | Insecticides reported as effective | Place | References |
|---|---|---|---|---|---|
| 1 | Celery | avermectin (abamectin) 0.17 kg a.i./ ha, thiocyclam 0.56 kg a.i./ ha and cyromazine 0.84 kg a.i./ ha | Cyromazine | USA | Grafius and Hayden (1988) |
| 2 | Green house vegetables | buprofezin at 0.75 g a.i./10 l | | Belgium and Sicily | Veire and Vacante (1988) |
| 3 | Gerbera | isoxathion, acephate, thiocyclam, cartap hydrochloride, cyromazine and flufenoxuron | | Japan | Saito *et al.* (1993) |
| 4 | Peas | cyfluthrin (0.005%), dimethoate (0.03%) and endosulfan (0.035%) | | Gujarat, India | Jyani *et al.* (1995) |
| 5 | Castor | chlorpyriphos (1.5%) at 3 g/ kg | | Hyderabad, India | Murthy and Prasad (1996) |
| 6 | Tomato | cypermethrin 25 EC (0.01%), endosulfan 35 EC (0.07%), dimethoate 30 EC (0.03%), dichlorvos 76 EC (0.07%), deltamethrin 2.8 EC (0.001%), malathion 50 EC (0.05%) and phorate 10 G at 1kg/ ha | cypermethrin, endosulfan and dimethoate | Maharashtra, India | Pawar *et al.* (1996) |

| Sl. No. | Crop | Insecticides tested | Insecticides reported as effective | Place | References |
|---|---|---|---|---|---|
| 7 | Tomato | fipronil 0.3% G, carbosulfan 5% G and carbofuran 3% G), bifenthrin 2.5% EC, fenpropathrin 10% EC, acephate 75% SP, pyriproxyfen 10% EC, fipronil 5% SC, imidacloprid 10% SL, carbosulfan 20% EC, methamidophos 60% SC, cypermethrin or phosphalone | bifenthrin, fenpropathrin, acephate and fipronil | Thailand | Rushtapakornchai and Petchwichit (1996) |
| 8 | Cotton | phosalone and chlorpyriphos | | Tamil Nadu, India | Jeyakumar and Uthamasamy (1997) |
| 9 | Tomato | cyromazine | cyromazine at 225g a.i./ha | Gujarat, India | Patel *et al.* (1998) |
| 10 | Watermelon | imidacloprid (0.25 ml/l), acephate (1g/l), oxydemeton-methyl (2ml/l), phosphamidon (0.5ml/l), methomyl (3ml/l), DDVP (1ml/l) and monocrotophos (1 ml/l) | Imidacloprid followed by acephate | Karnataka, India | Patil *et al.* (1999) |

| Sl. No. | Crop | Insecticides tested | | Place | References |
|---|---|---|---|---|---|
| 11 | Bhindi | Vertimec 1.8 EC (abamectin) applied at 20 g a.i./ ha | | Tamil Nadu, India | Logiswaran and Bhuvaneswari (2000) |
| 12 | Green bean | spinosad | | Tunisia | Gahbiche (2001) |
| 13 | Tomato | malathion (0.05%), DDVP (0.05%) and avermectin (0.01%) | avermectin (0.01%) | West Bengal, India | Chaudhuri and Senapathi (2001) |
| 14 | Watermelon | imidacloprid 200 SL @ 0.25 ml/l, acephate 75 SP @ 1 g/l, oxydemeton methyl 25 EC @ 2 ml/l, phosphamidon 85 WSC @ 0.5 ml/l, methomyl 12.5 SL at 1 ml/l and DDVP 76 EC @ 1 ml/l and monocrotophos 36 SL @ 2ml/l | imidacloprid and acephate at 25, 50 and 75 days after sowing | Karnataka, India | Patil *et al.* (2001) |
| 15 | Tomato | granular or wettable powder clothianidin, nitenpyram and acephate flufenoxuron EC | Clothianidin WP | Japan | Ozawa *et al.* (2002) |

| Sl. No. | Crop | Insecticides tested | Insecticides reported as effective | Place | References |
|---|---|---|---|---|---|
| 16 | Tomato | abamectin (Vertimec 1.8 EC) at 5.00, 7.00 and 10.00 g a.i./ha, Fluvalinate 25 EC at 37.50 g a.i./ha, profenofos 50 EC at 500 g a.i./ha, polytrin C 44 EC (cypermethrin+ profenofos) at 440 g a.i./ha | Abamectin at 10 g a.i./ha | Maharashtra, India | Walunj *et al.* (2002) |
| 17 | Tomato | bensultap and cyromazine | Bensultap @ 3 kg/ha | U.K. | Civelek and Weintraub (2003) |
| 18 | French bean | phosphalone, chlorpyriphos+ cypermethrin, triazophos, endosulfan, fenobucarb and monocrotophos | Triazophos, chlorpyriphos + cypermethrin and phosphalone | Orissa, India | Saradhi and Patnaik (2003) |
| 19 | Tomato | endosulfan (0.07%) + NSKE (3%), methomyl (0.05%) + NSKE, deltamethrin (0.028%) + honge oil, endosulfan, methomyl, deltamethrin, profenofos (0.05%) | Deltamethrin + honge oil followed by deltamethrin alone and methomyl+NSKE | Benagaluru, India | Reddy and Kumar (2004) |

| Sl. No. | Crop | Insecticides tested | Insecticides reported as effective | Place | References |
|---|---|---|---|---|---|
| 20 | Tomato | colfas (40% ethion+5% cypermethrin), Achook, Kuchala | | Uttar Pradesh, India | Nath and Singh ( 2006) |
| 21 | Gerbera | confidor 200 SL (imidacloprid), trigard 75 WP (cyromazine), vertimec (abamectin), sumialfa 5 FL (esfenvalerate) in | Confidor 200 SL in irrigation water, Trigard 75 WP and Vertimec | Croatia | Paradikovic *et al.* (2006) |
| 22 | Tomato and french bean | phosalone (0.07% a.i.), chlorpyriphos + cypermethrin (0.055%), triazophos (0.05%), endosulfan (0.07%), fenobucarb (0.10%) and monocrotophos (0.08%) | chlorpyriphos + cypermethrin followed by triazophos | Bhubaneswar, India | Saradhi and Patnaik (2006) |
| 23 | Tomato | Abamectin (0.002%) followed by Spinosad (0.005%) and endosulfan | | Maharashtra, India | Ramesh and Ukey (2007) |
| 24 | Tomato | imdacloprid 17.8 EC (0.004%), thiamethoxam 25 WG (0.003%), quinalphos 25 EC (0 .05%), spinosad 45 EC (0.01%) and endosulfan 35 EC (0.05%) | Spinosad followed by thiamethoxam and imidacloprid | Nagpur, India | Wankhede *et al.* (2007) |

| Sl. No. | Crop | Insecticides tested | Insecticides reported as effective | Place | References |
|---|---|---|---|---|---|
| 25 | Chrysanthemum | Wilt-Pruf® and VaporGard® | | Canada | Conroy *et al.* (2008) |
| 26 | Castor | carbaryl 50 WP (0.2%), endosulfan 35 EC (0.05%), triazophos 40 EC (0.05), spinosad 45 SC (0.018%) and fipronil 5 SC (0.01%) | spinosad 45 SC (0.018%) | Solapur, India | Akashe *et al.* (2009) |
| 27 | Onion | chlorpyriphos+BPMC | | Philippines | Arida *et al.* (2009) |
| 28 | Cowpea | chlorpyriphos 20 EC (0.05%), triazophos 40 EC (0.04%), endosulfan 35 EC (0.07%) malathion 50 EC (0.05%), dichlorvos 76 EC (0.05%) and monocrotophos 36 SL (0.04%) | Chlorpyriphos followed by triazophos | Dharwad, India | Ganapathy *et al.* (2010) |
| 29 | Tomato | spinosad 45 SC (84 g a.i./ha) cypermethrin (60 g a.i./ha), lambda cyhalothrin (15 g a.i./ha) and chlorpyriphos (200 g a.i./ha) | | Ludhiana, India | Sharma and Chandel (2011) |

| Sl. No. | Crop | Insecticides tested | Insecticides reported as effective | Place | References |
|---|---|---|---|---|---|
| 30 | Tomato | cyazypyr 10% OD imidacloprid 17.8 SL and fipronil 5% SC | | West Bengal, India | Mandal (2012) |
| 31 | Green house vegetables | abamectin, chlorpyriphos, cyromazine, indoxacarb and spinosad | Chlorpyriphos, indoxacarb | Serbia | Saryazdi *et al.* (2012) |
| 32 | Tomato | profenofos 50 EC (1ml/l), endosulfan 35 EC (2ml/l), dimethoate 50 EC (1.5 ml/l), profenofos 40% + cypermethrin 4% (0.6 ml/l), cypermethrin 25 EC (0.3 ml/l), imidacloprid 600 FS (0.6 g/l), thiamethoxam 20 SG (0.6g/l) | profenofos 40% + cypermethrin 4% | Uttar Pradesh, India | Rai *et al.* (2013) |
| 33 | Cucumber | cartap hydrochloride. imidacloprid, thiamethoxam and spinosad. | | Pune, India | Hanumappa and Chavan (2013) |

# Materials and Methods

# 3. MATERIALS AND METHODS

The present study on the "Biotic agents for the management of American serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera:Agromyzidae)" was carried out at the Department of Agricultural Entomology and Instructional farm, College of Horticulture, Vellanikkara, Kerala Agricultural University, during 2011 - 2013. The study included a survey for the collection of indigenous natural enemies of *L. trifolii*, evaluation of entomopathogenic nematodes (EPNs) in the laboratory, pot culture and field for their efficacy against *L. trifolii*. The compatibility of EPNs with commonly used insecticides was also assessed. The materials used for the study and the methods followed are described hereunder.

## 3.1 Collection of indigenous natural enemies of *Liriomyza trifolii*

### 3.1.1 Survey

Purposive surveys were conducted during the months of January to March, 2011 to collect the indigenous natural enemies of *L. trifolii* when infestation of *L. trifolii* was high in Kerala. Roving surveys were conducted in cowpea (*Vigna unguiculata* (L.)), ash gourd (*Benincasa hispida* Thunb.), snake gourd (*Trichosanthes cucumerina* L.), bitter gourd (*Momordica charantia* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai)) and pumpkin (*Cucurbita moschata* Duchesne) growing fields of three districts, namely, Ernakulam, Kottayam and Thrissur. Two block panchayats were selected from each district panchayats and from each block panchayats, two locations were surveyed. The fields which were not sprayed with insecticides were only selected for the surveys as the application of insecticides reduced the emergence of parasitoids which were the major natural enemies of *L. trifolii*. The infested leaves were collected from the vegetable crops in polythene covers and were brought to the laboratory. In the laboratory infested leaves were

examined for the presence of parasitized maggots in the leaves. Details of the locations and crops surveyed are given in Table 4.

**Table 4. Details of the locations and crops surveyed for collection of natural enemies of *Liriomyza trifolii***

| District panchayats | Block panchayats | Locations | Crops surveyed |
|---|---|---|---|
| Thrissur | Pazhayannur | Elanad | Cowpea |
| | | Pazhayannur | Bitter gourd |
| | Ollukkara | Madakkathara Vellanikkara | Cowpea, snake gourd, bitter gourd, watermelon |
| | | Pananchery | Snake gourd |
| Ernakulam | Vadavukodu | Kadakkanad Mazhuvannur | Cowpea, snake gourd |
| | | Mangalathunada | Cowpea |
| | Mulanthuruth | Mulanthuruthy | Cowpea |
| | | Thiruvangulam | Ash gourd |
| Kottayam | Madapally | Paippad | Cowpea, pumpkin |
| | | Nalukody | Cowpea |
| | Pallom | Puthupally | Ash gourd |
| | | Manganam | Cowpea |

### 3.1.2 Entomopathogens of *Liriomyza trifolii*

#### 3.1.2.1 Isolation of entomopathogens from *Liriomyza trifolii*

Infested leaves were examined under the microscope to locate dead maggots. The leaf mines containing the dead maggots were carefully opened by using a sharp needle and the dead maggots were taken. The cadavers were then surface sterilized using sodium hypochlorite (1%) for 30 seconds. They were washed with sterile

distilled water for three times and were kept over a sterilized tissue paper for drying. After drying these cadavers were placed on PDA medium (Annexure I) in Petri dishes and kept for incubation till the development of bacterial or fungal colony.

### 3.1.2.2 Pathogenicity of the isolated microorganisms

Leaf bits containing the maggots of *L. trifolii* were cut and inoculated with the microbial suspension by leaf spread method for testing the pathogenicity of isolated microorganisms. Observations were taken till the death or pupation of the treated maggots.

### 3.1.3 Parasitoids of *Liriomyza trifolii*

The parasitization of *L. trifolii* on cowpea, ash gourd, snake gourd, pumpkin, bitter gourd and watermelon was studied. Infested leaves of the above crops collected during the survey were examined for the presence or larvae or pupae of any parasitoid (Plate 1). The per cent parasitization on *L. trifolii* was calculated for the crop plants surveyed from different districts, according to the formula given below.

$$\text{Per cent parasitization} = \frac{\text{Number of parasitized maggots} \times 100}{\text{Total number of maggots collected}}$$

### 3.1.4 Predators of *Liriomyza trifolii*

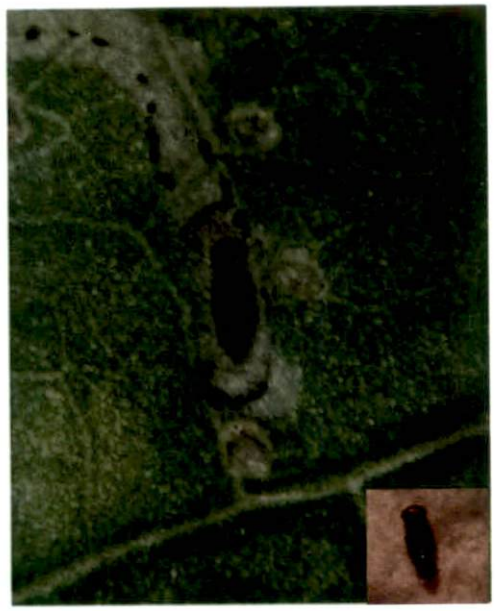
The *L. trifolii* infested vegetable fields surveyed from Thrissur, Ernakulam and Kottayam districts were carefully examined for the presence of predators and were recorded.

### 3.1.5 Extent of damage by *Liriomyza trifolii*

The extent of infestation of *L. trifolii* from six vegetable crops grown in Madakkathara and Vellanikkara of Thrissur district was estimated. Ten infested plants from each crop were randomly selected in the crop fields. From each plant, 15 leaves were selected at random for taking observations on damage. Scoring was done according to the infested area present on each leaf as given below in a 0 – 4 scale.

`Plate 1. Mines containing parasitized maggots of *Liriomyza trifolii*

a) Larva of the endoparasitoid



b) Pupa of endoparasitoid

**Table 5. Scoring for *Liriomyza trifolii* infestation on leaf**

| Per cent leaf area infested | Score | Infestation intensity |
|---|---|---|
| 0 | 0 | No infestation |
| 1 - 15 | 1 | Low infestation |
| 16 - 30 | 2 | Medium infestation |
| 31 - 50 | 3 | High infestation |
| > 51 | 4 | Severe infestation |

The infestation index was also calculated as given below (Wheeler, 1969).

$$\text{Infestation index} = \frac{\text{Sum of all scores}}{\text{Number of scores} \times \text{Maximum score}} \times 100$$

### 3.1.6 Emergence of parasitoids from *Liriomyza trifolii* on different crops

Periodic collection of dead insects was made from the fields surveyed. Infested leaves containing healthy and parasitized larvae were collected. Observations were made on the number of healthy and parasitized larvae.

For the emergence of parasitoids, the leaves containing parasitized larvae were placed in polythene covers having pin holes and kept undisturbed in the laboratory. The parasitoids emerged were collected in test tubes and killed using ethyl acetate. The parasitoids emerged from different crops were counted and kept separately. The dead parasitoids were preserved in 70 per cent ethanol for identification.

For estimating the per cent emergence of parasitoids from each crop, infested leaves containing 120 parasitized larvae were collected and the leaves were placed in polythene covers with pinholes. Three replications were maintained for each crop at a rate of 40 parasitized larvae/ replication. The per cent parasitoid emergence was calculated as follows.

$$\text{Per cent parasitoid emergence} = \frac{\text{Number of parasitoids emerged}}{\text{Total number of parasitized larvae}} \times 100$$

### 3.1.7 Parasitism of *Liriomyza trifolii* in different vegetable crops

The parasitoids emerged from each vegetable crops were sorted and number of parasitoid species was recorded. The rate of parasitism of *L. trifolii* in different vegetable crops in Thrissur district was estimated.

## 3.2 Laboratory evaluation of the entomopathogenic nematodes against *Liriomyza trifolii*

Microorganisms isolated from the field collected larval cadavers were observed as non-pathogenic to the larvae of *L. trifolii*. Hence the soil isolated EPNs were evaluated for their efficacy against *L. trifolii*. Laboratory rearing of *L. trifolii* was done to get sufficient number of healthy larvae for the evaluation.

### 3.2.1 Laboratory rearing of *Liriomyza trifolii*

#### 3.2.2.1 Rearing in polythene bags

Cowpea seeds (var. Anaswara) were sown in plastic pots (Plate 2a). When the seedlings were nine days old, they were covered with polythene covers having pin holes. Adults of *L. trifolii* were released in 2 ♀: 1 ♂ ratio into the covers and these were tied tightly to the plastic pot to prevent the escape of adult flies. The pinholes in the polythene covers facilitated aeration and helped in preventing the accumulation of moisture inside the polythene covers. One per cent honey solution mixed with Vitamin E was given as food for the adult flies. Female flies oviposited on the leaves. In order to get more larvae, the seedlings were changed once in two days. The development of mines was observed. The prepupae emerged from the mines pupated inside the polythene covers within few hours. The pupae were collected with the help of camel hair brush and placed in small glass vials, and kept at room temperature till adult emergence. The adults emerged were again released to cowpea seedlings to

**Plate 2. Rearing of *Liriomyza trifolii***

a) Polythene cages


b) Rearing cage

maintain the laboratory culture of the insect. Diluted honey solution mixed with Vitamin E was given as food for the adults. Cotton pieces soaked in this solution were placed inside the polythene bags.

### 3.2.2.2 Rearing cage

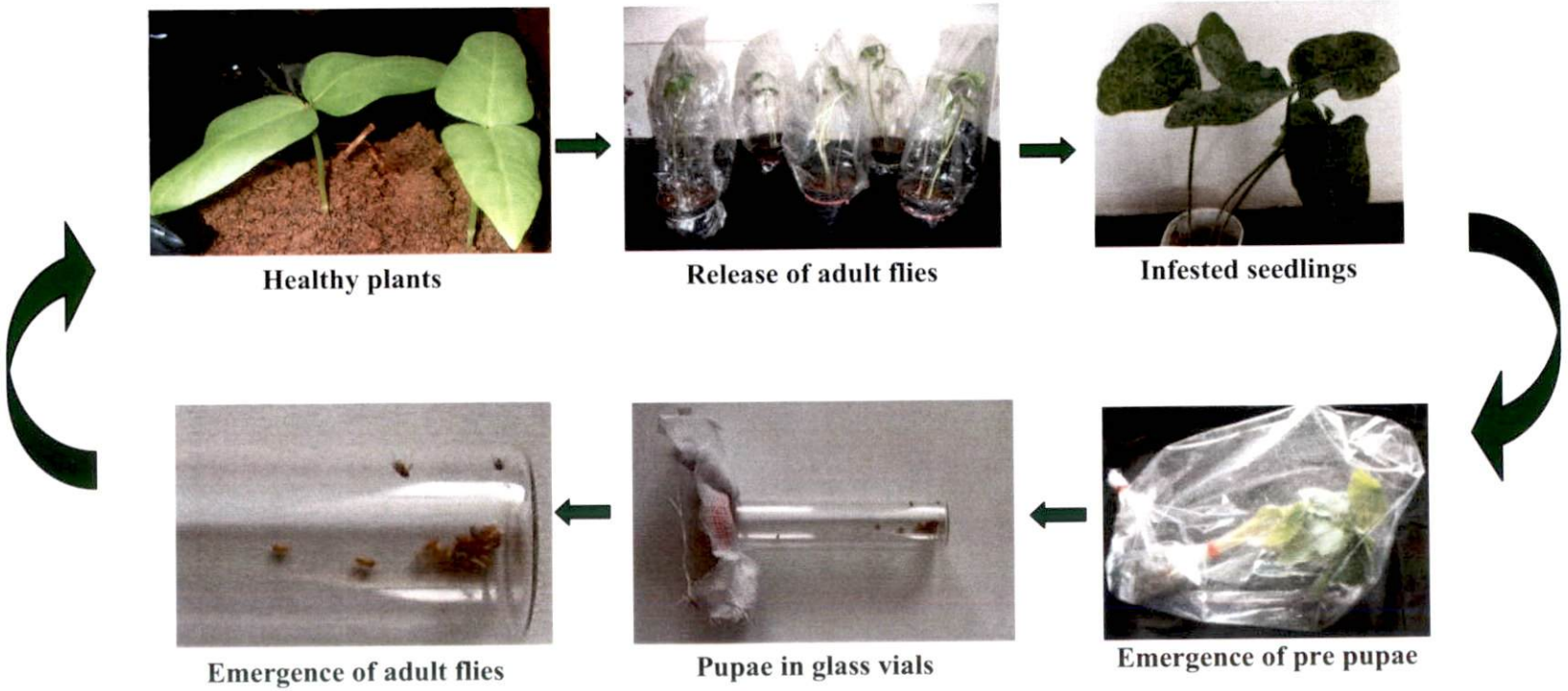
A low cost rearing cage of size 40×30×30 $cm^3$ was fabricated with cardboard, polythene sheet and mull cloth (Plate 2b) to rear *L. trifolii* during the off season when population of leaf miner adults was very low in the field. The front side of cage was provided with a round opening of 17 cm diameter and a muslin cloth sleeve was stitched and pasted around the opening. This cloth sleeve opening was used for handling the insects and plants inside the cage. The distal end of cloth sleeve was kept closed while not in use. A window of size 22×13 $cm^2$ was cut on the sides of the cage and was closed by fixing muslin cloth of suitable size.

On the back side, two rectangular windows of 14 cm length and 12.5 cm breadth were cut and were covered with plastic sheet with pin holes for aeration and also for the entry of sunlight. The top of the cage was covered with plastic sheets with pin holes to provide aeration and sunlight. The joints of the cage were carefully sealed from inside by cello tapes to prevent the escape of adult flies.

Cowpea seeds (var. Anaswara) were sown in disposable cups of 6 cm height and 6.5 cm diameter. Three fourth of the cup was filled with potting mixture prepared in the ratio 1:1:1 with soil, sand and cow dung. Three seeds were sown in each cup. Nine days old seedlings were used for the culturing of *L. trifolii*. After the adult emergence, the flies were separated as males and females. Ten to fifteen pairs of newly emerged adults were released in to the rearing cage for oviposition.

Steps in the mass rearing of *L. trifolii* are given in Plate 3.

Plate 3. Steps in the mass rearing of *Liriomyza trifolii*



### 3.2.2 Soil isolation and purification of entomopathogenic nematodes

#### 3.2.2.1 Rearing of Greater wax moth, *Galleria mellonella*

The last instar larvae of *G. mellonella* reared in the laboratory in artificial diet (Annexure II) was used as the trap insect to isolate EPNs from soil.

#### 3.2.2.2 Collection of soil samples

Soil samples were collected from two locations each from each district panchayats. From each location, four sites were selected and samples were collected from three spots in each site. Soil samples were collected after removing the top soil with a shovel from a depth of 10 to 20 cm. Samples were collected from undisturbed area where the agricultural operations were not carried out. The details of the sites of collection of soil samples are given in Table 6. Soil samples were collected in polythene bags and were labelled properly. The soil samples were brought to the laboratory for the isolation of EPNs.

**Table 6. Details of the collection of soil samples**

| Districts | Locations | Sites | Type of field | Number of samples |
|---|---|---|---|---|
| Thrissur | Kannara | 4 | Uncultivated | 12 |
| | Vellanikkara | 4 | Uncultivated | 12 |
| Ernakulam | Kadakkanad | 4 | Uncultivated | 12 |
| | Peruvamuzhi | 4 | Uncultivated | 12 |
| Kottayam | Puthupally | 4 | Uncultivated | 12 |
| | Manganam | 4 | Uncultivated | 12 |

### 3.2.2.3 Isolation and extraction of entomopathogenic nematodes from soil samples

Soil samples were brought to the laboratory and were transferred to plastic jars for the isolation of EPNs. The soil samples were baited with last instar larvae of *G. mellonella* and were covered with muslin cloth to facilitate aeration and to prevent the escape of larvae (Plate 4). Pieces of muslin cloth were placed on the top of soil also to prevent larvae from coming out of the soil samples and to ensure contact with third stage juveniles of EPNs, if any, in the soil in the containers. The mortality of *G. mellonella* was observed for one week. The dead insects were collected daily from the soil. After surface sterilization with sodium hypochlorite (1%), cadavers were transferred to dry filter paper kept in Petri dishes for incubation.

After two to three days, the dead larvae were transferred to White's trap (White, 1927) for emergence of nematodes (Plate 5). For harvesting infective juveniles (IJs), watch glass with the cadavers was removed and extraction was carried out by adding 50 ml of sterile distilled water or 0.1 per cent formalin. Watch glass was replaced after the harvesting of IJs. Extraction of nematodes was done daily until the production was stopped (3 - 4 days).

The harvested nematodes were allowed to settle in a beaker. The supernatant was decanted and more sterile distilled water was added till the suspension became clean (3 - 4 times). The IJs were stored in sterilized soil and also in aqueous suspension provided with an aquarium aerator.

### 3.2.3 Pathogenicity studies of entomopathogenic nematodes to *Liriomyza trifolii*

The isolated EPNs (3.2.1.3) were tested for their pathogenicity to the larvae of *L. trifolii*. Different doses of EPNs, *viz*., 10, 20, 30, 40 and 50 IJs/ maggot were tested on ten numbers each of second instar maggots of *L. trifolii* in penicillin vials. Leaf bits containing the maggots were cut and placed in penicillin vials. Mortality of the maggots was recorded after 12 h.

**Plate 4. Isolation of entomopathogenic nematodes from soil**

**Collected soil sample**

**Soil baited with *Galleria mellonella***

**Covered jars**

**Plate 5. White's trap for the extraction of entomopathogenic nematodes**

White's trap technique

Emerging infective juveniles

### 3.2.4. Identification of entomopathogenic nematodes

The isolated EPNs were identified from Division of Nematology, Indian Agricultural Research Institute, New Delhi.

### 3.2.5 Bioefficacy of entomopathogenic nematodes against *Liriomyza trifolii*

#### 3.2.5.1 Mass multiplication of isolated entomopathogenic nematodes

Mass multiplication of the EPNs was done on last instar larvae of greater wax moth, *G. mellonella*. The laboratory rearing of *G. mellonella* was carried out in artificial diet (Annexure II) (Singh, 1994). The fifth instar larvae were used for the multiplication of EPNs.

Two millilitre of EPN suspension containing 1000 IJs (@ 500 IJs/ ml) was applied on Whatman No. 1 filter paper placed in the Petri dish. Ten healthy larvae were released to this Petri dish. After 24 h, dead larvae were separated and were kept on a dry filter paper and incubated for two days. On the third day, larvae were transferred to White's trap for the extraction of EPNs for conducting further studies.

#### 3.2.5.2 Maintenance of the soil isolated EPNs

The soil isolated EPNs were maintained in sterilized soil and in sterile distilled water. The extraction of nematodes was done using distilled water and the extracted nematodes were mixed with the soil in plastic jar, when the storage was done in soil.

Entomopathogenic nematodes were also stored in distilled water with intermittent aeration and change of water in glass beakers. The EPNs could live in distilled water when aeration was provided. The nematodes were maintained healthy and virulent by inoculating healthy larvae of *G. mellonella* once in three weeks.

#### 3.2.5.3 Bioefficacy of entomopathogenic nematodes against *Liriomyza trifolii*

The soil isolated EPNs were tested for their efficacy against *L. trifolii* in the laboratory along with *Steinernema bicornutum* obtained from Banana Research Station, Kannara, Thrissur and *Heterorhabditis indica* obtained from Indian

Cardamom Research Institute (ICRI), Myladumpara, Idukki. Five different doses, *viz.*, 10, 15, 20, 25 and 30 IJs/ maggot were tested and three replications were maintained. The IJs were counted individually and placed on to the small circular filter paper disc placed at the bottom of the penicillin vials. Cowpea leaf bit containing healthy maggots were placed on the filter paper at a rate of one maggot per vial (Plate 6). Three replications were maintained for each treatment and ten maggots were used per replication. Observation on mortality of maggots was taken at 12, 18, 24 and 30 h after treatment using a binocular stereoscope. The data obtained was statistically analyzed by using SPSS 17.00 software.

### 3.2.6 Determination of median lethal concentrations

The observations on mortality of the larvae of *L. trifolii* taken at different intervals were used to work out $LC_{50}$, $LT_{50}$ and $LT_{90}$ values.

## 3.3 Pot culture evaluation of entomopathogenic nematodes

The most effective EPN obtained in the laboratory studies was further evaluated against *L. trifolii* in a pot culture experiment. When the leaf miner infested plants were kept in open field condition, parasitization was observed. Hence the pot culture experiment was carried out in polyhouse of AINP on Agricultural Acarology, Department of Agricultural Entomology, College of Horticulture, Thrissur to prevent the parasitization of maggots. The experiment was carried out in Completely Randomized Design with seven treatments and three replications (Plate 7). Cowpea seedlings which were artificially infested inside the rearing cages were planted in pots. Seedlings were selected in such a way that the number of mines per pot was ten numbers. Number of live maggots present in each pot was accurately recorded. The dose of EPNs was based on the $LC_{90}$ value estimated in the laboratory. The details of treatments applied are given in Table 7. Observations on the mortality of larvae were taken for five days after treatment. Statistical analysis of the data obtained was

**Plate 6. Laboratory evaluation of entomopathogenic nematodes against *Liriomyza trifolii***

**Penicillin vials containing *Liriomyza trifolii* and EPNs**

**Plate 7. Pot culture evaluation of entomopathogenic nematodes against *Liriomyza trifolii***


a) Polyhouse - Chamber 1

b) Polyhouse - Chamber 2

carried out by using SPSS 17.00 software. Other natural enemies of *L. trifolii* occurred in polyhouse were also recorded.

### 3.3.1 Assessment of strength of entomopathogenic nematode suspension

The concentration of EPNs in the stock solution was assessed using a counting dish and a tally counter. After the harvest of EPNs from the cadavers, the nematode suspension was made into a known aliquot and was mixed thoroughly. Fifty microlitre (µl) of nematode suspension was drawn out from the stock solution and placed in the nematode counting dish. It was diluted with five millilitre (ml) distilled water for the ease of counting. This was kept under the microscope and the counting was done using a tally counter. The required number of EPNs was taken by adjusting the quantity of nematode suspension taken from the stock solution.

**Table 7. Treatments in pot culture evaluation of entomopathogenic nematodes against *Liriomyza trifolii***

| Treatment | Dose | Source/ Manufacturer |
|---|---|---|
| $T_1$ : *Steinernema carpocapsae* Isolate -1 (foliar application) | 16 IJs/-maggot | Isolated from soil (Kannara, Thrissur) |
| $T_2$ : *Steinernema bicornutum* (foliar application) | 30 IJs/ maggot | BRS, Kannara |
| $T_3$ : *Heterorhabditis indica* (foliar application) | 32 IJs/ maggot | ICRI, Myladumpara |
| $T_4$ : Formulation of EPN, *H. indica* (soil application) (Soldier®) | 8 lakhs IJs/ pot | Multiplex Biotech Ltd., Bengaluru |
| $T_5$ : *Beauveria bassiana* (foliar application) | $1\times10^7$ spores/ ml | AICRP on BCCP & W, College of Horticulture, Thrissur |
| $T_6$ : Azadirachtin 1 EC @ 0.005% (foliar application) | 5 ml/ l | P.J. Margo Private Ltd., Tumkur |
| $T_7$ : Untreated control | | |

## 3.4 Field efficacy of entomopathogenic nematodes against *Liriomyza trifolii*

### 3.4.1 Site of the study

The most effective EPN observed in the pot culture study was further evaluated for its bioefficacy against *L. trifolii* in the field by conducting a field experiment at the Instructional Farm, College of Horticulture, Kerala Agricultural University during November to December, 2013.

The experiment was laid out in Randomized Block Design (Plate 8) with five treatments and four replications. Cowpea (var. Anaswara) plants were raised at a spacing of 30×60 cm in plots of size 2×2 $m^2$ and number of plants per plot was 18 at a rate of six plants/ row. Spacing and fertilizer recommendations were given as per Package of Practices Recommendations (P.O.P.) (KAU, 2011). Pre treatment counts were recorded after the infestation of *L. trifolii.* Details of treatments are given in Table 8.

The most effective EPN, *H. indica* was tested with a commercial formulation of *H. indica*, the P.O.P recommended insecticide - azadiractin and a commonly used newer generation insecticide - fipronil along with an untreated control. The treatments were applied to the second instar larvae of *L. trifolii* in the field.

Observations on the mortality of the maggots were recorded daily for five days after the treatment application. The number of dead maggots inside the mines was recorded. The data obtained was statistically analyzed by using M STAT software.

**Plate 8. Experimental field**

**Table 8. Treatments in the field evaluation of entomopathogenic nematodes against *Liriomyza trifolii* in cowpea**

| Treatments | Dose | Source /Manufacturer |
|---|---|---|
| $T_1$ : *Heterorhabditis indica* (foliar application) | 32 IJs/ maggot | ICRI, Myladumpara |
| $T_2$ : Formulation of EPN, *H. indica* (soil application) | 2 billion IJs/ ha | Multiplex Biotech Ltd., Bengaluru |
| $T_3$ : Fipronil 5 SC @ 0.002% (foliar application) | 0.4 ml/l | Bayer Crop Science, Thane (West), India. |
| $T_6$ : Azadirachtin 1 EC @ 0.005% (foliar application) | 5 ml/ l | P.J. Margo Private Ltd., Tumkur |
| $T_7$ : Untreated control | | |

### 3.5 Compatibility of entomopathogenic nematodes against insecticides

The compatibility of pot culture tested entomopathogenic nematodes, namely, *S. carpocapsae* Isolate - 1, *S. bicornutum* and *H. indica* with ten commonly used insecticides was tested under laboratory conditions. The insecticides used for the study are given in Table 9.

Freshly extracted EPNs were used for the study. Three replications were maintained for each treatment at a rate of 1000 IJs/ replication. The IJs were picked up individually and placed in small plastic bottles which contained small quantity of distilled water. This nematode suspension was made up to 10 ml. The quantity of insecticide (recommended concentration) required to make 10 ml insecticide solution was added to nematode suspension. Ten milliliters of distilled water containing 1000 IJs was kept as untreated control. The plastic bottles containing treated IJs and control were kept undisturbed at room temperature. Observations for mortality of IJs were taken at 24, 48 and 72 hours after treatment (HAT) under a stereomicroscope. The

whole solution from each replication was poured on to the counting dish and was observed for mortality. While taking observation, the nematodes which did not move even after prodding were considered as dead.

The per cent mortality was calculated according to the following formula.

$$\text{Per cent mortality of the IJs} = \frac{\text{Number of dead IJs}}{\text{Total number of IJs}} \times 100$$

The mortality occurred in control was corrected with Abbott's formula (Abbott, 1925).

$$\text{Corrected mortality (\%)} = \frac{T - C}{100 - C} \times 100$$

Where,

T = Per cent mortality in treatment

C = Per cent mortality in control

The data on the mortality of the IJs were statistically analyzed by using SPSS 17.00 software.

### 3.5.2 Virulence of entomopathogenic nematodes

Virulence of infective juveniles exposed to insecticides was tested in the laboratory for pathogenicity and ability for multiplication inside the host larva. Last instar larvae of *G. mellonella* were used for the study. The nematodes in the insecticide solutions were filtered through Whatman No. 1 filter paper which was made in the form of a cone. The nematodes were washed three times with distilled water to remove insecticide residues deposited on their body. The IJs were carefully washed out off the filter paper into plastic beakers and were kept suspended in distilled water for 24 h. After 24 h, 500 healthy IJs were introduced into filter paper

kept in the Petri dish to which healthy larvae of *G. mellonella* were released at a rate of five larvae/ Petri dish. Three replications were maintained for each treatment. The Petri dishes were kept undisturbed at room temperature. Observations were taken for larval mortality at 24 h interval upto 5 days. Dead larvae were separated and were placed on dried filter paper inside Petri dishes. Later the cadavers were transferred to White's trap for the extraction of nematodes as described earlier to assess the multiplication of insecticide treated EPNs inside the cadavers.

**Table 9. Insecticides used for the compatibility studies**

| Sl. No. | Generic name of insecticides | Trade name of insecticides | Strength of formulations | Per cent concentration | Chemical class | Mode of action |
|---|---|---|---|---|---|---|
| 1 | Chlorantraniliprole | Coragen | 18.5 SC | 0.005 | Phthalic acid diamide | Ryanodine receptor modulator |
| 2 | Buprofezin | Applaud | 25 SC | 0.04 | Chitin Synthesis Inhibitor | Inhibitors of chitin biosynthesis |
| 3 | Spinosad | Tracer | 45 SC | 0.009 | Spinosyn | Nicotinic acetylcholine receptor agaonist |
| 4 | Imidacloprid | Confidor | 200 SL | 0.006 | Neonicotinoid | Nicotinic acetylcholine receptor agaonist/ antagonist |
| 5 | Fipronil | Regent | 5 SC | 0.002 | Fiprole | GABA-gated chloride channel antagonist |
| 6 | Azadirachtin | Econeem plus | 1 EC | 0.005 | Neem based | Insect growth regulator |
| 7 | Chlorpyriphos | Calban | 20 EC | 0.05 | Organophosphate | Acetylcholine esterase inhibitor |
| 8 | Quinalphos | Ekalux | 25 EC | 0.05 | | |
| 9 | Dimethoate | Rogor | 30 EC | 0.04 | | |
| 10 | Malathion | Malathion | 50 EC | 0.10 | | |

# Results

# 4. RESULTS

The results of the study on "Biotic agents for the management of American serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)" carried out at the Department of Agricultural Entomology and Instructional farm, College of Horticulture, Vellanikkara during 2011 to 2013 are presented here.

## 4.1 Collection of indigenous natural enemies of *Liriomyza trifolii*

### 4.1.1 Survey

The survey carried out from different vegetable growing fields of Thrissur, Ernakulum and Kottayam districts of Kerala from January 2011 to March 2011 revealed the occurrence of various natural enemies, *viz*., entomopathogens, parasitoids and predators of *L. trifolii*.

### 4.1.2 Entomopathogens of *Liriomyza trifolii*

#### 4.1.2.1 Isolation of entomopathogens from *Liriomyza trifolii*

Six microorganisms were isolated from the cadavers of *L. trifolii* (Table 10). Five bacterial and a fungal isolate were obtained from the maggots of *L. trifolii* which infested cowpea (*Vigna unguiculata*), snake gourd (*Trichosanthes cucumerina*) and the weed plant, *Spilanthes calva* L. One species of fungus was obtained from the un emerged pupa of *L. trifolii*.

**Table 10. Microorganisms isolated from *Liriomyza trifolii***

| Sl. No. | Stage of *L. trifolii* | Microorganisms isolated | Colour | Crop | Place |
|---|---|---|---|---|---|
| 1 | Maggot | Bacterium | White | Cowpea | Thrissur |
| 2 | Maggot | | | Snake gourd | Thrissur |
| 3 | Maggot | | | *Spilanthes calva* | Thrissur |
| 4 | Maggot | Bacterium | Orange | Cowpea | Kottayam |
| 5 | Maggot | Bacterium | Red | *S. calva* | Thrissur |
| 6 | Pupa | Fungus | White | Snake gourd | Thrissur |

#### 4.1.2.2 Pathogenicity of isolated microorganisms

Pathogenicity of the isolated microorganisms was tested and is given in Table 11. The maggots treated with the isolated microorganisms could pupate. Healthy adults emerged from 92.5 per cent pupae. Hence the microorganisms isolated were considered as secondary pathogens developed on *L. trifolii* and were discarded.

### 4.1.3 Parasitoids of *Liriomyza trifolii*

#### 4.1.3.1 Extent of parasitization in *Liriomyza trifolii*

The survey revealed the occurrence of high amount of parasitism in the vegetable crops surveyed. The per cent parasitization of the larvae of *L. trifolii* estimated from different crops during the survey (January - March, 2011) conducted in three districts, namely, Thrissur, Ernakulam and Kottayam is presented in Table 12.

**Table 11. Effect of artificial inoculation of the isolated microorganisms on maggots of *Liriomyza trifolii***

| Sl. No. | Microorganisms obtained | Colour of colony | No. of maggots used | Per cent mortality | No. of maggots pupated | No. of pupae hatched |
|---|---|---|---|---|---|---|
| 1 | Bacterium | White | 20 | 0.00 | 20 | 18 |
| 2 | Bacterium | | 20 | | 20 | 16 |
| 3 | Bacterium | Orange | 20 | | 20 | 20 |
| 4 | Bacterium | Red | 20 | | 20 | 20 |
| 5 | Bacterium | | 20 | | 20 | 19 |
| 6 | Fungus | White | 20 | - | - | 18 |

**Table 12. Rate of field parasitism on maggots of *Liriomyza trifolii* on vegetable crops (January - March, 2011)**

| Month and year | Districts surveyed | Locations surveyed | Crops surveyed | No. of live mines/ plant (average)* | Per cent parasitism |
|---|---|---|---|---|---|
| January, 2011 | Thrissur | Madakkathara | Bitter gourd | 20.00 | 27.70 |
| | | Madakkathara | Watermelon | 10.00 | 21.24 |
| | Ernakulam | Kadakkanad | Cowpea | 15.00 | 28.94 |
| | | Mangalathunada | Cowpea | 10.00 | 42.10 |
| | Kottayam | Manganam | Ash gourd | 7.00 | 10.96 |
| | | Manganam | Cowpea | 5.00 | 46.78 |
| February, 2011 | Thrissur | Pananchery | Snake gourd | 23.00 | 28.27 |
| | | Elanad | Cowpea | 17.00 | 22.01 |
| | | Pazhayannur | Bitter gourd | 13.00 | 57.40 |
| | Ernakulam | Mazhuvannur | Snake gourd | 30.00 | 24.74 |
| | | Mulamthuruthy | Cowpea | 14.00 | 58.99 |
| | Kottayam | Paippadu | Cowpea | 4.00 | 28.80 |
| March, 2011 | Thrissur | Vellanikkara | Cowpea | 34.00 | 54.10 |
| | | Vellanikkara | Watermelon | 64.00 | 17.04 |
| | Ernakulam | Kadakkanad | Cowpea | 15.00 | 25.02 |
| | | Kadakkanad | Cowpea | 7.00 | 31.87 |
| | | Mulamthuruthy | Cowpea | 11.00 | 31.84 |
| | | Thiruvamkulam | Ash gourd | 21.00 | 27.48 |
| | Kottayam | Paippad | Pumpkin | 7.00 | 21.22 |
| | | Nalukody | Cowpea | 7.00 | 38.75 |
| | | Nalukody | Ash gourd | 17.00 | 46.80 |

*Mean of ten plants

Among the vegetable crops surveyed, the live larval mines varied in different crops and also in different locations. The highest number of mines per plant was observed in watermelon (64) in Vellanikkara (Thrissur Dt.) followed by cowpea (34) in Vellanikkara (Thrissur Dt.) and snake gourd in Mazhuvannur (Ernakulam Dt.). The per cent parasitism ranged from 10.96 to 46.78 per cent in January. Highest parasitism (46.78%) of *L. trifolii* larvae was observed from cowpea field of Manganam (Kottayam Dt.) followed by cowpea in Mangalathunada (Ernakulam Dt.) (42.1%). In February, per cent parasitism ranged from 22.01 to 58.99. The highest parasitism was observed in cowpea at Mulamthuruthy (58.99%) followed by bitter gourd (*Momordica charantia*) field in Pazhayannur (Thrissur Dt.) (57.4%). The parasitism ranged from 17.04 to 46.82 per cent in March, with the highest parasitism observed from cowpea (54.10%) in Vellanikkara (Thrissur Dt.) followed by ash gourd in Nalukody (Kottayam Dt.) (46.82%).

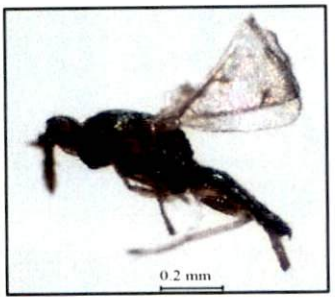
### 4.1.3.2 Identification of parasitoids of *Liriomyza trifolii*

The parasitoids obtained during the survey were identified and presented in Table 13 and Plate 9. Nine species of parasitoids belonging to two families (Eulophidae and Braconidae) were obtained from *L. trifolii.* The identification of the parasitoids was done by Dr. T.C. Narendran, Coordinator, AICP on Taxonomy & Capacity Building (MoEF), Zoological Survey of India, Kozhikode and Dr. P.M. Sureshan, Scientist, Western Ghat Regional Centre, Zoological Survey of India, Kozhikode. The parasitoids collected from the different vegetable crops are given in Table 14.

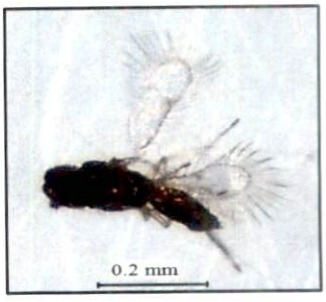
The eulophids, *Closterocerus* spp. (sp. 1 and sp. 2) were obtained from different fields in Thrissur, Ernakulam and Kottayam districts. *Chrysonotomyia* sp. was also observed from the three districts surveyed. The hymenopteran parasitoids, *Closterocerus* sp. 1, *Closterocerus* sp. 2 and *Chrysonotomyia* sp. were observed as

**Plate 9. Parasitoids of *Liriomyza trifolii***

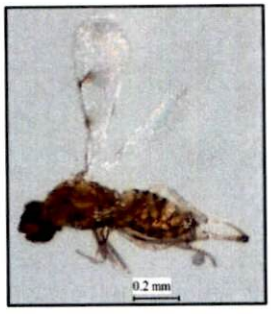

1. ***Closterocerus* sp. 1**



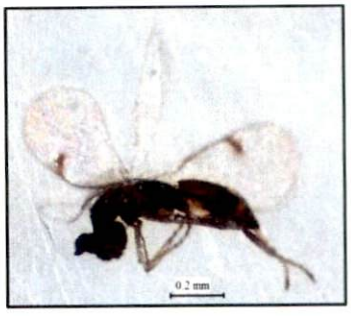

2. ***Closterocerus* sp. 2**




3. ***Chrysonotomyia* sp.**



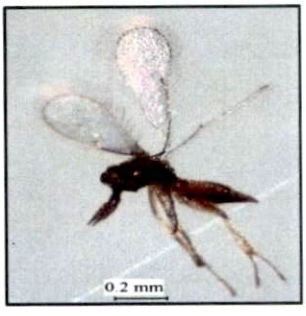

4. ***Cirrospilus acadius***



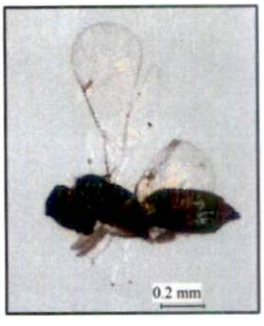

5. ***Cirrospilus brevicorpus***


6. ***Aprostocetus* sp.**


7. ***Toxares* sp.**




8. ***Tetrastichus* sp.**




9. **Unidentified species**

**Table 13. Parasitoids of *Liriomyza trifolii* obtained during the survey**

| Sl. No. | Parasitoids obtained | Type of parasitoids | Families | Order |
|---|---|---|---|---|
| 1 | *Closterocerus* sp. 1 | Larval | Eulophidae | Hymenoptera |
| 2 | *Closterocerus* sp. 2 | | | |
| 3 | *Chrysonotomyia* sp. | | | |
| 4 | *Cirrospilus brevicorpus* Shafee & Rizvi | | | |
| 5 | *Tetrastichus* sp. | | | |
| 6 | Unidentified sp. belonging to subfamily Entedoninae | | | |
| 7 | *Toxares* sp. | Larval-pupal | Braconidae | |
| 8 | *Cirrospilus acadius* Narendran | Larval | Eulophidae | |
| | *Aprostocetus* sp. | | | |

**Table 14. Parasitoids recorded from selected vegetable crops in three districts**

| Sl. No. | Hymenopteran parasitoids | Districts | | |
|---|---|---|---|---|
| | | Thrissur | Ernakulam | Kottayam |
| 1 | *Closterocerus* spp. (Family Eulophidae) | Cowpea, snake gourd, watermelon, bitter gourd | Cowpea, snake gourd, pumpkin, ash gourd | Cowpea, pumpkin, ash gourd |
| 2 | *Chrysonotomyia* sp. (Family Eulophidae) | Cowpea, snake gourd, Watermelon, bitter gourd | Cowpea, snake gourd, pumpkin, ash gourd | Cowpea, pumpkin, ash gourd |
| 3 | Unidentified species (Family Eulophidae) | Cowpea, watermelon | Ash gourd, cowpea | Ash gourd, cowpea |
| 4 | *Toxares* sp. (Family Braconidae) | Snake gourd | - | - |
| 5 | *Tetrastichus* sp. (Family Eulophidae) | Cowpea | - | - |
| 6 | *Cirrospilus brevicorpus* Shafee & Rizvi (Family Eulophidae) | Cowpea | - | - |
| 7 | *Cirrospilus acadius* Narendran (Family Eulophidae) | Tomato | - | - |

the dominant parasitoids of *L. trifolii* from the three districts surveyed. The braconid, *Toxares* sp. was obtained only from snake gourd in Thrissur District. The eulophid, *C. acadius* was recorded only on tomato plants in Thrissur district. *Tetrastichus* sp. was collected only from the polyhouse and was not obtained from any of the vegetable grown in the field as parasitoids of *L. trifolii*. An unidentified species belonging to the subfamily Entedoninae (Family Eulophidae) was also present on parasitized larvae obtained from cowpea, ash gourd and watermelon (*Citrullus lanatus*).

In addition to the above parasitoids, *Aprostocetus* sp. was obtained from the weed plant, *Spilanthes calva* and was not observed in any of the nearby crop plants. All parasitoids obtained in the study were solitary larval, endoparasitoids except *Toxares* sp. which was larval - pupal.

### 4.1.4 Predators of *Liriomyza trifolii*

Predatory ants (Family Formicidae) were observed to feed on the maggots of *L. trifolii* inside the mines. These small ants used to take maggots from the mines by tearing off the mines (Plate 10a). Flies belonging to family Dolichopodidae (Order Diptera) were also observed in the poly house (Plate 10b).

### 4.1.5 Extent of damage by *Liriomyza trifolii*

One hundred and fifty leaves were selected to study the severity of damage. Scoring was done based on the per cent leaf area infested in different vegetable crops surveyed. According to the scores, infestation index was worked out. The per cent emergence of parasitoids from the six vegetable crops collected from Madakkathara and Vellanikkara, Thrissur district was also worked out and is given in Table 15.

**Plate 10. Predators of *Liriomyza trifolii***

a) **Predatory ant feeding on the maggots of *Liriomyza trifolii***

b) **Predatory fly (Diptera: Dolichopodidae)**

**Table 15. Severity of damage caused by *Liriomyza trifolii* and per cent emergence of parasitoids of *Liriomyza trifolii* from different crops**

| Sl. No. | Crops | Infestation index (%) | Per cent emergence of parasitoids |
|---|---|---|---|
| 1 | Ash gourd | 55.00 | 48.33 |
| 2 | Cowpea | 45.00 | 59.17 |
| 3 | Snake gourd | 42.50 | 51.67 |
| 4 | Water melon | 35.00 | 43.75 |
| 5 | Pumpkin | 25.00 | 46.43 |
| 6 | Bitter gourd | 25.00 | 38.89 |

The intensity of infestation varied in different crops. Among the six crops surveyed from two locations of Thrissur district, namely, Madakkathara and Vellanikkara, the highest infestation index was observed in ash gourd (55%) followed by cowpea (45%). The older leaves were preferred more than the younger leaves. The lowest infestation (25%) was observed for pumpkin (*Cucurbita moschata*) and bitter gourd.

Four different species of parasitoids of *L. trifolii* were obtained from the vegetable crops. The highest emergence of the parasitoids was obtained from cowpea (59.17%) followed by snake gourd (51.67%) and the lowest emergence were recorded from bitter gourd (38.89%).

### 4.1.6 Parasitism of *Liriomyza trifolii* in different vegetable crops

The rate of parasitism of *L. trifolii* in different vegetable crops in Thrissur district was estimated and is presented in Table 16.

**Table 16. Rate of parasitism of *Liriomyza trifolii* in different vegetable crops**

| Sl. No. | Hymenopteran parasitoids (Family Eulophidae) | Rate of parasitism (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Ash gourd | Snake gourd | Pumpkin | Watermelon | Bitter gourd | Cowpea |
| 1 | *Closterocerus* spp. | 42.50 | 50.00 | 35.71 | 28.13 | 27.78 | 38.33 |
| 2 | *Chrysonotomyia* sp. | 5.00 | 1.67 | 10.71 | 6.25 | 11.11 | 7.50 |
| 3 | Unidentified species | 0.83 | - | - | 9.38 | - | 10.00 |
| 4 | *Cirrospilus brevicorpus* Shafee & Rizvi | - | - | - | - | - | 3.33 |

In ash gourd, highest parasitism was obtained with *Closterocerus* spp. contributing 42.5 per cent which was followed by *Chrysonotomyia* sp. (5%). The most abundant species present on bitter gourd was *Closterocerus* spp. causing 27.78 per cent parasitism followed by *Chrysonotomyia* sp. (11.11%). In cowpea, the major species was *Closterocerus* spp. (38.33%) followed by the unidentified species (10%). *Closterocerus* spp. was the most abundant parasitoid in pumpkin also followed by *Chrysonotomyia* sp. comprising of 35.71 per cent and 10.71 per cent parasitism of *L. trifolii,* respectively. However, about 50 per cent parasitism was accounted by *Closterocerus* spp. in snake gourd, followed by *Chrysonotomyia* sp. (1.67%). In watermelon, *Closterocerus* spp., an unidentified species and *Chrysonotomyia* sp. were the parasitoids obtained accounting for 28.13, 9.38 and 6.25 per cent parasitism, respectively.

## 4.2 Laboratory evaluation of native entomopathogenic nematodes (EPNs) against *Liriomyza trifolii*

### 4.2.1 Mass rearing of *Liriomyza trifolii* in rearing cage

The laboratory rearing of *L. trifolii* was done on nine days old cowpea plants in rearing cages. Maximum number of larval mines observed from an infested plant (Plate 11) was 30 and a total of 520 maggots were obtained within four days from 45 seedlings after two days of release of adult flies.

### 4.2.2 Isolation of entomopathogenic nematodes from soil

Soil isolated EPNs were mass multiplied on the larvae of *Galleria mellonella* and tested for their effectiveness against *L. trifolii* in the laboratory.

A total of 72 soil samples were collected from three different districts namely, Thrissur, Ernakulam and Kottayam districts and four samples yielded EPNs. The details of the isolated EPNs are given in Table 17.

**Plate 11. Mass multiplication of *Liriomyza trifolii* in rearing cage**

**a) Cowpea seedlings inside the rearing cage**

**b) Mines on infested plants from rearing cage**

**Table 17. Details of the entomopathogenic nematodes isolated from soil**

| District | Locations | Type of field | Accession Number of EPN |
|---|---|---|---|
| Thrissur | Kannara | Uncultivated | EPN Isolate - 1<br>EPN Isolate - 2 |
| | Vellanikkara | Uncultivated | EPN Isolate - 3<br>EPN Isolate - 4 |

Four soil samples from Kannara and Vellanikkara of Thrissur district yielded entomopathogenic nematodes. Accession numbers *viz*., EPN Isolate - 1, 2, 3 and 4 were given to these four entomopathogenic nematodes isolated.

### 4.2.2. Identification of entomopathogenic nematodes

The EPNs isolated from soil namely, EPN Isolate - 1 and EPN Isolate - 2 (Kannara, Thrissur district), EPN Isolate - 3 and EPN Isolate - 4 (Vellanikkara, Thrissur district) were identified by Dr. (Mrs.) Sudershan Ganguly, Principal Scientist, Division of Nematology, Indian Agricultural Research Institute, New Delhi who confirmed all isolates as *Steinernema carpocapsae*.

### 4.2.3 Pathogenicity of entomopathogenic nematodes isolated from soil

All the soil isolated EPNs were identified as *S. carpocapsae*. These were tested for pathogenicity to maggots of *L. trifolii* and were found positive.

### 4.2.4 Bioefficacy of entomopathogenic nematodes against *Liriomyza trifolii*

Bioefficacy of the soil isolated EPNs, namely, *S. carpocapsae* Isolate - 1, 2, 3 and 4 was tested along with *Steinernema bicornutum* and *Heterorhabditis indica* under laboratory conditions.

The maggots inside the leaf mines and the pre pupae emerged from mines were killed by the infective juveniles (IJs) present on the treated filter paper. Nematodes

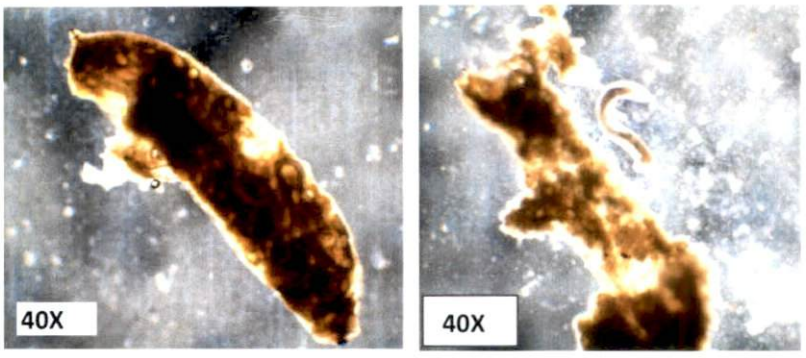
were observed inside the dead maggots of *L. trifolii* (Plates 12). The colour of the infected maggots changed to dark brown to black (Plate 13(a)). Pupae from infected larvae had a shrivelled appearance (Plate 13(b)). The pupae of *L. trifolii* were not infected by EPNs. The mortality caused by EPN isolates increased with increase in the concentration of IJs. The mortality of the maggots of *L. trifolii* at different time intervals, *viz.*, 12, 18, 24 and 30 hours after treatment (HAT) were recorded and per cent mortality was calculated. The data were statistically analyzed and presented in Tables 18 to 21.

The mortality caused by *S. carpocapsae* Isolate - 1 increased from 30 per cent $T_1$ (10 IJs/ maggot) to 83.33 per cent in $T_5$ (30 IJs/ maggot) at 12 HAT. The mortality caused by application of 10 IJs ($T_1$), 15 IJs ($T_2$) and 20 IJs/ maggot ($T_3$) was statistically on par. No significant difference was observed among the treatments, $T_2$, $T_3$ and $T_4$ also. The higher concentrations namely, $T_3$ (20 IJs/ maggot), $T_4$ (25 IJs/ maggot) and $T_5$ (30 IJs/ maggot) were also equal in effectiveness against *L. trifolii*.

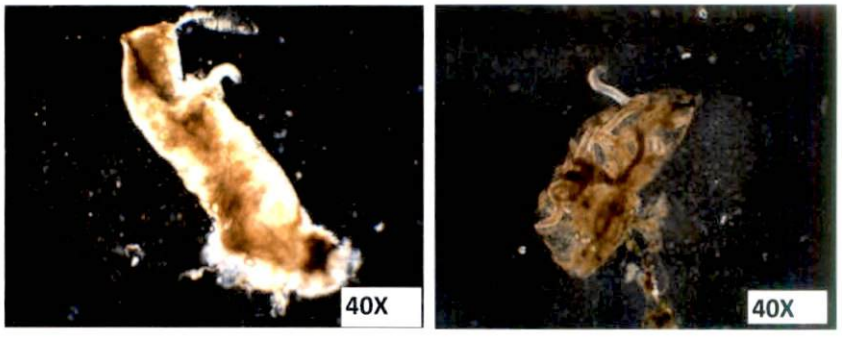
*S. carpocapsae* Isolate - 2 caused mortality ranging from 0.00 per cent in $T_1$ to 56.67 per cent to *L. trifolii* in $T_5$. A minimum of 15 IJs were required to cause mortality in 12 h. Highest mortality (56.67%) was recorded from the highest concentration (30 IJs/ maggot) applied. No significant difference was observed between the concentrations from $T_1$ to $T_4$. But the higher concentration, $T_5$ (30 IJs/ maggot) was significantly superior to all other treatments tested.

Mortality of *L. trifolii* larvae caused by *S. carpocapsae* Isolate - 3 ranged from 13.33 to 66.67 per cent. No significant difference was observed between the treatments applied and all treatments were on par.

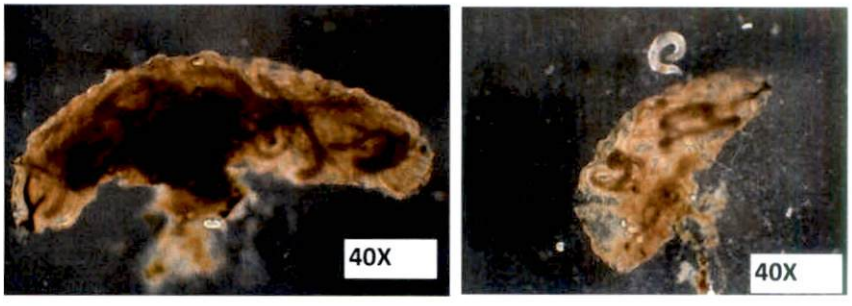
**Plate 12. Entomopathogenic nematodes inside the maggots of *Liriomyza trifolii***



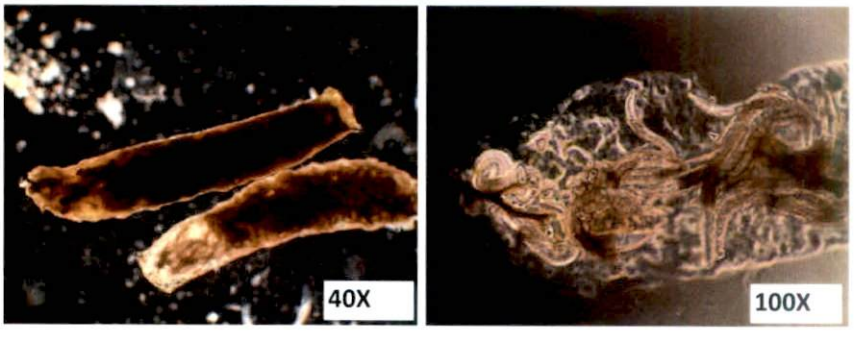

a) *Steinernema carpocapsae* Isolate – 1




b) *S. carpocapsae* Isolate – 2



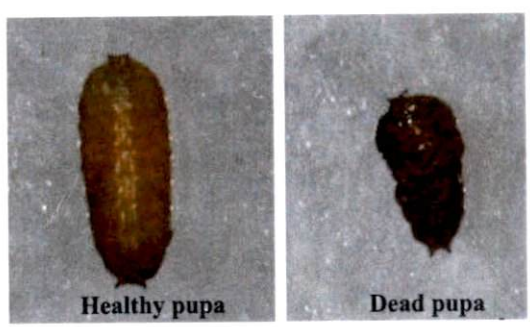

c) *S. carpocapsae* Isolate – 3




d) *S. carpocapsae* Isolate – 4

**Plate 13. Infection by entomopathogenic nematodes**


a) Maggots of *Liriomyza trifolii*




b) Prepupae of *L. trifolii*

**Table 18. Mortality of *Liriomyza trifolii* caused by entomopathogenic nematodes at 12 HAT**

| Treatments | Per cent mortality | | | | | |
|---|---|---|---|---|---|---|
| | *Steinernema carpocapsae* | | | | | |
| | Isolate - 1 | Isolate – 2 | Isolate – 3 | Isolate – 4 | *S. bicornutum* | *H. indica* |
| $T_1$ - 10 IJs/ maggot | 30.00[a] (5.47) | 0.00[a] (0.71) | 13.33[a] (3.67) | 13.33[a] (3.67) | 3.33[a] (1.55) | 6.67[a] (2.39) |
| $T_2$ - 15 IJs/ maggot | 40.00[ab] (6.04) | 13.33[ab] (3.16) | 10.00[a] (2.83) | 20.00[a] (4.43) | 13.33[ab] (3.16) | 3.33[a] (1.55) |
| $T_3$ - 20 IJs/ maggot | 60.00[abc] (7.76) | 26.67[ab] (4.45) | 23.33[a] (4.71) | 23.33[a] (4.86) | 36.67[c] (6.05) | 10.00[a] (3.24) |
| $T_4$ - 25 IJs/ maggot | 66.67[bc] (8.18) | 40.00[ab] (5.42) | 13.33[a] (3.67) | 46.67[b] (6.80) | 26.67[bc] (5.19) | 26.67[b] (5.19) |
| $T_5$ - 30 IJs/ maggot | 83.33[c] (9.15) | 56.67[b] (7.42) | 6.66[a] (2.39) | 66.67[b] (8.19) | 33.33[bc] (5.72) | 73.33[c] (8.56) |

Number of insects used – 10 maggots/ replication

Values in parentheses are SQRT transformed values

HAT - Hours after treatment ; *S. bicornutum – Steinernema bicornutum* ; *H. indica – Heterorhabditis indica*

Means followed by same letters are not statistically different

**Table 19. Mortality of *Liriomyza trifolii* caused by entomopathogenic nematodes at 18 HAT**

| Treatments | Per cent mortality | | | | | |
|---|---|---|---|---|---|---|
| | *Steinernema carpocapsae* | | | | *S. bicornutum* | *H. indica* |
| | Isolate - 1 | Isolate – 2 | Isolate – 3 | Isolate – 4 | | |
| $T_1$ - 10 IJs/ maggot | 50.00[a] (7.08) | 6.67[a] (2.39) | 20.00[a] (4.53) | 23.33[a] (4.86) | 10.00[a] (3.24) | 6.67[a] (2.39) |
| $T_2$ - 15 IJs/ maggot | 70.00[b] (8.35) | 46.67[ab] (6.44) | 23.33[a] (4.86) | 26.67[a] (5.19) | 36.67[b] (5.99) | 13.33[a] (3.67) |
| $T_3$ - 20 IJs/ maggot | 86.67[bc] (9.32) | 40.00[ab] (5.40) | 40.00[b] (6.27) | 46.67[b] (6.86) | 40.00[b] (6.33) | 16.67[a] (4.09) |
| $T_4$ - 25 IJs/ maggot | 96.67[c] (9.85) | 50.00[ab] (6.90) | 56.67[b] (7.55) | 56.67[b] (7.54) | 36.67[b] (6.08) | 40.00[b] (6.33) |
| $T_5$ - 30 IJs/ maggot | 96.67[c] (9.85) | 73.33[b] (8.56) | 53.33[b] (7.33) | 76.67[c] (8.78) | 56.67[b] (7.51) | 90.00[c] (9.50) |

Number of insects used – 10 maggots/ replication

Values in parentheses are SQRT transformed values

HAT - Hours after treatment ; *S. bicornutum – Steinernema bicornutum* ; *H. indica – Heterorhabditis indica*

Means followed by same letters are not statistically different

**Table 20. Mortality of *Liriomyza trifolii* caused by entomopathogenic nematodes at 24 HAT**

| Treatments | Per cent mortality | | | | | |
|---|---|---|---|---|---|---|
| | *Steinernema carpocapsae* | | | | | |
| | Isolate - 1 | Isolate – 2 | Isolate – 3 | Isolate – 4 | *S. bicornutum* | *H. indica* |
| $T_1$ - 10 IJs/ maggot | 73.33[a] (8.54) | 26.67[a] (4.99) | 63.33[a] (7.95) | 53.33[a] (7.29) | 43.33[a] (6.57) | 16.67[a] (4.04) |
| $T_2$ - 15 IJs/ maggot | 90.00[b] (9.48) | 73.33[b] (8.43) | 83.33[ab] (9.11) | 53. 33[a] (7.29) | 53.33[b] (8.15) | 16.67[a] (3.93) |
| $T_3$ - 20 IJs/ maggot | 100.00[b] (10.00) | 66.67[b] (7.96) | 80.00[ab] (8.91) | 80.00[b] (8.93) | 65.66[b] (8.16) | 23.33[a] (4.81) |
| $T_4$ - 25 IJs/ maggot | 100.00[b] (10.00) | 70.00[b] (8.30) | 80.00[ab] (8.91) | 96.67[b] (9.83) | 80.00[bc] (8.89) | 53.33[b] (7.27) |
| $T_5$ - 30 IJs/ maggot | 96.67[b] (9.83) | 93.33[b] (9.65) | 100.00[b] (10.00) | 86.67[b] (9.28) | 93.33[c] (9.66) | 100.00[c] (10.00) |

Number of insects used – 10 maggots/ replication

Values in parentheses are SQRT transformed values

HAT - Hours after treatment ; *S. bicornutum – Steinernema bicornutum* ; *H. indica – Heterorhabditis indica*

Means followed by same letters are not statistically different

**Table 21. Mortality of *Liriomyza trifolii* caused by entomopathogenic nematodes at 30 HAT**

| Treatments | Per cent mortality | | | | | |
|---|---|---|---|---|---|---|
| | *Steinernema carpocapsae* | | | | | |
| | Isolate - 1 | Isolate – 2 | Isolate – 3 | Isolate – 4 | *S. bicornutum* | *H. indica* |
| $T_1$ - 10 IJs/ maggot | 73.33[a] (8.54) | 50.00[a] (7.05) | 63.33[a] (7.95) | 53.33[a] (7.29) | 63.33[a] (7.95) | 16.67[a] (4.04) |
| $T_2$ - 15 IJs/ maggot | 90.00[b] (9.48) | 93.33[b] (9.65) | 83.33[b] (9.11) | 53.33[a] (7.29) | 83.33[bc] (9.10) | 20.00[a] (4.37) |
| $T_3$ - 20 IJs/ maggot | 100.00[b] (10.00) | 86.67[b] (9.31) | 83.33[b] (9.11) | 80.00[b] (8.93) | 70.00[ab] (8.35) | 33.37[a] (5.67) |
| $T_4$ - 25 IJs/ maggot | 100.00[b] (10.00) | 80.00[b] (8.93) | 83.33[b] (9.11) | 96.67[b] (9.83) | 86.67[bc] (9.29) | 63.37[b] (7.93) |
| $T_5$ - 30 IJs/ maggot | 96.67[b] (9.83) | 96.67[b] (9.83) | 100.00[b] (10.00) | 86.67[b] (9.28) | 93.33[c] (9.66) | 100.00[c] (10.00) |

Number of insects used – 10 maggots/ replication

Values in parentheses are SQRT transformed values

HAT - Hours after treatment ; *S. bicornutum – Steinernema bicornutum* ; *H. indica – Heterorhabditis indica*

Means followed by same letters are not statistically different

In the case of *S. carpocapsae* Isolate - 4, $T_1$ (10 IJs/ maggot), $T_2$ (15 IJs/ maggot), $T_3$ (20 IJs/ maggot) were statistically on par and were inferior to $T_4$ (25 IJs/ maggot and $T_5$ (30 IJs/ maggot). The mortality caused by *S. carpocapsae* Isolate - 4 ranged from 13.33 per cent to 66.67 per cent.

The mortality caused by *S. bicornutum* ranged from 3.33 per cent to 36.67 per cent. The lower concentrations applied, namely, 10 IJs/ maggot and 15 IJs/ maggot were on par. $T_2$ (15 IJs/ maggot) was on par with $T_4$ (25 IJs/ maggot) and the higher concentration, $T_5$ (30 IJs/ maggot). Application of $T_3$ (25 IJs/ maggot) was significantly superior from the two lower concentrations tested, namely, $T_1$ (10 IJs/ maggot) and $T_2$ (15 IJs/ maggot).

Mortality of maggots of *L. trifolii* caused by *H. indica* ranged from 6.67 to 73.33 per cent. The lower concentrations, namely, $T_1$ (10 IJs/ maggot), $T_2$ (15 IJs/ maggot) and $T_3$ (20 IJs/ maggot) were on par. $T_4$ (25 IJs/ maggot) and $T_5$ (30 IJs/ maggot) were significantly superior from all other treatments tested.

Hence among the EPNs evaluated at 12 HAT, *S. carpocapsae* Isolate - 1 caused highest mortality of 83.33 per cent at 12 h after treatment.

There was increase in the mortality caused by the EPNs tested at 18 HAT. In *S. carpocapsae* Isolate - 1, the mortality ranged from 50.00 to 96.67 per cent. There was significant difference in the treatments. The treatment, $T_1$ (10 IJs/ maggot) was significantly inferior to all other doses tested. The application of 15 IJs/ maggot ($T_2$) and 20 IJs/ maggot ($T_3$) were on par. No significant difference in mortality was observed between the higher concentrations, $T_3$ (20 IJs/ maggot), $T_4$ (25 IJs/ maggot) and $T_5$ (30 IJs/ maggot).

*S. carpocapsae* Isolate - 2 produced 6.67 to 73.33 per cent mortality to the larvae of *L. trifolii* at different concentrations. The first four treatments, namely, $T_1$, $T_2$, $T_3$ and $T_4$ were on par. The treatments, $T_2$, $T_3$, $T_4$ and $T_5$ were also statistically on par.

The mortality caused by *S. carpocapsae* Isolate - 3 was comparatively less and it ranged from 20.00 to 53.33 per cent. No significant difference was observed between the lower concentrations, $T_1$ and $T_2$. The higher concentrations, 20, 25 and 30 IJs/ maggot were on par and were significantly superior to $T_1$ and $T_2$.

*S. carpocapsae* Isolate - 4 produced slightly higher mortality than Isolate - 3 and it ranged from 23.33 per cent at the lowest concentration, $T_1$ (10 IJs/ maggot) and the highest mortality of 76.67 per cent at the highest concentration, $T_5$ (30 IJs/ maggot). $T_1$ and $T_2$ were on par. No significant difference was observed between the treatments, $T_3$ and $T_4$. The highest concentration, $T_5$ (30 IJs/ maggot) was significantly superior to all other treatments.

Mortality caused by *S. bicornutum* ranged from 10.00 to 56.67 per cent and almost similar in effectiveness to Isolate - 4. The lowest concentration, $T_1$ (10 IJs/ maggot) was significantly inferior to all other treatments, which were on par in effectiveness.

*Heterorhabditis indica* caused 6.67 to 90.00 per cent mortality to the larvae of *L. trifolii*. The first three concentrations, namely, $T_1$, $T_2$ and $T_3$ caused lower mortalities compared to other EPNs tested. $T_5$ (30 IJs/ maggot) was significantly superior to other treatments and caused mortality as observed with Isolate - 1.

Considering the mortalities caused by the different EPNs, *S. carpocapsae* Isolate - 1 ranked first in mortality ranging from 50 in the lowest concentration to 96.67 in the highest concentration.

At 24 HAT, the mortality caused by *S. carpocapsae* Isolate - 1 ranged from 73.33 to 96.67 per cent. The lowest concentration, $T_1$ (10 IJs/ maggot) was significantly inferior to all other concentrations applied. A dose of 20 IJs/ maggot and above caused cent per cent mortality.

*S. carpocapsae* Isolate - 2, caused a per cent mortality ranging from 26.67 to 93.33 to *L. trifolii* larvae. As in the case of *S. carpocapsae* Isolate - 1, the lowest

concentration of *S. carpocapsae* Isolate - 2 was significantly inferior to all other concentrations tested.

The mortality produced by *S. carpocapsae* Isolate - 3 ranged from 63.33 to 100 per cent. The lowest concentration of 10 IJs/ maggot was significantly inferior to the highest concentration of 30 IJs/ maggot. Treatments $T_2$ (15 IJs/ maggot), $T_3$ (20 IJs/ maggot) $T_4$ (25 IJs/ maggot) and $T_5$ (30 IJs/ maggot) were on par. The four lower concentrations were statistically on par.

*S. carpocapsae* Isolate - 4 caused mortality ranging from 53.33 to 96.67 per cent. No significant difference was observed among the two lower concentrations, namely, $T_1$ (10 IJs/ maggot) and $T_2$ (15 IJs/ maggot). The three higher concentrations were on par and were significantly superior to the two lower concentrations.

In the case of *S. bicornutum*, the mortality ranged from 43.33 to 93.33 per cent. The treatments, $T_2$ (15 IJs/ maggot), $T_3$ (20 IJs/ maggot) and $T_4$ (25 IJs/ maggot) were on par. No significant difference was observed between the two higher concentrations, namely, $T_4$ (25 IJs/ maggot) and $T_5$ (30 IJs/ maggot) also.

The mortality caused by *H. indica* ranged from 16.67 to 100.00 per cent. Compared to the four isolates of *S. carpocapsae* and *S. bicornutum*, except the higher concentration of 30 IJs/ maggot, other treatments resulted in less mortality. The lower concentrations, $T_1$ (10 IJs/ maggot), $T_2$ (15 IJs/ maggot) and $T_3$ (20 IJs/ maggot) were on par. The treatments, $T_4$ (25 IJs/ maggot) and $T_5$ (30 IJs/ maggot) were significantly superior to $T_1$, $T_2$ and $T_3$.

Among the EPNs tested against *L. trifolii* larvae inside the mines, the lowest concentration which caused 100 per cent mortality was with that of *S. carpocapsae* Isolate - 1.

At 30 HAT, *S. carpocapsae* Isolate - 1, 3 and *H. indica* only caused 100 per cent mortality. Higher larval mortalities caused by other EPNs were 96.67 per cent in *S. carpocapsae* isolate - 2 and 4 and 93.33 per cent in *S. bicornutum*. The lowest

concentration at which 100 per cent mortality observed was in 20 IJs/ maggot in Isolate - 1, 30 IJs/ maggot in Isolate - 3 and *H. indica*. All isolates of *S. carpocapsae* and *S. bicornutum* caused a minimum of 50 per cent mortality at the lowest concentration tested (10 IJs/ maggot). However in *H. indica*, 25 IJs/ maggot was required to cause 50 per cent mortality.

### 4.2.5 Determination of median lethal concentrations

The cumulative mortality obtained after 12, 18, 24 and 30 HAT were subjected to Probit Analysis (SPSS 17.00) to determine the $LC_{50}$ and $LC_{90}$ values for different time intervals and $LT_{50}$ at different concentrations.

#### 4.2.5.1 Determination of $LC_{50}$ and $LC_{90}$

The $LC_{50}$ and $LC_{90}$ values estimated by Finney's method of Probit analysis are given in Table 22 and Table 23.

**Table 22. $LC_{50}$ values (IJs) of EPNs at different time intervals**

| Sl. No. | Treatments | Time intervals | | | |
|---|---|---|---|---|---|
| | | 12h | 18h | 24h | 30 h |
| 1 | *Steinernema carpocapsae* Isolate - 1 | 17.64 | 9.82 | 1.79 | 1.79 |
| 2 | *S. carpocapsae* Isolate - 2 | 27.50 | 22.56 | 13.95 | 4.64 |
| 3 | *S. carpocapsae* Isolate - 3 | 25.69 | 25.69 | 2.71 | 3.52 |
| 4 | *S. carpocapsae* Isolate - 4 | 25.99 | 21.52 | 10.06 | 10.06 |
| 5 | *S. bicornutum* | 35.07 | 27.30 | 11.73 | 11.73 |
| 6 | *Heterorhabditis indica* | 27.72 | 24.51 | 21.99 | 20.71 |

The $LC_{50}$ values obtained for *S. carpocapsae* Isolate - 1 were 17.64, 9.82, 1.79 and 1.79 at 12 h, 18 h, 24 h and 30 h respectively. These values were less when compared to all other EPNs studied.

At 12 HAT, *S. carpocapsae* Isolate - 1 showed the lowest $LC_{50}$, followed by isolates 3, 4, 2, *H. indica* and *S. bicornutum*.

At 18 HAT, the lowest $LC_{50}$ was observed in *S. carpocapsae* Isolate - 1. Similar trend was shown at 24 HAT also. However very low $LC_{50}$ value (2.71) was exhibited by *S. carpocapsae* Isolate - 3.

At 30 HAT, *S. bicornutum* had the lowest $LC_{50}$ (1.65) which was closely followed by Isolate - 1 (1.79).

**Table 23. $LC_{90}$ values (IJs) of entomopathogenic nematodes at different time intervals**

| Sl. No. | Treatments | Time intervals | | | |
|---|---|---|---|---|---|
| | | 12h | 18h | 24h | 30 h |
| 1 | *Steinernema carpocapsae* Isolate - 1 | 35.23 | 22.02 | 15.61 | 15.61 |
| 2 | *S. carpocapsae* Isolate - 2 | 40.09 | 39.33 | 30.32 | 24.78 |
| 3 | *S. carpocapsae* Isolate - 3 | 49.14 | 49.14 | 23.00 | 22.01 |
| 4 | *S. carpocapsae* Isolate - 4 | 42.18 | 38.82 | 27.50 | 27.50 |
| 5 | *Steinernema bicornutum* | 59.04 | 51.59 | 29.64 | 28.88 |
| 6 | *Heterorhabditis indica* | 37.98 | 34.12 | 32.16 | 30.51 |

$LC_{90}$ value at 24 HAT for *S. carpocapsae* Isolate - 1 (15.61 h) was the lowest among all EPNs. $LC_{90}$ values varied from 59.04 to 35.23 at 12 HAT, 22.02 to 51.59 at

18 HAT, 15.61 to 32.16 at 24 HAT and 15.61 to 30.51 at 30 HAT in the five EPNs tested.

Among the soil isolated EPNs, *S. carpocapsae* Isolate - 1 from Kannara, Thrissur (Dt.) showed low $LC_{50}$ and $LC_{90}$ values at all time intervals. Hence *S. carpocapsae* Isolate - 1 was selected as the most effective isolate among those isolated from soil for conducting further experiments.

### 4.2.5.2. Determination of $LT_{50}$

The time required to cause 50 per cent mortality to the larvae of *L. trifolii* was estimated for different doses by Finney's method of Probit analysis and was given in Table 24.

**Table 24. $LT_{50}$ values of entomopathogenic nematodes at different doses of infective juveniles/ maggot**

| Sl. No. | Treatments | $LT_{50}$ (h) | | | |
|---|---|---|---|---|---|
| | | 10 IJs/ maggot | 15 IJs/ maggot | 20 IJs/ maggot | 25 IJs/ maggot |
| 1 | *Steinernema carpocapsae* Isolate - 1 | 18.32 | 13.46 | 10.84 | 10.20 |
| 2 | *S. carpocapsae* Isolate - 2 | 29.49 | 19.28 | 19.39 | 16.64 |
| 3 | *S. carpocapsae* Isolate - 3 | 24.33 | 21.08 | 17.72 | 18.74 |
| 4 | *S. carpocapsae* Isolate - 4 | 26.67 | 26.54 | 18.66 | 13.88 |
| 5 | *Steinernema bicornutum* | 26.67 | 21.03 | 19.37 | 18.76 |
| 6 | *Heterorhabditis indica* | 55.72 | 47.09 | 39.35 | 23.08 |

IJs - Infective juveniles

$LT_{50}$ values of all the EPNs varied at the different concentrations studied. The $LT_{50}$ values of all EPNs decreased with increase in the concentrations applied (number of IJs/ maggot).

*S. carpocapsae* Isolate - 1 had the lowest $LT_{50}$ values at all concentrations, when treated at 10, 15, 20 and 25 IJs/ maggot, resulting in 18.32, 13.46, 10.84 and 10.20 h, respectively. The $LT_{50}$ value for *S. bicornutum* at 10 IJs/ maggot was 26.67 h and the values decreased to 18.76 h at 25 IJs/ maggot. At 10 IJs/ maggot, $LT_{50}$ value of *H. indica* was 55.72 which decreased to 23.08 at 25 IJs/ maggot. *H. indica* took more time to cause 50 per cent mortality at all concentrations tested.

### 4.3 Pot culture evaluation of native entomopathogenic nematodes

Evaluation of the most effective EPN isolated from the soil (*S. carpocapsae* Isolate - 1) along with *S. bicornutum* and *H. indica* was done in infested potted seedlings under polyhouse conditions. The mortality caused by various treatments applied is given in Table 25. Application of nematodes was done at 16 IJs/ maggot for *S. carpocapsae* Isolate - 1, at 30 IJs/ maggot for *S. bicornutum* and at 32 IJs/ maggot for *H. indica* which was fixed based on the $LC_{90}$ value based on laboratory experiment.

Among the treatments given as foliar spray, $T_6$ (azadirachtin @ 0.005%) caused the highest mortality of 84.51 per cent showing higher effectiveness in controlling the maggots of *L. trifolii*. $T_6$ was significantly superior to all other treatments tested. Even though all the treatments other than $T_6$ were on par, *H. indica* ($T_3$) caused higher mortality (18.98%). It was followed by *S. carpocapsae* Isolate - 1 ($T_1$) which caused 15.55 per cent mortality to the larvae of *L. trifolii* present inside mines and *S. bicornutum* with 9.99 per cent. Soil application of the commercial formulation of *H. indica* was also found to have less effect in controlling the population of *L. trifolii* in the polyhouse causing 7.28 per mortality to the pre pupae of *L. trifolii* which dropped from cowpea leaves to soil for pupation. Discolouration

**Table 25. Effect of entomopathogenic nematodes on *Liriomyza trifolii* (pot culture evaluation)**

| Treatments | Per cent mortality (5 DAT) |
|---|---|
| $T_1$ : *Steinernema carpocapsae* Isolate - 1 @ 16 IJs/ maggot (foliar application) | $15.55^a$ (3.81) |
| $T_2$ : *Steinernema bicornutum* @ 30 IJs/ maggot (foliar application) | $9.99^a$ (3.07) |
| $T_3$ : *Heterorhabditis indica* @ 32 IJs/ maggot (foliar application) | $18.98^a$ (4.40) |
| $T_4$: Formulation of EPN, *H. indica* @ 8 lakhs IJs /pot (soil application) (Soldier)® | $7.28^a$ (2.78) |
| $T_5$ : *Beauveria bassiana* @ $1\times10^7$ spores/ml (foliar application) | $0.00^a$ (0.71) |
| $T_6$ : Azadirachtin 1% @ 0.005% (foliar application) | $84.51^b$ (9.17) |
| $T_7$ : Untreated control | $0.00^a$ (0.71) |

Values in parentheses are SQRT transformed values

DAT – Days after treatment

Means followed by same letters are not statistically different

was observed in the dead maggots in all the treatments (Plate 14). The cadavers appeared reddish in the case of $T_3$ (*H. indica*) which was the characteristic colour of infection by *H. indica*. The application of *Beauveria bassiana* caused no mortality to the maggots of *L. trifolii* inside the leaf mines and the emergence of all maggots as pre pupae were observed. As *B. bassiana* was ineffective for controlling the maggots of *L. trifolii* inside the mines, it was substituted with fipronil at 0.002 per cent which was commonly used for control of *L. trifolii,* for field evaluation.

### 4.4. Field efficacy of entomopathogenic nematodes against *Liriomyza trifolii*

The field evaluation of foliar application of *H. indica* was conducted along with fipronil at 0.002%, azadirachtin at 0.005% and soil application of EPN formulation of *H. indica* for their efficacy against *L. trifolii*. The mortality of maggots of *L. trifolii* obtained is given in Table 26.

In the first spraying, fipronil at 0.002% ($T_3$) caused the highest mortality of 91.52 per cent to the second instar larvae of *L. trifolii* present inside the leaf mines which was followed by azadirachtin at 0.005% ($T_4$) causing 90.15 per cent mortality. $T_3$ was on par with $T_4$ and these were statistically superior to all other treatments used for evaluation. Foliar application of *H. indica* caused 59.59 per cent mortality to the larvae of *L. trifolii* which was on par with $T_5$ (control) and $T_2$. This showed that the foliar application of *H. indica* and soil application of *H. indica* were less effective in controlling *L. trifolii* at field level.

In the second spraying also, fipronil at 0.002% ($T_3$) was the most effective treatment in controlling the larval stage of *L. trifolii* causing a mortality of 86.09 per cent followed by azadirachtin at 0.005% ($T_4$) causing 81.69 per cent mortality. Statistically $T_3$ and $T_4$ were on par. Foliar application of *H. indica* was significantly superior to $T_2$ and $T_5$. $T_2$ was inferior to all other treatments evaluated at field level.

From the two sprayings, fipronil at 0.002% was observed as the most effective in causing highest mortality to the second instar larvae of *L. trifolii* followed by

**Plate 14. Pot culture evaluation of entomopathogenic nematodes against *Liriomyza trifolii***

$T_1$ - ***Steinernema carpocapsae*** **Isolate – 1**

$T_2$ - ***Steinernema bicornutum***

$T_3$ – ***Heterorhabditis indica***

$T_4$ - Formulation of ***H. indica***

$T_5$ - ***Beauveria bassiana***

$T_6$ - Azadirachtin

**Control**

**Table 26. Mortality of *Liriomyza trifolii* in different treatments in the field trial**

| Treatments | Per cent mortality (5 DAT) | |
|---|---|---|
| | **First spraying (09.12.2013)** | **Second spraying (20.12.2013)** |
| $T_1$ : *Heterorhabditis indica* @ 32 IJs/ maggot (foliar application) | $59.59^b$ | $57.30^b$ |
| $T_2$ : Formulation of EPN, *H. indica* @ 2 billion IJs/ ha (soil application) (Soldier)® | $44.11^b$ | $39.87^c$ |
| $T_3$ : Fipronil 5 SC @ 0.002% (foliar application) | $91.52^a$ | $86.09^a$ |
| $T_4$ : Azadiractin 1% @ 0.005% (foliar application) | $90.15^a$ | $81.69^a$ |
| $T_5$ : Untreated control | $55.38^b$ | $37.15^c$ |
| CD value | 24.69 | 11.67 |

DAT – Days after treatment

Means followed by same letters are not statistically different

azadirachtin at 0.005%. Soil application of formulation of *H. indica* was not effective in controlling the larval stages of *L. trifolii* which were present on the leaves. Foliar application of *H. indica* was observed to be slightly effective in the field condition.

## 4.5 Compatibility of entomopathogenic nematodes with insecticides

Laboratory studies were conducted for the compatibility of EPNs, *viz.*, *S. carpocapsae* Isolate - 1, *S. bicornutum* and *H. indica* for their tolerance to insecticides.

### 4.5.1 Compatibility of *Steinernema carpocapsae* Isolate - 1 with insecticides

Response of the IJs of *S. carpocapsae* to insecticides at tested doses taken at 24 h, 48 h and 72 h is presented in Table 27.

At 24 HAT, quinalphos at 0.05% caused 90.18 per cent mortality to the IJs of *S. carpocapsae* which was followed by chlorpyriphos at 0.05% causing 86.17 per cent mortality of IJs. Buprofezin at 0.04% caused low mortality of 2.84 per cent. Quinalphos and chlorpyriphos were statistically on par and showed the incompatibility with IJs of *S. carpocapsae*. Buprofezin was superior to quinalphos and chlorpyriphos showing slightly higher compatibility with IJs. Only 0.50 per cent mortality was effected when the IJs were treated with the botanical insecticide, azadirachtin at 0.005% which showed the compatibility of IJs with azadirachtin.

At 48 HAT, chlorantraniliprole at 0.005% and malathion at 0.1% were on par showing its high compatibility with IJs after two days. These insecticides were superior to dimethoate at 0.04%, fipronil at 0.002%, imidacloprid at 0.006% and azadirachtin which caused slightly higher mortality of IJs of *S. carpocapsae*. Application of quinalphos at 0.05% caused 95.99 per cent mortality on the second day which was followed by chlorpyriphos resulting in 87.60 per cent mortality. Quinalphos followed by chlorpyriphos at 0.05% were inferior to all other treatments.

**Table 27. Mortality caused by different insecticides to *S. carpocapsae* Isolate - 1**

| Sl. No. | Insecticides tested with concentration | Corrected mortality at different time intervals (%) | | |
|---|---|---|---|---|
| | | 24 HAT | 48 HAT | 72 HAT |
| 1 | Buprofezin 25 SC @ 0.04% | 2.84$^{b}$ (1.77) | 16.79$^{c}$ (4.15) | 35.40$^{c}$ (5.60) |
| 2 | Spinosad 45 SC @ 0.009% | 0.64$^{a}$ (1.06) | 0.74$^{b}$ (1.11) | 4.68$^{b}$ (2.20) |
| 3 | Chlorpyriphos 20 EC @ 0.05% | 86.17$^{c}$ (9.31) | 87.60$^{d}$ (9.39) | 92.87$^{d}$ (9.66) |
| 4 | Quinalphos 25 EC @ 0.05% | 90.18$^{c}$ (9.52) | 95.99$^{e}$ (9.82) | 96.17$^{d}$ (9.83) |
| 5 | Dimethoate30 EC @ 0.04% | 0.33$^{a}$ (0.89) | 0.43$^{ab}$ (0.95) | 0.50$^{a}$ (0.99) |
| 6 | Fipronil 5 SC @ 0.002 % | 0.17$^{a}$ (0.81) | 0.23$^{ab}$ (0.85) | 1.17$^{a}$ (1.28) |
| 7 | Chlorantraniliprole 18.5 SC @ 0.005% | 0.07$^{a}$ (0.75) | 0.07$^{a}$ (0.75) | 0.17$^{a}$ (0.82) |
| 8 | Malathion 50 EC @ 0.1% | 0.10$^{a}$ (0.77) | 0.10$^{a}$ (0.77) | 0.23$^{a}$ (0.85) |
| 9 | Imidacloprid 200 SL @ 0.006% | 0.33$^{a}$ (0.90) | 0.57$^{ab}$ (1.02) | 0.77$^{a}$ (1.11) |
| 10 | Azadirachtin 1 EC @ 0.005% | 0.30$^{a}$ (0.89) | 0.50$^{ab}$ (0.98) | 0.47$^{a}$ (0.99) |

Number of infective juveniles (IJs) used – 1000 IJs/ replication

Values in parentheses are SQRT transformed values

Means followed by same letters are not statistically different

HAT – Hours after treatment

At 72 HAT, dimethoate at 0.04%, fipronil at 0.002%, chlorantraniliprole, malathion at 0.1%, imidacloprid at 0.006% and azadirachtin at 0.005% were on par showing their high compatibility with IJs of *S. carpocapsae*. Quinalphos and chlorpyriphos which caused 96.17 and 92.87 per cent mortality, respectively were statistically inferior to all other treatments followed by spinosad at 0.009% and buprofezin.

Out of the ten insecticides tested, for their compatibility with *S. carpocapsae* Isolate – 1, most of the insecticides were found compatible with the bio agent except quinalphos and chlorpyriphos and to little extent buprofezin causing 2.84 per cent mortality at 24 HAT and increasing to 35.4 per cent at 72 HAT.

No reduction was observed in the virulence of IJs survived in the insecticide solution when allowed for infection in *G. mellonella*. Normal multiplication as compared to control was observed in the case of IJs. All the cadavers were brown to black in colour which was the characteristic of *Steinernema* sp. (Plate 15a).

### 4.5.2 Compatibility of *Steinernema bicornutum* with insecticides

The effect of different insecticides tested against *S. bicornutum* is given in Table 28.

At 24 HAT, spinosad, chlorpyriphos, dimethoate, fipronil, chlorantraniliprole, imidacloprid and azadirachtin were on par and superior to all other treatments showing their high compatibility with the IJs of *S. bicornutum*. Buprofezin caused 2.38 per cent mortality to the IJs and was inferior to all other treatments except malathion and quinalphos. Quinalphos produced the highest mortality of 14.41 per cent to the IJs at 24 HAT.

Quinalphos caused the highest mortality of 17.5 per cent mortality to the IJs at 48 HAT and was highly inferior to all other treatments followed by malathion effecting 5.32 per cent mortality.

**Plate 15.** **Infection of *Galleria mellonella* larvae with entomopathogenic nematodes**

a) **Infection of *Steinernema carpocapsae* Isolate – 1**

b) **Infection of *Steinernema bicornutum***

c) **Infection of *Heterorhabditis indica***

**Table 28. Mortality caused by different insecticides to *Steinernema bicornutum***

| Sl. No. | Insecticides tested with concentration | Corrected mortality at different time intervals (%) | | |
|---|---|---|---|---|
| | | 24 HAT | 48 HAT | 72 HAT |
| 1 | Buprofezin 25 SC @ 0.04% | 2.38 [b] (1.70) | 2.37[b] (1.70) | 2.55[b] (1.74) |
| 2 | Spinosad 45 SC @ 0.009% | 0.23[a] (0.85) | 0.50[a] (0.99) | 1.28[ab] (1.32) |
| 3 | Chlorpyriphos 20 EC@ 0.05% | 0.10[a] (0.77) | 0. 50[a] (0.99) | 1. 41[ab] (1.37) |
| 4 | Quinalphos 25 EC @ 0.05% | 14.41[d] (3.86) | 17.50[d] (4.23) | 99.93[d] (10.02) |
| 5 | Dimethoate30 EC @ 0.04% | 0.13[a] (0.79) | 0.50[a] (0.98) | 0.60 [a] (1.03) |
| 6 | Fipronil 5 SC @ 0.002 % | 0.20[a] (0.84) | 0.35[a] (0.92) | 0.80 [a] (1.12) |
| 7 | Chlorantraniliprole 18.5 SC @ 0.005% | 0.27[a] (0.87) | 0.50[a] (0.99) | 1.62[ab] (1.40) |
| 8 | Malathion 50 EC @ 0.1% | 5.23[c] (2.40) | 5.32[c] (2.40) | 10.19[c] (3.27) |
| 9 | Imidacloprid 200 SL @ 0.006% | 0.10[a] (0.77) | 1.44[b] (1.38) | 1.44[ab] (1.39) |
| 10 | Azadirachtin 1 EC @ 0.005% | 0.33[a] (0.91) | 0.53[a] (1.01) | 0.78[a] (1.21) |

Number of infective juveniles (IJs) used – 1000 IJs/ replication

Values in parentheses are SQRT transformed values

Means followed by same letters are not statistically different

HAT – Hours after treatment

After three days, highest mortality of 99.93 per cent was observed from quinalphos expressing its high incompatibility with IJs of *S. bicornutum* followed by malathion. Dimethoate, fipronil and azadirachtin were highly superior to all other treatments than spinosad, chlorpyriphos, chlorantraniliprole and imidacloprid showing their high compatibility with the IJs of *S. bicornutum*.

The EPN, *S. bicornutum* was observed to be compatible with all the insecticides tested except quinalphos. Mortality was also observed to IJs of *S. bicornutum* when treated with malathion. At 72 h, lowest mortality of IJs was obtained in the case of dimethoate and fipronil.

Mortality caused to the last instar larvae of *G. mellonella* showed the efficiency of retaining the virulence and pathogenicity of IJs even after the treatments with insecticides. The cadavers were brownish coloured (Plate 15b). During extraction with White's trap, IJs emerged from the cadavers showing their ability for multiplication inside cadaver.

### 4.5.3 Compatibility of *Heterorhabditis indica* with insecticides

The mortality caused to the IJs of *H. indica* is given in Table 29.

At 24 HAT, *H. indica* was observed to be incompatible with quinalphos causing 30.51 per cent mortality to IJs. Buprofezin and spinosad caused 2.71 and 1.95 per cent mortality respectively, to the IJs. After one day of the application, all the insecticides were on par and were compatible with IJs of *H. indica* except buprofezin and spinosad which caused low level mortalities and quinalphos which caused comparatively higher mortality to the IJs of *H. indica*.

The same pattern was observed after 48 HAT. The mortality caused by quinalphos increased from 30.51 (24 HAT) to 35.2 per cent (48 HAT). Mortality of the IJs was observed to increase in all the treatments after two days.

**Table 29. Mortality caused by different insecticides to *Heterorhabditis indica***

| Sl. No. | Insecticides tested with concentration | Corrected mortality at different time intervals (%) | | |
|---|---|---|---|---|
| | | 24 HAT | 48 HAT | 72 HAT |
| 1 | Buprofezin 25 SC @ 0.04% | 2.71[b] (1.78) | 3.10[b] (1.89) | 4.3[b] (2.20) |
| 2 | Spinosad 45 SC @ 0.009% | 1.95[b] (1.53) | 2.78[b] (1.80) | 3.37[b] (1.94) |
| 3 | Chlorpyriphos 20 EC @ 0.05% | 0.20[a] (0.84) | 0.33[a] (0.91) | 0.53[a] (1.01) |
| 4 | Quinalphos 25 EC @ 0.05% | 30.51[c] (5.56) | 35.20[c] (5.97) | 39.60[c] (6.33) |
| 5 | Dimethoate30 EC @ 0.04% | 0.37[a] (0.92) | 0.57[a] (1.02) | 0.64[a] (1.05) |
| 6 | Fipronil 5 SC @ 0.002 % | 0.24[a] (0.86) | 0.34[a] (0.91) | 0.40[a] (0.95) |
| 7 | Chlorantraniliprole 18.5 SC @ 0.005% | 0.14[a] (0.80) | 0.24[a] (0.85) | 0.27[a] (0.87) |
| 8 | Malathion 50 EC @ 0.1% | 0.17[a] (0.81) | 0.24[a] (0.86) | 0.57[a] (1.03) |
| 9 | Imidacloprid 200 SL @ 0.006% | 0.17[a] (0.81) | 0.40[a] (0.95) | 0.60[a] (1.05) |
| 10 | Azadirachtin 1 EC@ 0.005% | 0.20[a] (0.84) | 0.33[a] (0.91) | 0.53[a] (1.01) |

Number of infective juveniles (IJs) used – 1000 IJs/ replication

Values in parentheses are SQRT transformed values

Means followed by same letters are not statistically different

HAT – Hours after treatment

At 72 HAT, mortality caused by quinalphos increased from 35.2 (48 HAT) to 39.6 per cent (72 HAT). Quinalphos was inferior to other treatments followed by buprofezin and spinosad which caused 4.31 and 3.37 per cent mortality to IJs of *H. indica*.

On comparing the period of observation, the lowest mortality was observed with chlorantraniliprole expressing high compatibility of *H. indica* while incompatible with quinalphos. Slight mortality was also observed when the IJs were treated with buprofezin and spinosad.

Virulence and pathogenicity of IJs were evident from its capability to cause mortality to the last instar larvae of *G. mellonella*. Cadavers were brownish red in colour (Plate 15c) which is a characteristic of *Heterorhabditis* sp. IJs emerged when the cadavers were kept in White's trap.

The studies conducted for the compatibility of the IJs of *S. carpocapsae* Isolate - 1, *S. bicornutum* and *H. indica* revealed the high toxicity of quinalphos to IJs of *S. carpocapsae* Isolate - 1, *S. bicornutum* and moderate toxicity to IJs of *H. indica*. Chlorpyriphos was observed to be highly toxic to the IJs of *S. carpocapsae* Isolate - 1 from 24 HAT itself. Other insecticides were observed to be slightly or less toxic to the IJs of EPNs.

# Discussion

# 5. DISCUSSION

The present study entitled "Biotic agents for the management of American serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)" was undertaken to identify the natural enemies associated with different stages of the pest in Kerala for its utilization in the management of the pest. The results obtained in the study conducted during 2011 to 2013 are discussed below.

## 5.1 Survey for the collection of indigenous natural enemies of *Liriomyza trifolii*

### 5.1.1 Isolation of entomopathogens from *Liriomyza trifolii*

Six microorganisms, *viz.*, five isolates of bacteria and one species of a fungus were isolated from the cadavers of *L. trifolii*. However, Koch's postulates could not be proved with the isolated microorganisms. Hence the obtained microorganisms were considered to be non pathogenic secondary pathogens and hence not proceeded further.

Perusal of literature also shows that entomopathogens have not been isolated so far from *L. trifolii*. Only the well known entomopathogenic fungi were evaluated against *L. trifolii*. Hence the present study was continued with the parasitoids and entomopathogenic nematodes as the natural enemies of *L. trifolii*.

### 5.1.2 Collection of parasitoids of *Liriomyza trifolii*

Surveys conducted in three districts of Kerala for the collection of indigenous parasitoids of *L. trifolii* revealed the presence of nine hymenopteran parasitoids as the natural enemies of *L. trifolii* from different vegetable crops. The rate of parasitism and the species composition differed in various vegetable crops.

### 5.1.3 Parasitization of *Liriomyza trifolii*

The rate of parasitism ranged from 10.96 to 46.78, 22.01 to 58.99 and 17.04 to 54.1 per cent in January, February and March, respectively in different crops. During the survey period, higher parasitization was observed in the month of February followed by March. Smitha (2003) also obtained the highest parasitization of *L. trifolii* during the months of December, January and February. The per cent parasitization of *L. trifolii* varied in different crops. This is in agreement with the findings of Robert *et al.* (2012) who opined that host plant species could affect the behavior and attributes of parasitoids, such as host searching, oviposition and offspring fitness and also reported a significant interaction effect for host plant and *Liriomyza* species on parasitism and host feeding.

In India, the larval parasitism was reported as 34 to 49 per cent on castor, 19.4 per cent on cowpea, 18.7 per cent on tomato, 14.4 per cent in marigold and 8.4 per cent on ridge gourd (Kapadia and Parmar, 1997), while 30 to 40 per cent on vegetables in Senegal (Neuenschwander *et al.*, 1987), 75 per cent on watermelon in Hawai (Johnson, 1987). Schuster and Wharton (1993) recorded 15.5 to 28.8 per cent larval parasitism and 12.6 to 51.8 per cent larval - pupal parasitism of *Liriomyza* spp. on fresh market tomato in Florida.

### 5.1.4 Parasitoids of *Liriomyza trifolii*

Nine hymenopteran parasitoids, namely, *Cirrospilus acadius*, *Cirrospilus brevicorpus*, *Closterocerus* sp. 1, *Closterocerus* sp. 2, *Toxares* sp., *Chrysonotomyia* sp., *Aprostocetus* sp., *Tetrastichus* sp. and an unidentified sp. belonging to subfamily Entedoninae were observed to parasitize *L. trifolii*. All the parasitoids belonged to the family Eulophidae except *Toxares* sp. (Family Braconidae). Prieto and Ullola (1982) reported that about 92 per cent of the parasitoids attacking *L. trifolii* larvae were eulophids.

### 5.1.4.1 *Closterocerus* sp.

In the present study, *Closterocerus* spp. (species 1 and 2) were the predominant parasitoids of *L. trifolii.* These were observed from all the six vegetable crops studied and the rate of parasitism ranged from 27.78 to 50 per cent in the six vegetable crops surveyed.

About 74 species of *Closterocerus* were reported worldwide. Two species of *Closterocerus* were already reported as parasitoids of *L. trifolii* from India, namely, *C. agromyzae* (Regi *et al.*, 2003) in Kerala and *C. indica* (Bhat *et al.*, 2009) in J & K. *Closterocerus agromyzae* was reported as a parasitoid of *L. trifolii* on cowpea earlier from Kerala (Regi *et al.*, 2003). Bhat *et al.* (2009) recorded *C. indica* from vegetables from Jammu and Kashmir.

In the present study, *Closterocerus* spp. were recorded from all the vegetable crops studied, namely, cowpea (*Vigna unguiculata*), ash gourd (*Benincasa hispida*), snake gourd (*Trichosanthes cucumerina*), bitter gourd (*Momordica charantia*), watermelon (*Citrullus lanatus*) and pumpkin (*Cucurbita moschatae*).

Of the several species of *Closterocerus* reported outside India on *L. trifolii*, *Closterocerus cinctipennis* was recorded from USA (Chandler, 1982), *Closterocerus* sp. in chrysanthemum from Colombia (Prieto and Ullola, 1982). *Closterocerus formosus* in Iran (Talebi *et al.*, 2005; Hesami *et al.*, 2009) and *C. cinctipennis* in Mexico (Escoboza *et al.*, 2010).

*Closterocerus* spp. was also reported as the parasitoids of other species of *Liriomyza*. *Closterocerus* sp. was reported on *Liriomyza sativae* was reported from Venezuela (Issa and Marcano, 1994) and *C. cinctipennis* on *L. sativae* from USA (Chandler, 1982) and Mexico (Escoboza *et al.*, 2010).

*Closterocerus* sp. was obtained as a parasitoid of *L. trifolii* in the present study. However, several other workers have reported parasitization in a wide range of hosts by *Closterocerus* sp. *Closterocerus splendens* was recorded as natural enemy of

*Promecotheca* sp. from some parts of Tropical Asia (Ferriere, 1933). In addition to *L. trifolii, C. agromyzae* was recorded as the larval parasitoid of pea leaf miner, *Phytomyza atricornis* from India (Gokulpure, 1972). However, considering the higher per cent parasitism by *Closterocerus* sp. on majority of the vegetable crops surveyed, this group can be further studied for its suitability in applied biocontrol programme against *L. trifolii* in Kerala.

**5.1.4.2 *Chrysonotomyia* sp.**

*Chrysonotomyia* spp. were distributed worldwide and about 171 species have been recorded so far under this genus. Parasitism of *Chrysonotomyia* spp. on *L. trifolii* was reported earlier (Trumble, 1981; Bourdouxhe, 1982; Neuenschwander *et al.*, 1987; Chandler *et al.*, 1988; Patel and Schuster, 1992; Sahein and El-Maghraby, 1993; Kapadia, 1995; Men *et al.*, 1998; Galande and Ghorpade, 2007).

*Chrysonotomyia* sp. was obtained as the predominant species next to *Closterocerus* spp. in the present study. As in the case of *Closterocerus* spp., *Chrysonotomyia* sp. was also obtained from the six vegetable crop surveyed, namely, cowpea, ash gourd, snake gourd, pumpkin, watermelon and bitter gourd with parasitism from 1.67 to 11.11 per cent. *Chrysonotomyia rexia* was reported as a parasitoid of *L. trifolii* from Kerala (Regi *et al.*, 2003; Smitha, 2003). *Chrysonotomyia* sp. was reported as parasitoids of *L. trifolii* from Jammu and Kashmir (Bhat *et al.*, 2009).

Other species of *Chrysonotomyia* recorded from *L. trifolii* were *C. formosa* from Italy (Bene and Rumine, 1985) and Guam (Schreiner *et al.*, 1986; Bene, 1989), *C. punctiventris* from USA (Lynch and Johnson, 1987) and *C. okazakii* from Taiwan (Chin and Chih, 1998; Chin and Chih, 2001)

*Chrysonotomyia formosa* was also reported from other insects like *Chromatomyia horticola* and *C. syngenesiae* as natural enemies from Italy

(Bene, 1989). Parasitization of *Liriomyza sativae* by *Chrysonotomyia* sp. was reported from Venezuela (Issa and Marcano, 1994) and *Chrysonotomyia chlorogaster* from Turkey (Yabas and Ulubilir, 1995). Parasitization of agromyzid leaf miners by *Chrysonotomyia smaragdula* (Cikman and Uygun, 2003) was described from Turkey.

**5.1.4.3 *Cirrospilus* sp.**

In the present study, *Cirrospilus acadius* and *C. brevicorpus* were obtained as parasitoids of *L. trifolii* from tomato and cowpea, respectively. Perusal of literature showed no earlier reports on the parasitization by *C. acadius* and *C. brevicorpus* on *L. trifolii* from India. Hence these two parasitoids are being reported for the first time from India. Only a few specimens of *C. acadius* were obtained in this study and rate of parasitism of *C. brevicorpus* was 3.33 per cent.

One hundred and fifty two species of the genus *Cirrospilus* were reported from different parts of the world. From India, *Cirrospilus variegatus* and *C. ambiguous* were reported to parasitize *L. trifolii* (Kapadia, 1995).

*Cirrospilus ambiguous* was mentioned as parasitoid of *L. trifolii* from Taiwan (Chin and Chih, 1998) while *C. vittatus* from Jordan (Al-Ghabeish and Allawi, 2001), Turkey (Cikman and Uygun, 2003) and Iran (Talebi *et al.*, 2005; Asadi *et al*, 2006) and *Cirrospilus* sp. from Egypt (Fadl and El-Khawas, 2009).

The parasitization of *C. vittatus* on other agromyzids like *Chrysonotomyia horticola* and *C. syngenesiae* was also reported (Bene, 1989) in Tuscany while *C. vittatus* on *L. sativae* (Talebi *et al.*, 2005; Asadi *et al*, 2006) in Iran.

Apart from the dipteran leaf miners, *Cirrospilus* spp. were reported to parasitize okra leaf miner, *Trachys* sp. (Coleoptera: Buprestidae) (Rawat and Jakhmola, 1969) and pea leaf miner, *Phytomyza atricornis* (Diptera: Agromyzidae) in India (Gokulpure, 1973) while *C. quadristriatus* on citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) from India (Batra and Sandhu, 1981; Rao and Shivankar, 2002) and mango leaf weevil, *Rhynchaenus*

*mangiferae* (Fabricius) (Coleoptera: Curculionidae) in India (Peter and Balasubramanian, 1984).

**5.1.4.4 *Aprostocetus* spp.**

*Aprostocetus* spp. are widely distributed with about 764 species reported from different parts of the world. In the present study, *Aprostocetus* sp. was obtained as a larval parasitoid of *L. trifolii* from the weed plant, *Spilanthes* sp. So far there are no reports on the parasitization of *L. trifolii* by *Aprostocetus* sp. from India. Hence this could be considered as the first report as a parasitoid of *L. trifolii* from India. However, the parasitization of *Aprostocetus obtusae*, on *Melanagromyza obtusa* (Diptera: Agromyzidae) was reported from India by Narendran *et al.* (2005).

Several species of *Aprostocetus* were recorded from insects belonging to different Orders. Iyer (1942) reported *Aprostocetus krishnieri* as an important internal parasitoid of the amaranthus stem boring weevil, *Hypolixus truncatulus* and *Aprostocetus* (*Tetrastichus*) *sokolowskii* on *Plutella xylostella* from India (Patel and Patel, 1968).

Kishore *et al.* (1977) reported the parasitization of sorghum midge, *Contarinia sorghicola* (Coq.)), by *Aprostocetus* sp. *Aprostocetus purpureus* was described as natural enemies of *Planococcus* spp., *Coccus viridis* and *Ferrisia virgata* (Reddy *et al.*, 1990). *Aprostocetus neglectus* was reported from parasitized San Jose scale, *Quadraspidiotus perniciosus* (Rawat and Pawar, 1992) and *Aprostocetus niger* as parasitoids of gall insect, *Trioza fletcheri minor* (Singh *et al.*, 1995). Oriental mealybug, *Planococcus lilacinus* (Homoptera: Pseudococcidae) was also reported as a host of *A. purpureus* (Mani, 1995) and *A. purpureus* was also recorded as hyperparasitoid on exotic parasitoid, *Leptomastix dactylopii* parasitizing citrus mealybug, *Planococcus citri* (Krishnamoorthy and Mani, 1996). Kausalya *et al.* (1997) reported the parasitization of *Aprostocetus gala* and *A. coimbatorensis* on sorghum midge, *Stenodiplosis sorghicola* also from India.

Hayat *et al.* (2003) collected *Aprostocetus bangaloricus*, *Aprostocetus santalinus* as parasitoids from Coccoidea (Homoptera) attacking sandalwood, *Santalum album* from India. *Aprostocetus* sp. was also reported from *Rastrococcus iceryoides* (Das and Sahoo, 2005), eucalyptus gall wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae) (Vastrad *et al.*, 2009; Kavithakumari *et al.*, 2010).

The genus *Aprostocetus* had wide host range and hence this may not be a good parasitoid for *L. trifolii*.

**5.1.4.5 *Tetrastichus* sp.**

*Tetrastichus* sp. was recorded as a parasitoid of *L. trifolii* infesting cowpea from the polyhouse in the present study. It could not be collected from any of the vegetable crops in the open field.

The genus *Tetrastichus* has 518 species worldwide. Jagannatha (1994) also reported *Tetrastichus* sp. as larval parasitoids of *L. trifolii* from India. Several species of *Tetrastichus* were recorded as polyphagous parasitoids attacking the oothecae of cockroach, lac insect, scales, leaf hoppers and midges. Oothecae of two Indian species of cockroach were attacked by *T. hagenowii* and *T. asthenogmus* (Boucek *et al.*, 1979; Narashimham, 1984). Parasitization of *Tetrastichus purpureus* [*Aprostocetus purpureus*] was reported from lac insect, *Kerria lacca* (Srivastava and Mehra, 1980; Subbarayudu and Maheswar, 1998) and scale insect (*Melanaspis glomerata*) from India (Jadhav and Varma, 2001). *Tetrastichus diplosidis* was reported as a larval ecto-parasite of the sorghum ear head midge, *Contarinia sorghicola* from India (Thontadarya *et al.*, 1985). Parasitization of sugarcane leaf hopper, *Pyrilla perpusilla* by *Tetrastichus pyrillae* and *T. gala* were reported from India (Gholap and Chandele, 1985).

### 5.1.4.6 *Toxares* sp.

*Toxares* sp. obtained from the pupae of *L. trifolii* collected from snake gourd, is being reported for the first time as no earlier report of the species on the host.

In India, *Toxares shigai* was reported to parasitize *Mysus persicae* (Stary and Ghosh, 1975) while *Toxares deltiger* on *Aphis citricola, Brachycaudus helichrysi, Capitophorus hippophaes, Myzus ornatus, M. persicae, Metopolophium euryae* and *Schizaphis rotundiventris* (Stary and Ghosh, 1978), *Toxares shigai* on *Aphis farinosa* (Takada and Rishi, 1980), on *Brachycaudus helichrysi* (Stary and Ghosh, 1975), on *Chaitophorus leucomelas* (Takada and Rishi 1980) and on *M. persicae* (Stary and Ghosh, 1975).

In Japan, *Toxares macrosiphophagum* was recorded from *Aphis gossypii* (Takada, 1992).

Since *Toxares* sp. was commonly recorded as an aphid parasitoid, it also cannot be considered as an efficient parasitoid on *L. trifolii.*

### 5.1.5 Extent of damage by *Liriomyza trifolii*

The highest infestation index was observed for ash gourd (55%) followed by cowpea (45%) in the present study. Snake gourd, water melon, pumpkin and bitter gourd showed infestation indices of 42.5, 35, 25 and 25 per cent, respectively. Ash gourd, cowpea, snake gourd and watermelon were highly preferred by *L. trifolii.* This was in agreement with Smitha (2003) who reported severe infestation of leaf miner on cowpea, ash gourd and pumpkin. Pest surveillance studies conducted by Kerala Horticultural Development Programme also showed severe infestation of *L. trifolii* on the above mentioned crops (KHDP, 1998).

Ash gourd was observed as the highly preferred crop. This corroborates with the finding of Smitha (2003) who also reported the high susceptibility of ash gourd among cucurbits to *L. trifolii.* The bottom leaves were damaged more than upper

leaves by the larval stages of *L. trifolii*. The cotyledons were damaged more. The tender leaves were free from infestation. Higher sugar and nitrogen content of the cotyledons would enhance the infestation by *L. trifolii*. According to Ananthakrishnan (1992) sugar acted as feeding stimulants and larvae fed more voraciously on plant parts containing highest concentration of sugars. The chlorophyll content was also higher in cotyledons (Terman, 1977). Feeding activity and fecundity was also reported to be higher with increase in nitrogen content of leaf (Mikenberg and Ottenheim, 1990).

### 5.1.6 Emergence of parasitoids of *Liriomyza trifolii*

More than fifty per cent emergence of the parasitoids was observed from cowpea (59.17%) and snake gourd (51.67%). Ash gourd recorded a per cent emergence of 48.33 per cent. Drying of the leaves or leaf bits containing the larval stages or early pupal stages of parasitoid might be the reason for reduction in emergence of parasitoids.

### 5.1.7 Parasitism of *Liriomyza trifolii* in different vegetable crops

The number of species of parasitoids emerged from larvae of *L. trifolii* and rate of parasitism varied in different vegetable crops. The highest number of species of parasitoids (4) was obtained from cowpea. These were *Closterocerus* spp. (38.33% parasitism), unidentified eulophid (10% parasitism), *Chrysonotomyia* sp. (7.5% parasitism) and *Cirrospilus brevicorpus* (3.33% parasitism). Three species of parasitoids, namely, *Closterocerus* spp. (42.5% parasitism), *Chrysonotomyia* sp. (5% parasitism) and an unidentified eulophid (0.83% parasitism) were obtained from ash gourd. In watermelon, three species of parasitoids, namely, *Closterocerus* spp. (28.13% parasitism), unidentified eulophid (9.38% parasitism) and *Chrysonotomyia* sp. (6.25% parasitism) were present. Two different species of parasitoids obtained from snake gourd were *Closterocerus* spp. (50%) and *Chrysonotomyia* sp. (1.67%).

Two species were observed in bitter gourd and pumpkin, namely, *Closterocerus* spp. and *Chrysonotomyia* sp.

The parasitoid species attacking *L. trifolii* present in different crops would depend upon the host plants. Host plants species were reported to have an influence on host searching and oviposition of parasitoids (Robert *et al.*, 2012) due to significant interaction effect for host plant and *Liriomyza* species on parasitism and host feeding.

The study on the rate of parasitism of *L. trifolii* infesting six vegetable crops grown in nearby places of Vellanikkara showed that *Closterocerus* spp. was the most abundant species causing 27.78 to 50 per cent of parasitism of *L. trifolii*. This was followed by *Chrysonotomyia* sp. which caused 1.67 to 11.11 per cent parasitism of *L. trifolii*. *Closterocerus* spp. and *Chrysonotomyia* sp. were obtained from the host insect infesting cowpea, ash gourd, pumpkin, bitter gourd, watermelon and snake gourd. Smitha (2003) reported that *Chrysonotomyia rexia* was the most abundantly occurring species from Thrissur district apart from *Gryon* sp. and *Bracon* sp. Corroborating the present study with Smitha (2003), a shift in the species composition of parasitoids in Thrissur district on *L. trifolii* could be realized.

Hence considering the proportion of different parasitoids, *Closterocerus* spp. could be considered as the commonly found parasitoid species and a strong candidate that could be utilized for the suppression of *L. trifolii* especially in enclosed conditions like polyhouse followed by *Chrysonotomyia* sp.

### 5.1.6 Predators observed on *Liriomyza trifolii*

During the survey to collect natural enemies of *L. trifolii*, adult flies belonging to the family Dolicopodidae were observed hovering over the leaves. Dolicopodids were recorded as predators of small insects. Rauf *et al.* (2000) recorded dolicopodids as capturing and killing agromyzid adults.

Small ants were observed searching cowpea leaves infested with leaf miner under polyhouse cultivation. These ants opened the mines with their mandibles and extracted the maggots from the leaf mines and fed on it. Prieto and Ullola (1982) recorded a ponerine ant (Formicidae: Ponerinae) attacking the larvae of *L. trifolii*.

## 5.2 Laboratory evaluation of native entomopathogenic nematodes (EPNs) against *Liriomyza trifolii*

### 5.2.1 Isolation of entomopathogenic nematodes from soil

Soil is reported as a natural reservoir of EPNs (Akhurst, 1986; Gaugler, 1988) offering excellent conditions for nematode survival and activity. Four numbers of EPN isolates were obtained from 72 soil samples collected from three districts, namely, Thrissur, Ernakulam and Kottayam. The isolated EPNs were identified as *Steinernema carpocapsae* Weiser. Native soil isolated entomopathogens were used in the present study as the indigenous isolates of EPNs only could provide more efficient biological control because of the adaptation to local climate and population regulators of insect pest as opined by Bedding (1990). The EPNs were reported to have been isolated from all continents (except Antarctica) and all regions of the world (Hominick, 2002; Adams *et al.*, 2006). A check list of insect parasitic nematodes of India (Gantait and Sanyal, 2007) showed that a total of 72 species under three orders were reported so far from India. This list does not contain *S. carpocapsae* from Kerala. Hence this forms a new report for Kerala.

### 5.2.2 Mass rearing of *Liriomyza trifolii*

Rearing of the test insect *L. trifolii* was done both in polythene bags and in a low cost rearing cage fabricated for the study. The rearing cage was used for the multiplication of *L. trifolii* during off season. About 520 maggots could be produced within six days of release when ten to fifteen pairs of newly emerged adults were released. In the rearing cage, more number of adult flies was released to large number

of healthy seedlings which was not possible when reared in polythene bags. More number of seedlings could be infested with less number of flies.

The rearing cage for *L. trifolii* was made with locally available materials and hence it was very cheap. The design of the cage was very simple providing better aeration and penetration of light. Since there was no restriction in the number of males and females per plant, they could mate freely and could produce more eggs. According to Kaspi and Parrella (2008) *L. trifolii* females could remate more than once during their life time. Female fecundity was positively correlated with copulation rate (Arnqvist and Nilsson, 2000).

### 5.2.3 Bioefficacy of entomopathogenic nematodes against *Liriomyza trifolii*

The four soil isolated EPNs, namely, *S. carpocapsae* Isolate - 1, 2, 3 and 4 were tested for their efficacy against *L. trifolii* at five different concentrations along with *S. bicornutum* and *Heterorhabditis indica*. All the EPNs caused mortalities which varied at different time intervals after inoculation. Mortality of *L. trifolii* maggots occurred before 12 h and was directly proportional to time and concentration. The use of EPNs belonging to families Steinernematidae and Heterorhabditidae against leaf miners were reported earlier (Harris *et al.*, 1990; Olthof and Broadbent, 1991). LeBeck *et al.* (1993) reported that all larval stages were susceptible to *L. trifolii*. Steinernematid and Heterorhabditid nematodes were reported to cause mortality ranging from 48 to 98 per cent to larvae of *L. trifolii* (Hara *et al.*, 1993).

Dead maggots were seen inside the mines. EPNs were reported to enter through oviposition sites made by adult females or through tear in the mines (Harris *et al.*, 1990; LeBeck *et al.*, 1993)

The soil isolated EPNs caused mortality in a range of 0.00 to 83.33 at 12 HAT, 6.67 to 96.67 at 18 HAT, 26.67 to 100 per cent at 24 HAT and 50.00 to 100.00 per cent at 30 HAT. The mortality caused by *S. bicornutum* varied from 3.33 to 36.67

per cent at 12 HAT, 10.00 to 56.67 per cent at 18 HAT, 43.33 to 93.33 per cent at 24 HAT and 63.33 to 93.33 at 30 HAT. This shows the efficacy of *S. carpocapsae* Isolate 1, 2, 3, 4 and *S. bicornutum* in causing mortality to *L. trifolii* larvae. The result is in agreement with Harris *et al.* (1990) who reported 64 per cent mortality to leaf miner larvae in the laboratory with *S. carpocapsae*. Variation in effectiveness was observed between *S. carpocapsae* Isolates and *S. bicornutum* in the laboratory. This might be due to the variation in the pathogenicity of the symbiotic bacteria associated with different species of genus *Steinernema* as reported by Akhurst and Boemare (1990).

*Heterorhabditis indica* caused a mortality of 3.33 to 73.33 at 12 HAT, 13.33 to 90.00 at 18 HAT, 16.67 to 100.00 per cent at 24 HAT and 16.67 to 100.00 per cent at 30 HAT. Olthof and Broadbent (1991) reported 76 and 90 per cent mortality to leaf miners in the laboratory with *H. bacteriophora*.

### 5.2.4 Determination of median lethal concentrations

The $LC_{50}$ and $LC_{90}$ values were estimated to express the potency of the soil isolated EPNs, *S. bicornutum* and *H. indica*. The concentration of the IJs required to cause 50 and 90 per cent mortality to *L. trifolii* larvae were the lowest for *S. carpocapsae* Isolate - 1 at all time intervals. *S. carpocapsae* Isolate - 2 (isolated from Kannara) showed $LC_{50}$ and $LC_{90}$ values higher than that of Isolate - 1. This variation could again be supported by the findings of Akhurst and Boemare (1990).

### 5.2.5 Determination of $LT_{50}$

The time taken in hours to cause 50 per cent mortality for all the EPNs was worked out at different concentrations. Compared to other isolates, *S. carpocapsae* Isolate - 1 had low $LT_{50}$ and $LC_{90}$ values at all time intervals.

## 5.3 Pot culture evaluation of native entomopathogenic nematodes

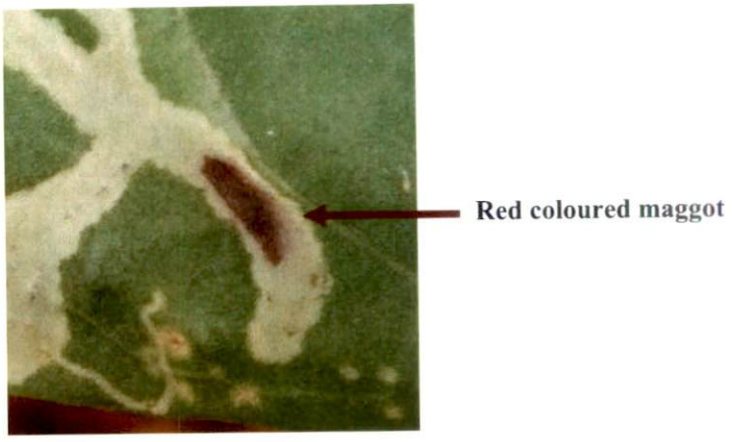
Pot culture evaluation of EPNs against *L. trifolii* was conducted with the selected soil isolate, *S. carpocapsae* Isolate - 1, *S. bicornutum*, *H. indica,* formulation of *H. indica*, *Beauveria bassiana* and the botanical insecticide, azadirachtin at 0.005% in the polyhouse. Pot culture experiments were conducted inside the polyhouse so as to prevent the natural parasitization of healthy maggots and the treatments were applied only once.

In the present study, *S. carpocapsae* Isolate - 1 caused 15.55 per cent mortality to the larvae of *L. trifolii* when applied at a concentration of 16 IJs/ maggot. The maggots infected with *Steinernema* spp. was brown coloured (Plate 16a). This was the characteristic of the symbiotic bacteria associated with particular species of EPNs. Olthof and Broadbent (1992) reported 53 to more than 83 per cent mortality to the larvae of leaf miners by *S. carpocapsae* in green house trials.

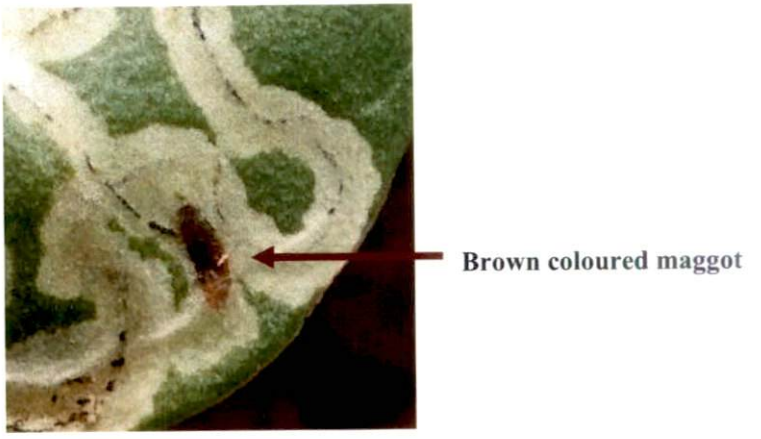
Application of *S. bicornutum* at 30 IJs/ maggot caused 9.99 per cent mortality to the larvae of *L. trifolii*. *H. indica* (@ 32 IJs/ maggot) caused 18.98 per cent mortality to the larvae of *L. trifolii*. The maggots killed by *H. indica* appeared red in colour inside the mines (Plate 16b).

The low mortality rates caused by the EPNs might be due to the lack of high humidity and occurrence of high temperature prevailing inside the polyhouse and also repeated application might be required to get satisfactory mortality. Maintenance of high relative humidity (above 90%) in the green house and moisture on the plants for at least six to eight hours after nematode applications was reported as critical for successful control leaf miners (Williams and Walters, 2000; Arthurs *et al.*, 2004). The best control of *L. trifolii* was reported to be achieved with two to four weekly applications of *S. carpocapsae* or *S. feltiae* at $1\times10^6$ IJs/ $m^2$ against second and the third instar larvae (LeBeck *et al.*, 1993; Williams and Walters, 2000).

**Plate 16. Maggots of *Liriomyza trifolii* infected with entomopathogenic nematodes**




**a) Maggot infected with *Heterorhabditis indica***




**b) Maggot infected with *Steinernema* spp.**

Application of the commercial formulation of *H. indica* had little effect on the control of *L. trifolii*. The larval stages of the leaf miners would be present on the leaves. Usually pre pupae of *L. trifolii* were dropped to the soil for pupation. But sometimes pupation occurred on the leaves and leaf axils. The pre pupae dropped to the soil could be killed by EPNs as it was reported as a susceptible stage (LeBeck *et al.*, 1993). The pupae were not reported to be attacked by the EPNs. The evaluation of the EPNs applied to the soil could not be observed correctly as the experiment was carried out in covered polybags to prevent the escape of emerging adult flies. The death of the flies could have occurred due to the high humidity and high temperature prevailed in the polybags.

In the present study, foliar application of *B. bassiana* was found ineffective in causing mortality to maggots of *L. trifolii*. Bordat *et al.* (1988) reported that *B. bassiana* was ineffective to puparia of *L. trifolii*.

Application of azadirachtin at 0.005% caused 84.51 per cent mortality to *L. trifolii* larvae and was significantly superior to all other treatments. Larew *et al.* (1985) reported significant mortality to the larvae and pupae of *L trifolii* with the application of 0.4 per cent crude neem extract. Knodel *et al.* (1986) reported that application of Margosan–O, commercial formulation of seed extract as foliar spray at 0.4, 0.84 and 1.25 per cent significantly reduced number of adults reared from treated plant. According to Sanderson *et al.* (1989) treatment with neem extracts (as Margosan-O) lasted long enough to disrupt the life cycle and was reported to reduce the chances of re-infestation from subsequent generations of the agromyzid.

All the maggots appeared dead within one day of application. This result is in agreement with the findings of Jeyakumar and Uthamasamy (1997) who reported Neem oil 50 EC (0.3%) (TNAU formulation) caused high larval mortality at 24 hours after treatment.

The pot culture evaluation conducted in the polyhouse indicated azadirachtin at 0.005% as the best treatment for the control of larval stages of *L. trifolii*. This was followed by the foliar application of *H. indica*.

## 5.4 Field efficacy of entomopathogenic nematodes against *Liriomyza trifolii*

In the field experiment, evaluation of the EPNs, *S. carpocapsae* Isolate -1, *H. indica*, formulation of *H. indica*, fipronil at 0.002% and neem formulation, azadirachtin at 0.005% were carried out against *L. trifolii*.

Fipronil and azadirachtin were significantly superior in controlling leaf miners, effecting more than 80 per cent mortality to the larvae. Rushtapakornchai and Petchwichit (1996) also reported significant control of *L. trifolii* with fipronil at 0.002%.

The effectiveness of neem extract and neem oil emulsion have been reported by Larew *et al.* (1985) and Jeyakumar and Uthamasamy (1997).

In the first spraying, foliar application of *H. indica* was on par with soil application of formulation of *H. indica.* But in the second spraying, foliar application of *H. indica* was superior to soil application of *H. indica* and untreated control.

The effect of the soil application of the commercial formulation of *H. indica* could not be assessed properly as emerging adults could not be collected and counted to see their emergence. EPNs present in soil might have caused mortality to the pre pupae. In the first and second sprayings, mortality of 44.1 per cent and 39.87 per cent was observed in the larval stages present inside the mines. This can be considered as natural mortality caused by parasitoids or by predators. Mortality of *L. trifolii* larvae was observed in control plots also (55.38% and 37.15%). The presence of immature stages of parasitoids was absent inside the dead maggots when observed under microscope. According to Heinz and Parrella (1989), *Diglyphus intermedius*, killed more hosts that it parasitized and killed larvae could be used for oviposition, host feeding or could be rejected. Patel *et al.* (2003) reported that when more hosts were

encountered, 35 per cent of the larvae were killed without oviposition. So the mortality caused to the maggots might be due to the feeding by adult parasitoids. The mortality can also be due to predators. Several predators, namely, mirids (Parrella *et al.*, 1982; Lucas and Alomar, 2002; Cantane *et al.*, 2004), ponerine ant (Prieto and Ullola, 1982), predaceous flies (Friedberg and Gijscoijt, 1984), predatory thrips (Arakaki and Okajima, 1998) and lynx spider in the family Oxyopidae (Prieto and Ullola, 1982) have been reported on *L. trifolii*.

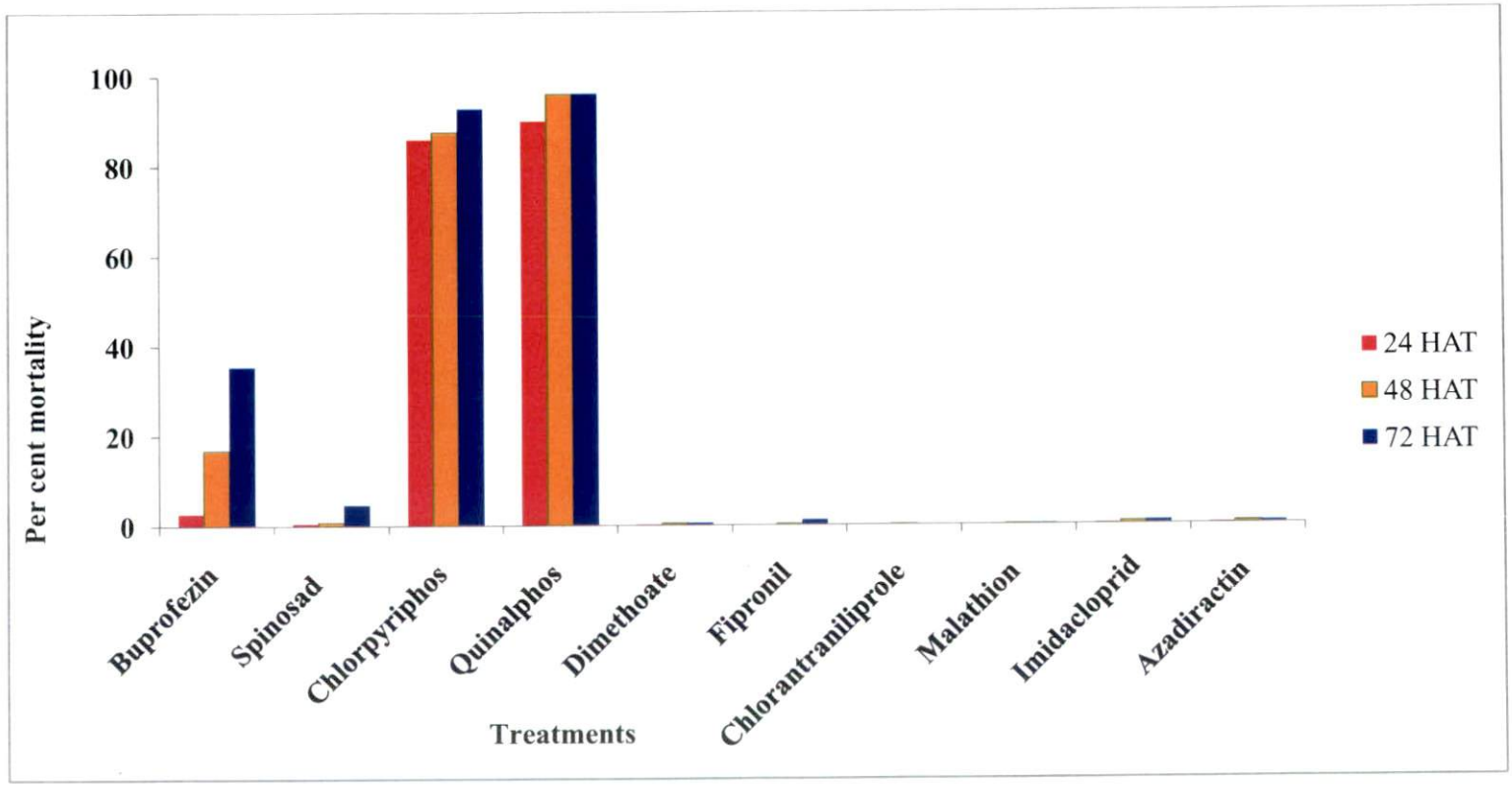
## 5.5 Compatibility of entomopathogenic nematodes with insecticides

Organophosphates (OP) and carbamates were considered as the most toxic chemical groups to steinernematids and heterorhabditids (Rovesti and Deseo, 1990; Gordon *et al.*, 1996). Hence in addition to the commonly used OP compounds, the newer molecules were also tested to find out their compatibility with the three entomopathogenic nematodes, namely, *Steinernema carpocapsae* Isolate - 1, *S. bicornutum* and *H. indica* in the laboratory by direct exposure method. The per cent mortality caused by different insecticides is shown in Fig. 1 to Fig. 3.

### 5.5.1 Compatibility of *S. carpocapsae* Isolate - 1 with insecticides

The compatibility of *S. carpocapase* Isolate - 2 with ten insecticides was evaluated in the laboratory (Fig. 1). Quinalphos at 0.05% and chlorpyriphos at 0.05% caused high mortality of 90.18 and 86.17 per cent respectively to IJs of *S. carpocapase* Isolate - 1 at 24 HAT and the mortality reached 96.17 and 92.87 per cent respectively after 72 h of treatment. In the present study, chlorpyriphos was observed as incompatible with the IJs of *S. carpocapsae* Isolate - 1. However, Negrisoli *et al.* (2010) and Seal *et al.* (2010) reported that chlorpyriphos was compatible with *S. carpocapsae*. The osmotically treated *S. carpocapsae* Strain All was also reported as compatible with chlorpyriphos (Xun *et al.*, 2012). The difference in the susceptibility of *S. carpocapsae* Isolate - 1 and *S. carpocapsae* Strain All to chlorpyriphos might be due to the difference in the strains. The compatibility of EPNs

**Fig. 1. Mortality caused by different insecticides to *S. carpocapsae* Isolate – 1**



was reported to depend upon strain specificity also in addition to species specificity (Laznik and Trdan, 2014).

Hussaini *et al.* (2001a) reported quinalphos as deleterious insecticide to some isolates of *Steinernema* and *H. indica* as survival and infectivity were impaired.

Buprofezin at 0.04% caused a mortality of 2.84 per cent at 24 HAT to 35.4 per cent at 72 HAT. This shows that buprofezin is moderately toxic to the IJs of *S. carpocapsae* Isolate - 1. Spinosad at 0.009% caused 4.68 per cent mortality to the IJs which was very low and negligible.

All other insecticides tested, namely, fipronil at 0.002%, chlorantraniliprole at 0.005%, malathion at 0.1%, imidacloprid at 0.006% and azadirachtin at 0.005% caused negligible mortality to the IJs and hence these were highly compatible with *S. carpocapsae* Isolate - 1. The compatibility of *S. carpocapsae* with malathion was reported by other workers also (Zang *et al.*, 1994; Gupta and Siddiqui, 1999; Xun *et al.*, 2012).

The compatibility of *S. carpocapsae* with fipronil, imidacloprid, azadirachtin and chlorantraniliprole was documented earlier (Xun *et al.*, 2012). Kulkarni *et al.* (2013) also reported the compatibility of *S. carpocapsae* with imidacloprid and spinosad. Spinosad at 0.009% caused very low per cent mortality of 4.68 at 72 HAT. A similar study conducted at Brazil by Negrisoli *et al.* (2010) revealing the compatibility of Tracer (spinosad) with *S. carpocapsae* under laboratory conditions is in accordance with present investigation.

Chlorantraniliprole at 0.005% caused mortality of 0.17 per cent which was the lowest compared to all insecticides tested expressing high compatibility of chlorantraniliprole at 0.005% with the IJs of *S. carpocapsae*. This corroborates with the result of Pino *et al.* (2013) who reported chlorantraniliprole as compatible with *S. carpocapsae*

The IJs of *S. carpocapsae* Isolate - 1, immersed in solution of dimethoate at 0.04% appeared as if dead with very little movement. Dimethoate is an organophosphorus compound. Reduction in the movement of IJs of *S. carpocapsae* was reported for organophosphorus compounds (Rovesti and Deseo, 1990).

This result shows that *S. carpocapsae* Isolate - 1 can be mixed with insecticides tested except quinalphos, chlorpyriphos and buprofezin.

### 5.5.2 Compatibility of *Steinernema bicornutum* with insecticides

The study on the compatibility of *S. bicornutum* with different insecticides (Fig. 2) showed that quinalphos at 0.05% was highly incompatible with the IJs of *S. bicornutum* and caused 99.93 per cent mortality at 72 HAT. The mortality was observed to be very low at 24 HAT and 48 HAT. But drastic increase in mortality was found at 72 HAT. All other insecticides were compatible with *S. bicornutum*.

The mortality caused by chlorpyriphos at 0.05% to *S. bicornutum* (1.41%) was observed to be very low when compared to its effect on the IJs of *S. carpocapsae* (92.87%). EPN species were reported to show difference in their susceptibility and sensitivity to different formulations of the same chemical pesticide (Grewal, 2002).

Malathion at 0.1% also caused low mortality of 5.23 per cent at 24 HAT to 10.19 per cent at 72 HAT to *S. bicornutum*.

The movement of the IJs of *S. bicornutum* was not affected while kept in the solution of dimethoate at 0.04% as observed for *S. carpocapsae*.

All other insecticides were observed to be compatible with IJs of *S. bicornutum* with negligible mortality. Hence all insecticides except quinalphos can be used together with *S. bicornutum*.

### 5.5.3 Compatibility of *Heterorhabditis indica* with insecticides

The compatibility of *H. indica* with ten insecticides (Fig.3) showed that all insecticides were compatible. Quinalphos at 0.05% caused 39.6 per cent mortality to

**Fig. 2. Mortality caused by different insecticides to *Steinernema bicornutum***

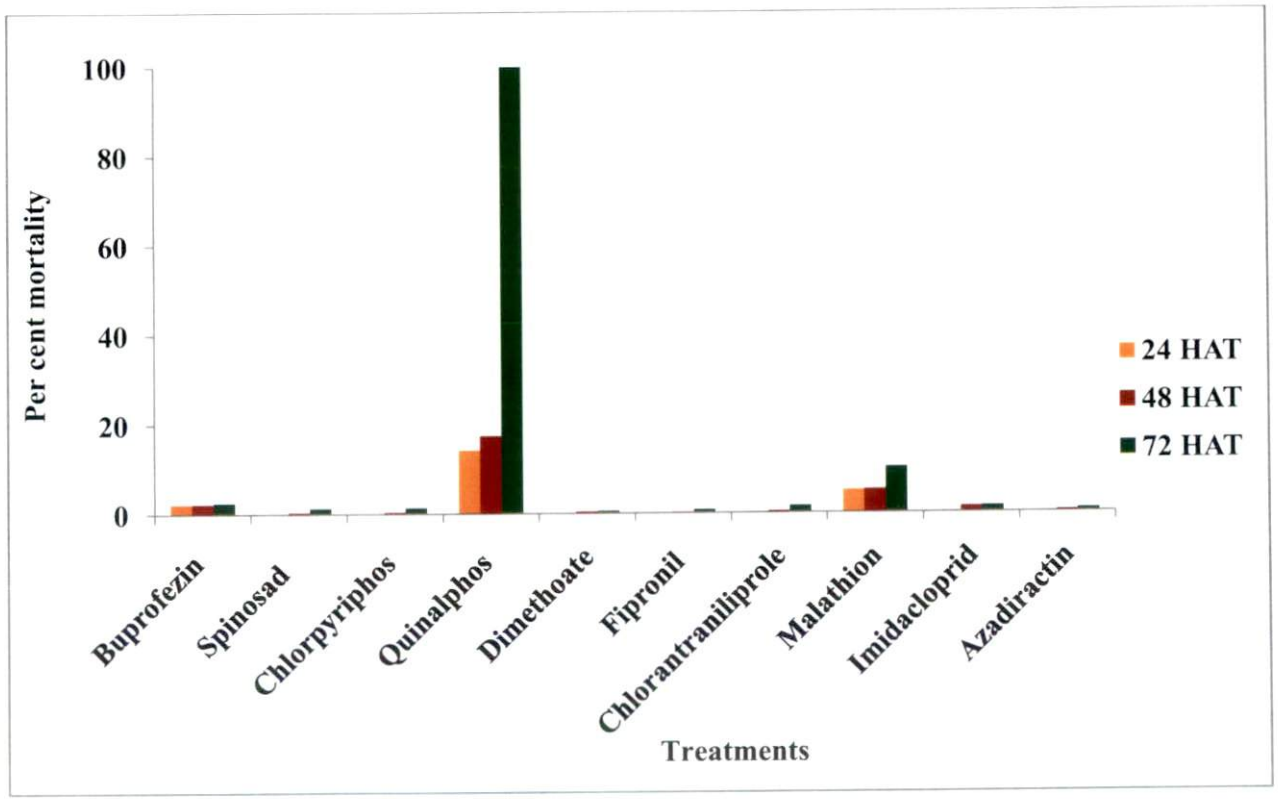
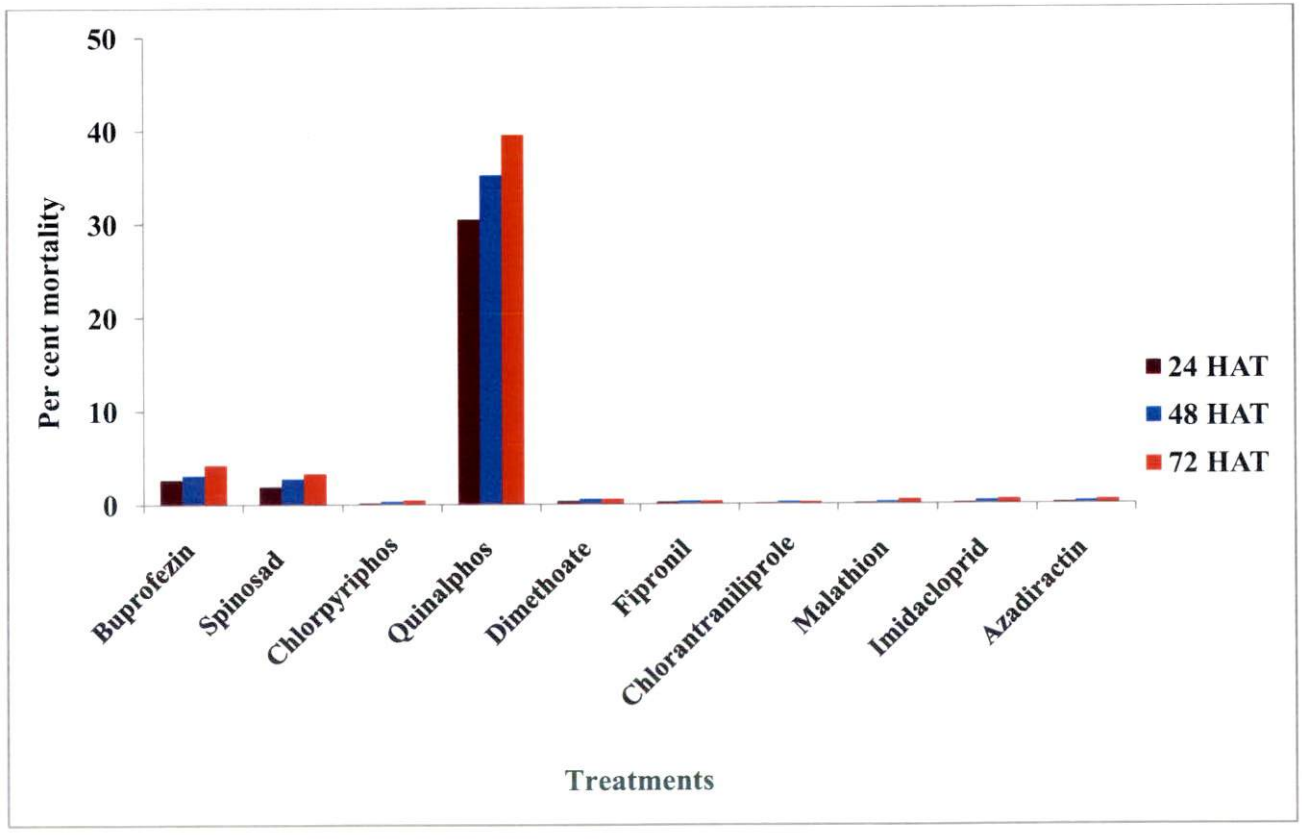


**Fig. 3. Mortality caused by different insecticides to *Heterorhabditis indica***



the IJs at 72 HAT. Prakash *et al.* (2011) reported that ICRI-18, a strain of *H. indica* showed five to 20 per cent mortality when treated with quinalphos. Hussaini *et al.* (2001a) proved quinalphos as deleterious to *H. indica* since survival and infectivity were impaired.

All other insecticides caused negligible mortality to *H. indica*. Buprofezin at 0.04% and spinosad at 0.009% caused 4.31 per cent and 3.37 per cent mortality respectively, to the IJs at even 72 HAT. As these two insecticides caused only less mortality, they could be considered as compatible with *H. indica*. Compatibility of *H. indica* with spinosad was reported earlier (Negrisoli *et al.*, 2010).

In contrast to the infectivity showed to the IJs of *Steinernema* sp., chlorpyriphos at 0.05% caused only 0.53 per cent mortality to *H. indica* at 72 HAT which could be considered as negligible. This was in agreement with the finding of Negrisoli *et al.* (2010) who reported chlorpyriphos as compatible with *H. indica*.

All other insecticides, namely, fipronil at 0.002%, chlorantraniliprole at 0.005%, malathion at 0.1%, imidacloprid at 0.006% and azadirachtin at 0.005% were compatible with *H. indica*.

Hence the compatibility study of *H. indica* showed that it is compatible with nine out of the ten insecticides tested and incompatible with quinalphos.

### 5.5.4 Virulence of the entomopathogenic nematodes

The IJs survived in the insecticides solutions for 72 h showed similar mortality with that of control even when the number of live IJs was less in number in a few insecticide-entomopathogenic nematode treatments. Emergence of the IJs was observed from all the parasitized larvae of *Galleria mellonella* L. This showed that the insecticides did not affect the infectivity and reproductive capacity of the IJs.

The compatibility study conducted with *S. carpocapsae* Isolate - 1, *S. bicornutum* and *H. indica* clearly showed that the insecticides affected the EPNs differently. Quinalphos is the most toxic insecticide to all EPNs.

*S. bicornutum* was incompatible with chlorpyriphos at 0.05%, while *H. indica* was compatible. Usually chlorpyriphos is being recommended for soil insects. Hence a combined application of *H. indica* is possible with chlorpyriphos.

# Summary

## SUMMARY


- Surveys were conducted in the vegetable fields of three districts, namely, Thrissur, Ernakulam and Kottayam from January to March, 2011 for the collection and identification of natural enemies associated with *Liriomyza trifolii*.
- Entomopathogens were not observed on any stages of *L. trifolii*
- Nine hymenopteran parasitoids, namely, *Closterocerus* sp. 1, *Closterocerus* sp. 2, *Chrysonotomyia* sp., *Cirrospilus brevicorpus* Shafee & Rizvi, *C. acadius* Narendran, *Tetrastichus* sp. and unidentified sp. (Subfamily Entedoninae) belonging to Eulophidae and *Toxares* sp. belonging to Braconidae were obtained as parasitoids of *L. trifolii*. *Aprostocetus* sp. was obtained from parasitized maggots of *L. trifolii* in weed plant, *Spilanthes calva* L.
- All parasitoids collected were solitary larval, endoparasitoids except *Toxares* sp. which was larval - pupal in nature
- Four species of parasitoids, *viz.*, *C. brevicorpus*, *C. acadius*, *Toxares* sp. and *Aprostocetus* sp. are reported for the first time in India as parasitoids of *L. trifolii*.
- *Closterocerus* spp. were the abundant parasitoids observed on *L. trifolii* followed by *Chrysonotomyia* sp.
- Per cent parasitism in the vegetable crops of three districts surveyed ranged from 10.96 to 46.78 in January, 22.01 to 58.99 in February and 17.04 to 46.82 in March

- A species of predatory fly (Family Dolichopodidae) and a species of small ants were observed as predators of adults and maggots of *L. trifolii,* respectively from the vegetable fields surveyed
- *Steinernema carpocapsae* was isolated for the first time from two locations in Kerala, namely, Kannara and Vellanikkara in Thrissur district
- *Steinernema carpocapsae* Isolate - 1 obtained from soil samples from Kannara was more effective against *L. trifolii* larvae with lowest $LC_{50}$ (1.79) and $LC_{90}$ (15.61) values (24 h)
- *Steinernema carpocapsae* Isolate - 1 showed low $LT_{50}$ values for all doses tested
- In pot culture experiment, azadirachtin 1 EC at 0.005% was the most effective treatment against *L. trifolii* followed by entomopathogenic nematode (EPN), *Heterorhabditis indica* at 32 IJs/ maggot
- In the field evaluation against *L. trifolii*, fipronil 5 SC at 0.002% was more effective followed by azadirachtin 1 EC at 0.005%
- Chlorantraniliprole 18.5 SC at 0.005% was found to be the most compatible insecticide with *S. carpocapsae* isolate - 1 causing only 0.17 per cent mortality to infective juveniles (IJs) at 72 hours after treatment (HAT). *Steinernema carpocapsae* Isolate - 1 was highly sensitive to quinalphos 25 EC at 0.05% and chlorpyriphos 20EC at 0.05%
- Dimethoate at 0.04% was the most compatible insecticide with *Steinernema bicornutum* and caused only 0.60 per cent mortality at 72 HAT and was followed by azadirachtin 1 EC at 0.005% with 0.78 per cent mortality to the IJs. Quinalphos 25 EC at 0.05% caused 99.93 per cent mortality at 72 HAT. *Steinernema bicornutum* was highly susceptible to quinalphos 25 EC at 0.05%

- *Heterorhabditis indica* was compatible with all insecticides tested except quinalphos 25 EC @ 0.05% which was moderately toxic resulting in 39.6 per cent mortality.
- Insecticides exposed and survived IJs could retain the virulence, pathogenicity and reproductive potential.

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# Annexure

## Annexure I

**Composition of PDA medium**

| | | |
|---|---|---|
| Potato | - | 200 g |
| Dextrose | - | 20 g |
| Agar | - | 20 g |
| Distilled water | - | 1 litre |

## Annexure II

**Diet of Greater wax moth, *Galleria mellonella* L.**

| | | |
|---|---|---|
| Wheat flour | - | 100 g |
| Wheat bran | - | 100 g |
| Glycerin | - | 175 ml |
| Milk powder | - | 100 g |
| Honey | - | 175 ml |
| Yeast | - | 50 g |
| Corn meal | - | 200 g |

# BIOTIC AGENTS FOR THE MANAGEMENT OF AMERICAN SERPENTINE LEAF MINER, *Liriomyza trifolii* (Burgess) (DIPTERA: AGROMYZIDAE)

By

**JYOTHI SARA JACOB**

**(2009-21-103)**

**ABSTRACT OF THE THESIS**

*Submitted in partial fulfilment of the requirements for the degree of*

## DOCTOR OF PHILOSOPHY IN AGRICULTURE

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# ABSTRACT

A study on "Biotic agents for the management of American serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)" was carried out at the Department of Agricultural Entomology, College of Horticulture, K.A.U., Vellanikkara during 2011-2013 with the objectives of collection and identification of indigenous natural enemies and to assess the pathogenicity of the entomopathogens to explore the feasibility of utilizing them for its management.

Surveys were conducted in the vegetable fields for the collection and identification of natural enemies associated with *L. trifolii* in three districts, namely, Thrissur, Ernakulam and Kottayam from January to March, 2011. The surveys revealed the occurrence of nine species of hymenopteran parasitoids. The per cent parasitism varied from 10.96 to 58.99 per cent among the crops surveyed. Three species of eulophids, namely, *Cirrospilus acadius* Narendran, *C. brevicorpus* Shafee & Rizvi *and Aprostocetus* sp. as well as the braconid, *Toxares* sp. are new reports for India. Among the parasitoids, *Closterocerus* spp. were the dominant group followed by *Chrysonotomyia* sp. All parasitoids were solitary, larval endoparasitoids except *Toxares* sp. which was larval-pupal in nature. One species each of small ants (Formicidae) and a dipteran fly (Dolichopodidae) were observed as predators on *L. trifolii*. In the study, no entomopathogens were observed from *L. trifolii*.

Considering the level of pesticide consumption in vegetable crops that undermine the potential of insect parasitoids and also that no entomopathogens could be observed during the survey, it was decided to evaluate entomopathogenic nematodes (EPNs) as biocontrol agents against *L. trifolii*.

Isolation of EPNs from 72 soil samples from Thrissur, Ernakulam and Kottayam districts yielded four isolates of *Steinernema carpocapsae*. Bioefficacy

studies carried out on these four isolates along with *Steinernema bicornutum* and *Heterorhabditis indica* showed that *S. carpocapsae* Isolate - 1 had the lowest $LC_{50}$, $LC_{90}$ and $LT_{50}$ values indicating their higher effectiveness against the maggots of the pest.

Pot culture study conducted to compare the potential of *S. carpocapsae* Isolate - 1 with other treatments showed that azadirachtin 1 EC at 0.005% was the most effective causing 84.51 per cent mortality to the maggots of *L. trifolii*. This was followed by the foliar application of *H. indica* at 32 infective juveniles (IJs)/ maggot which caused 18.98 per cent mortality. Application of *Beauveria bassiana* at $1\times10^7$ spores/ ml was not effective.

In the field evaluation, fipronil 5 SC at 0.002% was found to be the most effective treatment for controlling *L. trifolii* followed by azadirachtin 1 EC at 0.005%.

Compatibility of the IJs of the *S. carpocapsae* Isolate - 1, *S. bicornutum* and *H. indica* was studied with ten commonly used insecticides in the laboratory by direct exposure method. Chlorantraniliprole 18.5 SC at 0.005% was found to be the most compatible insecticide with *S. carpocapsae* isolate - 1 causing only 0.17 per cent mortality to IJs at 72 hours after treatment (HAT). Quinalphos 25 EC at 0.05% and chlorpyriphos20 EC at 0.05% were highly incompatible, causing 96.17 and 92.87 per cent mortality of the nematodes. Dimethoate 30 EC at 0.04% was the most compatible insecticide with *S. bicornutum* and caused only 0.60 per cent mortality at 72 HAT and was followed by azadirachtin 1 EC at 0.005% with 0.78 per cent mortality to the IJs. Quinalphos 25 EC at 0.05% caused 99.93 per cent mortality at 72 HAT. *Heterorhabditis indica* was compatible with all insecticides except quinalphos 25 EC at 0.05% which was moderately toxic resulting in 39.6 per cent mortality. The virulence, pathogenicity and multiplication of the survived IJs were not affected by the insecticide treatments.

Parasitoids and EPNs were observed as potential candidates for the management of *L. trifolii*. Hence future studies on the bio-ecology and mass production of dominant parasitoids and standardization of methods to improve the efficacy of EPNs are suggested for the successful control of *L. trifolii* in polyhouses as well as in the field.