



Research Note

Bio-efficacy of *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) against serpentine leafminer, *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) in oilseed crops

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ABSTRACT: Virulence and efficacy of Entomopathogenic Nematode (EPN), *Heterorhabditis bacteriophora* was tested against late instar larva of serpentine leafminer, *Liriomyza trifolii*, under laboratory conditions. Five different Infective Juvenile (IJs) concentrations (10, 30, 50, 70 and 100 IJ larva⁻¹) were used. The highest mortality rate caused by *H. bacteriophora* is 65.1% at 24 hours and 73.8% at 48 hours post inoculation at an inoculation concentration of 100 IJ larva⁻¹. The minimum concentration of 30 IJs per larva shown 37.1% and 52.2% mean larval mortality at 24 and 48 hours post inoculation, respectively. The virulence of *H. bacteriophora* (LC₅₀) was established at 54 IJs at 24 hours and 37.8 IJs at 48 hours for killing 50% of the larvae tested. The results of this study revealed the potential and scope of EPNs for their utilization in management of *L. trifolii* in oilseed crops.

KEYWORDS: Efficacy, *Heterorhabditis bacteriophora*, Late instar larva, serpentine leafminer

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INTRODUCTION

India is the 4th leading oilseeds producing country in the world and a wide variety of oilseeds (groundnut, rapeseed and mustard, sunflower, soybean, sesame, safflower, castor, linseed and niger) are grown in India. These crops are majorly grown under rainfed conditions and are more prone to damage caused by insect pests. Serpentine leafminer, *Liriomyza trifolii* (Burgess) is one of the predominant pest on a number of economically important crops including oilseeds. It is native to Southern United States of America and Central America. In India, *L. trifolii* was first reported in the 'Annual castor research workers' group meeting held at Hyderabad (DOR-1991), *L. trifolii* is a polyphagous pest, and it attacks 78 plant species belonging to 16 families (Srinivasan *et al.*, 1995). Damage is caused by the maggots, which feeds on the leaf mesophyll tissues causing leaf mines on the upper leaf surface. Heavy infestation causes drying and early dropping of leaves (Chandler and Thomas, 1983). Previous studies reported 70% yield loss in tomato (Zoebisch *et al.*, 1984), 15 to 70 % in French bean and 41% in cucumber (Krishna Kumar, 1998) caused by *L. trifolii* indicating the damage potential of this pest. The mines caused by the larva even act as entry points for pathogens making the plant susceptible to secondary infection. Eggs are laid by the adult females under

the leaf surface just below the epidermis. After hatching larva starts feeding on mesophyll cells and undergo further molting inside the leaf mine. The fourth instar larva is a non-feeding stage which comes out of the mine and falls into the soil for pupation. Many hymenopteran parasitoids attack leafminer present in leaf mines under natural conditions. The pre-pupal and pupa stages present in soil are very difficult to reach by natural enemies or even chemical pesticides. Another drawback with natural enemies is they are highly sensitive to chemical pesticides.

Entomopathogenic nematodes (EPNs) are biocontrol agents proven effective against a number of insect pests, especially on insects dwelling either fully or part of their lifecycle in soil. Use of entomopathogenic nematodes belonging to genera *Steinernema* and *Heterorhabditis* is one the biological control approach used against leafminer (Hara *et al.*, 1993). When compared to Steinernematids, Heterorhabditids has the capability to enter the host through natural openings as well as through host cuticle having high chance of infecting insects with soft cuticles like leafminers. Previous studies using *Steinernema* spp. and *Heterorhabditis* spp. against leafminer demonstrated significant larval mortality under laboratory conditions (Harris *et al.*, 1990; Le Beck *et al.*, 1993; Hara *et al.*, 1993; Jacob and Mathew,

2016) as well as reduction of leaf damage caused by larvae in greenhouse trials (Olthof and Broadbent, 1992; Williams and Walters, 2000). In this study, we aimed at utilizing the efficiency of EPNs against soil dwelling stages of serpentine leafminer. Hence, a laboratory study was carried out with *H. bacteriophora* to determine the potential of EPNs against serpentine leafminer, *L. trifolii* infecting castor.

One EPN species, *Heterorhabditis bacteriophora* was used in this study. The base culture of *H. bacteriophora* was procured from National Institute of Plant Health Management, Hyderabad. The infective juveniles (IJs) were multiplied on last instar larvae of the greater wax moth (*Galleria mellonella*) at 25 ± 1 °C for using in bioassay (Woodring and Kaya, 1988). The dead insects were placed on white's trap and emerged IJs were collected into distilled water (White, 1927). The nematode suspension was cleaned and stored at 20 ± 2 °C in B.O.D incubator. Before using for bioassay, the nematode suspension was left at room temperature for 2-3 hours for reviving the activity of nematodes and used for inoculation.

Source of *Liriomyza trifolii*

Culture of serpentine leafminer, *Liriomyza trifolii* maintained on potted castor plants (cv. DCH-177) in glass house was used for the experiment. Usually in castor, leafminer infestation occurs during early crop period especially on cotyledon leaves and lower leaves. Adult flies of serpentine leafminer were collected using aspirator from castor fields of ICAR-IIOR, Hyderabad and allowed for oviposition in potted castor plants at seedling stages (20 days after sowing). After multiplication, the required number of larvae for the different treatments were taken from the culture. For the bioassay, the infested leaves containing larvae were collected and observed for larval activity and size under stereomicroscope. Active and bigger larvae were selected and leaf portion containing the larva were cut and used directly in bioassay with *H. bacteriophora*.

Twelve well tissue culture plates were used to study virulence of *Heterorhabditis bacteriophora* on late instar larva of *L. trifolii* using five IJ concentrations of 10, 30, 50, 70, and 100 IJs larva⁻¹. Two tissue culture plate was used for each concentration. Whatman's filter paper (2.5 mm

diameter) was placed at bottom of each well and added with 100 µl volume of respective IJ concentrations to each well. One leafminer larva was placed in each well. For control, distilled water was added to one plate and larvae were placed in the well. All bioassay plates were sealed and incubated at 24 ± 2 °C in a BOD incubator. The plates were checked for larval mortality at 24, 48 and 72 hours. Cadavers were dissected under stereomicroscope to confirm the mortality as a result of EPN infection. The bioassay was repeated twice independently.

The experiment was carried out using completely randomized design and data was subjected to ANOVA to determine variance among the treatment means. CRD analysis was performed using WASP (Web Agri Stat Package) software developed by ICAR - Central Coastal Agricultural Research Institute. In addition, LC₅₀ values for *H. bacteriophora* were determined using the Probit analysis on mortality data (Finney, 1952).

In plate bioassay, *L. trifolii* larvae showed high susceptibility to *H. bacteriophora*. In general, the mortality of larvae increased with concentrations and also time of exposure. Among the five inoculation concentrations (10, 30, 50, 70 and 100 IJs), the larval mortality at 24h post inoculation at all concentrations ranged from 6.4% to 65.1% with significant differences in larval mortality between different IJ concentrations [$F=130.9$; CD (critical difference) = 11.578; $P<0.01$] (Fig. 1). Similarly, the overall mortality of larvae at 48h after treatment with *H. bacteriophora* ranged from 10.6% to 73.8% with mortality significantly different among the IJ concentrations used ($F=46.3$; CD=21.1; $P<0.01$) (Fig. 1). The calculated LC₅₀ value of *H. bacteriophora* at 24h is 54 and 37.8 at 48h (Table 1) indicating the susceptible nature of *L. trifolii* larvae to *H. bacteriophora* infection.

Our study provides an insight about the pathogenicity of *H. bacteriophora* to late instar larvae of serpentine leafminer, *L. trifolii*. The larvae were highly susceptible to *H. bacteriophora* with mortality of 6.4% to 73.8% at IJ concentrations @ 10, 30, 50, 70 and 100 IJs larva⁻¹. Further the LC₅₀ value (54 IJs) of *H. bacteriophora* also indicated that tested EPN strain is highly virulent to leafminer larva

Table 1. LC₅₀ values for *Heterorhabditis bacteriophora* against the larval stage of *Liriomyza trifolii* at 24 and 48 h post-infection

Hours after treatment	LC ₅₀ (95% fiducial limit)	Slope ± SE	Intercept±SE	Chi ² (df=3)	R ²
24	54.019(34.51-84.55)	1.932±0.006	1.652±0.01	0.749	0.975
48	37.86(23.98-59.77)	1.886±0.01	2.022±0.017	0.542	0.945

(Nguyen, 2008). Similar studies were performed by Lebeck *et al.* (1993) with *Steinernema carpocapsae* on different larval instars of *L. trifolii* and observed that second stage larva was highly susceptible to EPN infection than other instars and the IJs entered the larval mines through oviposition holes made by adults and through natural tears in mines and infected larvae through anus. In another study by Hara *et al.*, (1993), 20 different EPN species (*Heterorhabditis* spp. and *Steinernema* spp.) were evaluated for their efficacy against larvae of leafminer and observed larval mortality ranging from 32 to 80 %. No significant difference in virulence was observed among the strains. Further studies in green house experiment revealed that a relative humidity of > 92% caused > 65% leafminer larval mortality irrespective of the species tested. Similar observations were made by Williams and Walters (2000), when *S. feltiae* applied to leafminers of vegetables (*L. bryoniae* and *L. huidobrensis*) at high relative humidity (>90%), high larval mortality was observed than applying more nematodes at lower relative humidity. Jacob and Mathews (2016) evaluated the efficacy of *S. carpocapsae*, *S. bicornatum* and *H. indica* on *L. trifolii* larvae. *S. carpocapsae* was found virulent with low LC₅₀ (1.79 IJ/larva) in comparison with *S. bicornatum* (11.73 IJs larva⁻¹) and *H. indica* (21.99 IJs larva⁻¹). The mortality of *L. trifolii* larvae was directly proportional to time and concentration of IJs. Our study is in agreement with this observation that the increase in exposure time and concentration of EPN infective juveniles increased the mortality rate in leafminer larvae. Many of larvae started pupating within few hours after inoculation of IJs in our study, but mortality was observed even after pupation. It may be due to the quick action of EPNs and exposure to IJs during larval period. No doubt that EPNs are highly efficient in killing wide range of insect pests but their susceptibility to environmental stresses makes their utility questionable. Our laboratory study clearly depicted the potential of *H. bacteriophora* against *L. trifolii*. More research is to be carried out towards developing formulations which improves the performance of EPNs besides protecting them from fluctuations in the environment.

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