Potential of two indigenous strains of entomopathogenic nematodes, (*Steinernema feltiae* and *Heterorhabditis bacteriophora*) against the tomato leafminer (TLM), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), under laboratory and greenhouse environmental bioassay

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Research Article

Keywords: Biocontrol, Tomato, *T. absoluta*, *S. feltiae*, *H. bacteriophora*, Greenhouse, Environment

Posted Date: January 27th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1065129/v2>

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Abstract

The tomato leafminer (*Tuta Absoluta*) is an important pest of tomato crops which have features like; high reproductive potential, Invasive species are major threats to agronomic and natural ecosystems. Based on the pathogenicity of strains, only two isolates effectively show larvicidal activity. The native isolate was obtained from soil samples, collected from Rajgarh, Hamachi Pradesh, India. Petri dish bioassay use nematodes *S. feltiae* HR1 and *H. bacteriophora* HR2 species dose (0, 10, 20, 40, 80, 160/ IJs/cm²). The (%) 1st instar larval mortality was ranged from 24.15 to 85.38%. 2nd instar from 29.87 to 90.00%. 3rd instar from 24.15 to 90.00%. 4th instar from 31.53 to 90.00% and pupae stage mortality was ranged from 31.53 to 85.38%. Larvicidal activity after 48- and 72-hours exposure, the *S. feltiae* and *H. bacteriophora* (1.0, 1.30, 1.60, 1.90, 2.20/IJs/cm²) showed potent larvicidal activity with LC₅₀, LC₇₅ and LC₉₀ of all instars and pupa show high mortality. The strain inhibits the larval and pupal development 48 to 72 hr exposer time with LC₅₀ range from 05.42 to 23.67, LC₇₅ 20.29 to 83.12, LC₉₀ 16.52 to 98.89. Green house test is using the seam isolate of EPNs (HR1, HR2) on foliar application it caused by significant mortality results. These studies demonstrate the challenge for invasive species. The local indigenous strains of EPNs (*S. feltiae* HR1, *H. bacteriophora* HR2) as a good biocontrol agent against invasive pest of *T. absoluta*.

Key Message

- In the laboratory, the entomopathogenic nematode (EPN) *Steinernema feltiae*, and *Heterorhabditis bacteriophora* native isolate HR1, HR2, caused 90.00% above mortality in *T. absoluta*.
- In the greenhouse EPN isolate are caused 95% mortality in *Tuta absoluta* F₁ hybrid tomato crop plant.
- LC₅₀, LC₇₅, LC₉₀ ranged between 23.67 – 83.12 and 2.20 IJ/cm² in a period of 48–72 h.
- Under greenhouse conditions, a 3 foliar prying application of EPN with a mortality achieved significant % mortality of *T. absoluta*.
- Additionally, native EPNs isolate have enormous potential for the control of foliage pests and may become a powerful alternative for *T. absoluta* control.

Introduction

Invasive species are important threats to the ecological and agricultural systems. Tomato leafminer (TLM), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is native to South America. Outside its native place, the pest was detected for the first time in discovered in Spain in 2006 (Urbaneja et al., 2008), and since it quickly attacked 80 countries including India (CABI, 2018; CABI, 2020). The insect feeds on leaves, fruits, shoots, and apical shoots tomatoes can also cause 100 percent damage to plants in the absence of regulatory measures (Urbaneja et al., 2012; Balal et al., 2016; Biondi and Desneux, 2019). In India, the emergence of this insect was first carved with tomatoes in Pune and Bangalore in 2014 (Sridhar et al., 2014; Shashank et al., 2015), and since then it has spread to almost every part of the country tomato planting (Sridhar et al., 2014; Kalleshwaraswamy et al., 2015; Shashank et al., 2015; Balal et al., 2016; Sharma and Gavkare, 2017). In Himachal Pradesh, *T. absoluta* was first acquired in 2015 tomatoes in Nauni, Solan (Sharma and Gavkare, 2017). On problem-solving has emerged as a major threat to tomatoes in various parts of the state in open spaces and poly-houses. Previous reports indicate that in recent areas, *T. absoluta* quickly acquired a serious insect condition without drug abuse (Bielza, 2010; Desneux et al., 2010). *Tuta absoluta* control in particular based on pesticides and sometimes up to 14 times Insecticides while growing were needed (Campos et al., 2017; Han et al., 2019; Abbas et al., 2021; Waiba et al., 2021). Therefore, this insect is difficult to control chemically because the larvae live inside the mines and fruits where it is difficult to find pesticides. Biotic energy is high, spread speed again the ability to increase resistance to pesticides keeps pests at the bay challenge (Desneux et al., 2010; Ingegno et al., 2013; Roditakis et al., 2013; Roditakis et al., 2015). Invasive new invasive insect, tomato pinworm / small leaf, *T. Absoluta* was first recorded in Pune in a tomato plant that grew in a field and a thousand houses in 2014 and in southwestern India (Karnataka state) (ICAR 2015), and to our knowledge, there are no reports of this pest in eastern and northern and eastern India. Subsequently, the pest was reported on a farmer's farm in major tomato growing districts including Himachal Pradesh.

Entomopathogenic nematodes (EPNs) from the families Heterorhabditidae and Steinernematidae are soil organisms that bind to natural insects (Kaya and Gaugler 1993). These nematodes have evolved into interactions with bacteria in the genera *Photorhabdus* associated with *Heterorhabditis* spp., It is carried in the intestines of infectious hairs (IJs) (Bird and Akhurst 1983; Arthurs et al., 2004; Silva et al., 2002; Kasi et al., 2021). *Xenorhabdus* is connected to *Steinernema* spp. and is confined to a specific skin within the intestines of IJs. Nematodes receive their treatment by tracking insects (Lewis et al., 2006; Mansour and Biondi, 2021). After IJs have found a culprit, they infect it with an orifice such as the mouth, anus or edges or by entering the cuticle (especially in *Heterorhabditis* spp.). As soon as the IJs enter the host, they break down their outer cuticle (Sicard et al., 2004; Ben Husin and Port, 2021) and begin to absorb the hemolymph, causing the defiant to be released by extraction (*Steinernema* spp.) Or recurrence (*Heterorhabditis* spp.) (Martens et al., 2004; Martens and Goodrich-Blair 2005). The structure of nematodes - bacteria kill the host between 24 - 48h through septicemia or toxemia (Dowds and Peters 1971; Forst and Clarke 2002; Ndereyimana et al., 2020). Bacteria reminiscent of nematodes, which appear as IJs from extinct insect cadaver in search of new transmitters (Poinar 1990).

More than 100 species of EPNs have been identified worldwide (about 80% by steinernematid) and at least 13 of these species have been marketed (Shapiro-Ilan et al., 2014; Ndereyimana et al., 2019). In general, the natural violence against various insect species varies between EPN species. In addition, the differences between the types of EPNs in terms of demand management and tolerance of environmental conditions such as temperature and humidity may determine the field performance of EPNs (Martens et al., 2004; Noug, 2021). EPNs have been widely used in the control of economically important pests living in different habitats (Grewal et al., 2005; Susurluk, 2008). However, the formation of EPNs to delay the extinction or addition of supplements to increase leaf coverage and persistence of IJs have improved the use of EPNs in insect-infested animals (Williams and Walters 2000; Arthurs et al., 2004; Head et al., 2004; Kasi et al., 2021; Ben Husin and Port, 2021).

The objective of the present study was to provide fundamental information necessary for the utilization of indigenously isolated EPNs as biological control agents. The study dealt with 2 nematode species such as *S. feltiae* and *H. bacteriophora* and their pathogenicity against *Tuta absoluta* under laboratory and greenhouse conditions.

Materials And Methods

A *T. absoluta* colony was maintained on tomato plants under greenhouse conditions. The colony had been established from larvae collected in September 2020 from the vegetable science department from the tomato greenhouse in UHF, HP, India, that used *S. feltiae* and *H. bacteriophora* (Poinar) for the pest's management.

Rearing of tomato pinworm, *Tuta absoluta*

TLM larvae and pupae were collected in a place that holds full heat greenhouse. *T. Absoluta* was raised in a nursery greenhouse (Roditakis et al., 2013), at $26 \pm 2^\circ\text{C}$, $60 \pm 10\%$ RH, and L: D 8:16 photoperiod. These insects are grown in wooden cages covered with 80 mesh organdy cloth on tomato plants (*S. Lycopersicum* L.). Adults are fed 10% of the sugar solution in the oviposition cage.

Source of entomopathogenic nematodes

Two isolates of *S. feltiae* and *H. bacteriophora* were used in this study. The native isolate was obtained from soil samples, collected from Rajgarh, Hamachi Pradesh, India, using *G. mellonella* larvae as nematode traps. This isolate was cultured based on the method describe (Woodring and Kaya 1988) at $21 \pm 1^\circ\text{C}$ on the last instar larvae of *G. mellonella*. Infective juveniles (IJs) that emerged during the first ten days were collected from white traps stored at 4°C in distilled water for up to 14 days (Woodring and Kaya 1988). The nematodes were acclimatized at room temperature for about 30 min before being used in the experiments.

Effect of Nematode Concentration

Bioassays was performed in a petri dish (9 cm). Each unit is filled with 20 grams of sandy loam (Table 1). Soil moisture was adjusted to 7% (w / w). IJs were applied evenly on the sterilized sand surface at 0, 5, 10, 20, 40, 80, and 160 IJs / cm² in 1 ml of distilled water. Final sand moisture 10% (w / w). Ten t in each instar. The containers were kept at room temperature for 1 h before placing the entire larvae on the soil surface. There are four replicas for each concentration. Under controlled conditions the containers were kept in the growth chamber for 72 h, after which the larvae were separated from the surface through a fine sieve and held individually under controlled conditions until adulthood. Three days later, 25% of the dead larvae were randomly selected and dissected under a stereomicroscope to confirm nematode infection. The experiment was performed twice.

Larvicidal activity

Each nematode species was added at different concentrations (1.00, 1.30, 1.60, 1.90, 2.20/ mL⁻¹) into the 9 cm petri dish in triplicate with 2 ml of dechlorinated sterile water and 60 larvae of tested *Tuta absoluta* strains. The larvae were provided with young tomato leaves. One Petri plate without EPNs suspension was used as a control. After 24, 48, and 72 hr the number of dead larvae was calculated. The strains that killed more than 50% of the larvae were considered pathogenic (Morton and Garcia-del-Pino 2009; Kasi et al., 2021). Two nematode isolates were examined quantitatively for larvicidal activity against *T. absoluta*, using various concentrations of EPNs suspensions. The infected larvae were observed under a stereo zoom microscope for each concentration at 72 h exposure time.

Greenhous condition experimental fields

Study Site Selection

The experimental farm is located at an elevation of 1260 m above mean sea level with 30°52'N latitude and 77°11'E longitude with East-West orientation of poly-house which represents the mid-hill zone of Himalayas, HP, India. This is an ideal location for poly-house with various features like single door, side, and top vent, drip irrigation, fogging facility, and internal shading with 50 % green agro UV stabilized shade net.

Experimental material and layout plan

The tomato germplasm commercial market, F₁ Hybrid (BSS-816) were evaluated under a naturally ventilated poly-house having a 200 m² area. The experiment was conducted in a randomized block design (RBD) with four replications inside the modified naturally ventilated polyhouse (25 m × 10 m). Ten plants of the hybrid were planted at a spacing of 70 × 30 cm and trained on two stems in each replication.

Treatments

Table 1 Treatments used in the field experiment to control *Tuta absoluta* (M.)

Designation	Treatment description	Type of treatment
Native strain	<i>S. feltiae</i>	Entomopathogenic nematode
T ₁	1,00, 000 /IJ's / m ⁻²	Entomopathogenic nematode
T ₂	2,00,000 / IJ's / m ⁻²	Entomopathogenic nematode
T ₃	4,00,000 / IJ's / m ⁻²	Entomopathogenic nematode
Native strain	<i>H. bacteriophora</i>	
T ₄	1,00,000 / IJ's / m ⁻²	Entomopathogenic nematode
T ₅	2,00,000 / IJ's / m ⁻²	Entomopathogenic nematode
T ₆	4,00,000 / IJ's / m ⁻²	Entomopathogenic nematode
T ₇	Water	Untreated (Control)

Statistical Analysis

Insect mortality was control-corrected (Abbott 1925) and Arcsine transformed when required to meet assumptions of normality and homogeneity of variances. In all experiments, control-corrected mortality was subjected to one-factor analysis of variance (ANOVA)

$$(\% \text{ mortality in treatment} - \% \text{ mortality in control})$$

$$\text{Corrected mortality (\%)} = \frac{\text{ } }{100 - \% \text{ mortality in the control}} \times 100$$

The corrected percent mortality data thus obtained for different concentrations of *Tuta absoluta* (M.) at different concentrations were subjected to probit analysis as per the method given (Finney 1971). Concentration-mortality response data was conducted. Also, LSD ($P < 0.05$) values were calculated to differentiate means among treatments.

Results

Petri dish bioassay of *Tuta absoluta* life stages

bioassay of *T. absoluta* (1st instar)

The results revealed that the 1st instars larvae of the tomato leafminer were highly susceptible to two nematode species tested. After 72 h of treatment, there was an increase in (%) larval mortality in all treatments. The (%) larval mortality was ranged from 24.15 to 85.38 % (Fig 1). The highest % larval mortality was recorded in treatment with *H. bacteriophora* at 160 IJs/cm² were 85.38 % larval mortality was followed by *S. feltiae* at 160 IJs/cm² (80.76 %). The next best treatments in order of their efficacies were *H. bacteriophora* at 80 IJs/cm² (67.47 %), *S. feltiae* at 80 IJs/cm² (63.78 %) (Fig 1).

bioassay of *T. absoluta* (2nd instar):

There was no larval mortality observed in the untreated control. The (%) larval mortality was ranged from 29.87 to 90.00 %. After 72 h of treatment, the treatment *S. feltiae* at 160 IJs/cm² (90.00%) recorded the highest larval mortality of 2nd instars *T. absoluta* larvae and was found to be the most superior treatment. Which was followed by *H. bacteriophora* at 160 IJs/cm² (80.76%). The next best treatments in order of their efficacies were *S. feltiae* at 80 IJs/cm² (78.73%), *H. bacteriophora* at 80 IJs/cm² (72.08%). The (%) larval mortality was ranged from 24.15 to 90.00 % (Fig 2).

bioassay of *T. absoluta* (3rd instar):

Recorded highest larval mortality of 3rd instars *T. absoluta* larvae and found superior treatment. The highest percent larval mortality was recorded in treatment with *H. bacteriophora* at 160 IJs/cm² were 90.00% larval mortality was recorded which was followed by *S. feltiae* at 160 IJs/cm² (85.38%). The next best treatments in order of their efficacies were *H. bacteriophora* at 80 IJs/cm² (74.12%), *S. feltiae* at 80 IJs/cm² (69.50%) (Fig 2).

bioassay of *T. absoluta* (4th instar):

The (%) larval mortality was ranged from 31.53 to 90.00 %. Recorded highest larval mortality of 4th instars *T. absoluta* larvae and found to be most superior treatment. The highest percent larval mortality was recorded in treatment with *S. feltiae* at 160 IJs/cm² were 90.00% larval mortality was recorded which was

followed by *H. bacteriophora* at 160 IJs/cm²(80.76%) (Fig 4). The next best treatments in order of their efficacies were *S. feltiae* at 80 IJs/cm² (74.12%), *H. bacteriophora* at 80 IJs/cm² (67.84%) (Fig 4).

bioassay of *T. absoluta* (Pupa):

The (%) larval mortality was ranged from 31.53 to 85.38 %. Recorded highest larval mortality of pupae stage of *T. absoluta* larvae and found to be most superior treatment. The highest % larval mortality was recorded in treatment with *H. bacteriophora* at 160 IJs/cm² (85.38%) larval mortality was recorded which was followed by *S. feltiae* at 160IJs/cm² were 80.76%. The next best treatments in order of their efficacies were *H. bacteriophora* at 80 IJs/cm² (69.50%), *S. feltiae* at 80 IJs/cm² (67.47%) (Fig 5).

Bioassay of larvicidal activity

A toxicity assay was conducted to estimate the lethal concentration of entomopathogenic nematodes to following:

Larvicidal activity (1st instar)

1st instar larvae of *Tuta absoluta*. The LC₅₀, LC₇₅ and LC₉₀ for the *S. feltiae* (1.0, 1.30, 1.60, 1.90, 2.20/IJs/cm²), *H. bacteriophora* (1.0, 1.30, 1.60, 1.90, 2.20/IJs/cm²), 1st instar for 48 h were evaluated as LC₅₀ of 13.29 µg mL⁻¹, 23.67 IJs/cm² and LC₇₅ of IJs/cm², 75.64 IJs/cm², 78.77 IJs/cm², and LC₉₀ of IJs/cm², 31.64 IJs/cm², 32.46 IJs/cm² respectively. For 72 h exposure (Fig 6), LC₅₀ of 14.08 IJs/cm², 15.94 IJs/cm², LC₇₅ of 46.54 IJs/cm², 42.58 IJs/cm² and LC₉₀ of 16.52 IJs/cm², 10.07 IJs/cm² (Table 1).

Larvicidal activity (2nd instar)

2nd instar larvae of *Tuta absoluta*, for 48 h were evaluated as LC₅₀ of 14.27 IJs/cm², 13.47 IJs/cm² and LC₇₅ of IJs/cm², 48.42 IJs/cm², 55.26 IJs/cm², and LC₉₀ of IJs/cm², 45.40 IJs/cm², 96.81 IJs/cm², respectively. For 72 h exposure (Fig 7), LC₅₀ of 07.05 IJs/cm², 07.61 IJs/cm², LC₇₅ of 22.85 IJs/cm², 29.39 IJs/cm² and LC₉₀ of 65.81 IJs/cm², 65.14 IJs/cm² (Table 1).

Larvicidal activity (3rd instar)

3rd instar larvae of *Tuta absoluta*, for 48 h were evaluated as LC₅₀ of 19.29 IJs/cm², 08.55 IJs/cm² and LC₇₅ of IJs/cm², 83.12 IJs/cm², 40.47 IJs/cm², and LC₉₀ of IJs/cm², 50.44 IJs/cm², 98.89 IJs/cm², respectively. For 72 h exposure (Fig 8), LC₅₀ of 07.52 IJs/cm², 05.42 IJs/cm², LC₇₅ of 25.42 IJs/cm², 20.70 IJs/cm² and LC₉₀ of 26.03 IJs/cm², 69.19 IJs/cm² (Table 1).

Larvicidal activity (4th instar)

4th instar larvae of *Tuta absoluta*, for 48 h were evaluated as LC₅₀ of 09.32 IJs/cm², 13.90 IJs/cm² and LC₇₅ of IJs/cm², 48.17 IJs/cm², 60.10 IJs/cm², and LC₉₀ of IJs/cm², 74.18 IJs/cm², 76.43 IJs/cm², respectively. For 72 h exposure (Fig 9), LC₅₀ of 05.89 IJs/cm², 08.92 IJs/cm², LC₇₅ of 20.29 IJs/cm², 32.61 IJs/cm² and LC₉₀ of 61.71 IJs/cm², 56.63 IJs/cm² (Table 1).

Larvicidal activity (Pupae stage)

Pupae of *Tuta absoluta*, for 48 h were evaluated as LC₅₀ of 09.10 IJs/cm², 13.90 IJs/cm² and LC₇₅ of µg mL⁻¹, 41.75 IJs/cm², 60.10 IJs/cm², and LC₉₀ of IJs/cm², 78.47 IJs/cm², 98.43 IJs/cm², respectively. For 72 h exposure (Fig 10),

Table 1.
Log probit analysis of larvicidal activity of tested nematode strain against tomato pinworm *Tuta absoluta* larvae instars and pupa stage

Stage of Insect	Nematode sp.	Exposure Time (hrs)	LC ₅₀ (IJs/cm ²) 95% LCI-UCL	LC ₇₅ (IJs/cm ²) 95% LCI-UCL	LC ₉₀ (IJs/cm ²) 95% LCI-UCL	Intercept	Slope ± SE	χ ² value	P-value
1- instar	<i>S. feltiae</i>	48	13.29 (08.17 - 21.63)	31.64 (22.35 - 88.18)	75.64 (46.51 - 23.03)	-1.004	1.3 ± 0.22	1.05	0.012*
			72	14.08 (09.90 - 20.01)	46.54 (32.74 - 66.16)	16.52 (96.05 - 94.04)	-1.49	1.5 ± 0.24	3.10
1- instar	<i>H. bacteriophora</i>	48	23.67 (16.85 - 33.25)	32.46 (26.53 - 65.49)	78.77 (56.08 - 11.65)	-1.77	01 ± 0.23	1.49	0.002**
			72	15.94 (11.85 - 21.45)	42.58 (31.65 - 57.29)	10.07 (76.61 - 38.67)	-1.90	1.3 ± 0.25	4.41
2- instar	<i>S. feltiae</i>	48	14.27 (09.98 - 20.40)	48.42 (33.87 - 69.22)	45.40 (17.71 - 20.87)	-1.46	02 ± 0.24	2.30	0.004**
			72	07.05 (04.81 - 10.33)	22.85 (15.60 - 33.46)	65.81 (44.93 - 96.37)	-1.12	1.2 ± 0.26	1.83
2- instar	<i>H. bacteriophora</i>	48	13.47 (08.98 - 20.20)	55.26 (36.85 - 82.86)	76.81 (31.24 - 82.13)	-1.24	01 ± 0.23	0.83	0.006**
			72	07.61 (05.02 - 11.53)	29.39 (19.40 - 44.53)	65.14 (65.44 - 50.21)	-1.01	1.5 ± 0.25	0.86
3- instar	<i>S. feltiae</i>	48	19.29 (12.83 - 29.01)	50.44 (25.79 - 65.31)	83.12 (24.99 - 55.28)	-1.36	02 ± 0.23	0.27	0.004**
			72	07.52 (05.11 - 11.07)	25.42 (17.27 - 37.41)	26.03 (51.66 - 11.89)	-1.11	01 ± 0.25	2.06
3- instar	<i>H. bacteriophora</i>	48	08.55 (05.42 - 13.51)	40.47 (25.63 - 63.90)	78.89 (10.79 - 83.77)	-0.93	01 ± 0.23	1.99	0.018*
			72	05.42 (03.51 - 08.36)	20.70 (13.41 - 31.97)	69.19 (44.81 - 17.83)	-0.85	02 ± 0.26	1.85
4- instar	<i>S. feltiae</i>	48	09.32 (05.80 - 14.98)	48.17 (29.98 - 77.41)	74.18 (31.42 - 39.34)	-0.91	1.3 ± 0.23	0.46	0.018*
			72	05.89 (03.92 - 08.85)	20.29 (13.51 - 30.47)	61.71 (41.09 - 92.68)	-0.96	1.2 ± 0.26	1.53
4- instar	<i>H. bacteriophora</i>	48	13.90 (09.16 - 21.10)	60.10 (39.60 - 91.20)	76.43 (47.89 - 40.56)	-1.21	01 ± 0.23	2.18	0.007**
			72	08.92 (06.01 - 13.24)	32.61 (21.98 - 48.38)	56.63 (70.52 - 55.23)	-1.14	02 ± 0.24	1.02
Pupa	<i>S. feltiae</i>	48	09.10 (05.82 - 14.23)	41.75 (26.71 - 65.28)	78.47 (15.21 - 57.11)	-0.97	1.3 ± 0.23	2.57	0.015*
			72	08.83 (05.88 - 13.26)	33.89 (22.57 - 50.88)	60.68 (75.72 - 64.66)	-1.09	02 ± 0.24	1.66
Pupa	<i>H. bacteriophora</i>	48	13.90 (09.16 - 21.10)	60.10 (39.60 - 91.20)	81.43 (47.89 - 40.56)	-1.21	01 ± 0.23	2.18	0.007**
			72	09.23 (06.35 - 13.43)	31.11 (21.40 - 45.24)	72.83 (63.84 - 34.98)	-1.23	1.5 ± 0.25	5.12
<ul style="list-style-type: none">• EPNs suspensions were administered at a concentration of (IJs/cm²).• Lethal Concentration are calculated by Probit Methods.• Significant differences from control are indicated (**<i>P</i> < 0.01; *<i>P</i> < 0.05).									

-LC₅₀ of 08.83 IJs/cm², 09.23 IJs/cm², LC₇₅ of 33.89 IJs/cm², 31.11 IJs/cm² and LC₉₀ of 60.68 IJs/cm², 72.83 IJs/cm² were noted by using (OPSTAT) software, the best larvicidal activity was obtained during the 72 h of exposure. The *S. feltiae* and *H. bacteriophora*, strains showed potent larvicidal activity with low concentration even at 48 and 72 h of exposure to compare other strains (Table 1).

The mortality was determined through different concentrations for both 48 and 72 h exposure. The mortality rate depends on the concentration and exposure time. However, the highest mortality range was observing *H. bacteriophora* treatment at very low concentrations for 48 and 72 h. Even though *S. feltiae* showed slow mortality in 48 and 72 h exposure time, they restrained the larval development at the early pupal stage.

Efficacy of EPNs against *T. absoluta* in greenhouse condition

Data pertaining to the survival population of tomato leafminer *Tuta absoluta* on tomato one day before and 3rd, 7th 10th and 14th days after first spray was presented in (Table 2). The mean survival population of *T. absoluta* larvae one day before spray was ranged from 5.77 to 7.36 larvae/ plant. The pre-treatment data was found to be non-significant indicating the uniformity population of pest throughout the experimental plot. At 14th days of first spray slight increase in the larval population was observed in all the treated plots. The mean number survival populations of larvae were ranged from 1.31 to 2.13 larvae/plant. In untreated control plot it was recorded highest 4.09 larvae/plant. The treatment with *H. bacteriophora* at 1,00,000 IJ's/cm² (1.31 larvae/plant) was found be consistently effective against *T. absoluta* followed by *H. bacteriophora* at 2,00,000 IJ's/cm² (1.42 larvae/plant) which were found to be equally effective.

Treatment with *S. feltiae* at 1,00,000 IJ's/cm² (1.59 larvae/plant), *S. feltiae* at 2,00,000 IJ's/cm² (1.68 larvae/plant) which were the next in order of their efficacy and significant differences did not existed in remaining treatments.

Thus, overall results on efficacy indicated that *H. bacteriophora* at 1,00,000 IJ's/cm² (1.78 larvae/plant) was the most effective compared to other treatments in reducing the survival population of *T. absoluta*. The treatment with *S. feltiae* at 1,00,000 IJ's/cm² (1.97 larvae/plant) was the next best effective treatment. Followed by *H. bacteriophora* at 2,00,000 IJ's/cm² (2.00 larvae/plant) were next in order of efficacy. At 14th days of second spray slight increase in the larval population was observed in all the treated plots. The mean number survival populations of larvae were ranged from 1.18 to 1.51 larvae/plant. In untreated control plot it was recorded highest 4.24 larvae/plant. The treatment with *H. bacteriophora* at 2,00,000 IJ's/cm² (1.18 larvae/plant) was found be consistently effective against *T. absoluta* followed by *S. feltiae* at 2,00,000 IJ's/cm² (1.38 larvae/plant) which were found to be equally effective. Treatment with *H. bacteriophora* at 1,00,000 IJ's/cm² (1.45 larvae/plant), *H. bacteriophora* at 4,00,000 IJ's/cm² (1.48 larvae/plant) which were the next in order of their efficacy and significant differences did not existed in remaining treatments.

Thus the overall performance of the treatments indicated that the treatment with *H. bacteriophora* at 2,00,000 IJ's/cm² (1.60 larvae/ plant) was found most superior over *H. bacteriophora* at 1,00,000 IJ's/cm² (1.66 larvae/plant), *H. bacteriophora* at 4,00,000 IJ's/cm² (1.77 larvae/plant). Treatments with *S. feltiae* at 1,00,000 IJ's/cm² (1.84 larvae/plant), *S. feltiae* at 2,00,000 IJ's/cm² (1.85 larvae/plant), *S. feltiae* at 4,00,000 IJ's/cm² (2.06 larvae/plant) were found equally effective in reducing the *T. absoluta* population. At 14th days of third spray slight increase in the larval population was observed in all the treated plots. The mean number survival populations of larvae were ranged from 1.02 to 1.98 larvae/plant. In untreated control plot it was recorded highest 4.92 larvae/plant. The treatment with *H. bacteriophora* at 4,00,000 IJ's/cm² (1.02 larvae/plant) was found be consistently effective against *T. absoluta* followed by *H. bacteriophora* at 2,00,000 IJ's/cm² (1.09 larvae/plant) which were found to be equally effective. Treatment with *S. feltiae* at 2,00,000 IJ's/cm² (1.17 larvae/plant), *S. feltiae* at 1,00,000 IJ's/cm² (1.34 larvae/plant) and *S. feltiae* at 4,00,000 IJ's/cm² (1.53 larvae/plant), *H. bacteriophora* at 1,00,000 IJ's/cm² (1.98 larvae/plant) were found to be equally effective. However, the treatments with respectively as compared to 4.92 larvae/ plant in untreated control.

Thus, overall performance of the various treatments after third spray found that treatment with *H. bacteriophora* at 4,00,000 IJ's/cm² proved to be consistently most effective and superior over rest of the treatments and recorded the lowest larval population (1.17 larvae/ plant) compared to 4.48 larvae/plant recorded in untreated control. Treatment with *H. bacteriophora* at 2,00,000 IJ's/cm² (1.34 larvae/plant), was next best treatment in the order of efficacy. Significant differences do not exist among the treatments with *S. feltiae* at 2,00,000 IJ's/cm² (1.60 larvae/plant) and *S. feltiae* at 4,00,000 IJ's/cm² (1.65 larvae/plant) were found on par with each other. Whereas, the treatments with *S. feltiae* at 1,00,000 IJ's/cm² (1.83 larvae/plant), and *H. bacteriophora* at 2,00,000 IJ's/cm² (2.25 larvae/ plant) were found moderately effective in reducing the surviving *T. absoluta* population. The overall results of the present investigation after three spraying presented in (Table 20.4) revealed that among the entomopathogenic agents *H. bacteriophora* at 2,00,000 IJ's/cm² proved to be consistently effective against *T. absoluta* by recording a least larval population (1.64 Larvae/plant). The treatment with *H. bacteriophora* at 4,00,000 IJ's/cm² and *S. feltiae* at 1,00,000 IJ's/cm², *S. feltiae* at 2,00,000 IJ's/cm² also showed better results against *T. absoluta*.

Table 2.

Efficacy of entomopathogenic nematode against *Tuta absoluta* in tomato crop (overall effect of three sprays).

Treatments /(Isolates)	EPNs Dose IJs/m ²	Pre count	1 st Spray				Mean	2 nd Spray				Mean	3 rd Spray				Mean
			Mean survival population of <i>T. absoluta</i> larvae/plant					Mean survival population of <i>T. absoluta</i> larvae/plant					Mean survival population of <i>T. absoluta</i> larvae/plant				
			3 DAS	7 DAS	10 DAS	14 DAS		3 DAS	7 DAS	10 DAS	14 DAS		3 DAS	7 DAS	10 DAS	14 DAS	
<i>S. feltiae</i> (HR1)	1,00,000	05.77 (02.60)	02.41 (01.84)	02.16 (01.77)	01.72 (01.64)	01.59 (01.60)	01.97 (01.71)	02.15 (01.77)	01.92 (01.70)	01.79 (01.67)	01.51 (01.58)	01.84 (01.68)	02.66 (01.91)	01.70 (01.64)	01.63 (01.62)	01.34 (01.53)	01.83 (01.67)
<i>S. feltiae</i> (HR1)	2,00,000	06.52 (02.74)	02.67 (01.91)	02.36 (01.83)	02.05 (01.74)	01.68 (01.63)	02.19 (01.77)	02.12 (01.76)	02.10 (01.76)	01.80 (01.67)	01.38 (01.54)	01.85 (01.68)	01.83 (01.68)	01.76 (01.66)	01.64 (01.62)	01.17 (01.47)	01.60 (01.60)
<i>S. feltiae</i> (HR1)	4,00,000	06.90 (02.80)	02.74 (01.93)	02.40 (01.84)	02.34 (01.82)	02.07 (01.75)	02.38 (01.83)	02.17 (01.78)	02.12 (01.76)	02.12 (01.76)	01.83 (01.68)	02.06 (01.74)	01.58 (01.60)	01.74 (01.65)	01.77 (01.66)	01.53 (01.59)	01.65 (01.62)
<i>H. bacteriophora</i> (HR2)	1,00,000	07.20 (02.85)	02.317 (01.82)	02.06 (01.75)	01.44 (01.56)	01.31 (01.51)	01.78 (01.66)	01.87 (01.69)	01.56 (01.60)	01.77 (01.66)	01.45 (01.56)	01.66 (01.62)	02.59 (01.89)	02.31 (01.82)	02.12 (01.76)	01.98 (01.72)	02.25 (01.79)
<i>H. bacteriophora</i> (HR2)	2,00,000	06.51 (02.73)	02.577 (01.89)	02.19 (01.78)	01.84 (01.68)	01.42 (01.55)	02.00 (01.72)	01.74 (01.65)	01.81 (01.67)	01.68 (01.63)	01.18 (01.47)	01.60 (01.60)	01.64 (01.62)	01.43 (01.56)	01.23 (01.49)	01.09 (01.44)	01.34 (01.52)
<i>H. bacteriophora</i> (HR2)	4,00,000	06.21 (02.68)	02.64 (01.90)	02.26 (01.80)	02.14 (01.77)	02.13 (01.76)	02.29 (01.80)	01.74 (01.65)	01.83 (01.68)	02.05 (01.74)	01.48 (01.57)	01.77 (01.66)	01.41 (01.55)	01.17 (01.42)	01.08 (01.44)	01.02 (01.42)	01.17 (01.45)
Control	Water Spray	07.36 (02.88)	03.53 (02.12)	03.87 (2.20)	04.08 (02.25)	04.09 (02.25)	03.89 (02.20)	03.79 (02.19)	04.03 (02.24)	04.28 (02.29)	04.24 (02.28)	04.08 (02.25)	04.16 (02.27)	04.23 (02.28)	04.61 (02.37)	04.92 (02.43)	04.48 (02.33)
SE±		N/A	0.02	0.01	0.04	0.05		0.007	0.01	0.01	0.01		0.02	0.02	0.02	0.01	
CD at 5%		0.08	0.06	0.05	0.14	0.16		0.02	0.05	0.05	0.05		0.07	0.07	0.06	0.05	

DAS = Days after spray

*Figures in parentheses are transformed values $\bar{O}x + 0.5$

Discussion

In this study it is evident that *S. feltiae* and *H. bacteriophora* were able to kill *Tuta absoluta* larvae at various concentrations namely 1,00,000, 2,00,000 and 4,00,000 IJs/ cm². *Tuta absoluta* larvae mortality increased with increase in concentrations. In this case the highest concentration tested (4,00,000 IJs/ cm²) achieved the highest mortality under poly-house conditions. This mortality increase with increase in concentration can be attributed to large population of symbiotic bacteria released by EPNs when they penetrate the larvae as reported by (Eleftherianos *et al.* 2010; Han *et al.*, 2019). Steinernematid IJs retain *Xenorhabdus* symbionts within an intestinal vesicle, while *Photorhabdus* cells stick together in the anterior part of the Heterorhabditids gut and releases them upon invasion of an insect host (Dillman *et al.* 2012; Lankin *et al.*, 2021).

The third instar larvae were used; other studies revealed that EPNs were able to find and kill all the four larval instars inside or outside the leaf galleries (Batalla-Carrera *et al.*, 2010; Van Damme *et al.*, 2016; Kasi *et al.*, 2021). Found that *T. absoluta* larval stage was the most vulnerable to EPNs (Batalla-Carrera *et al.*, 2010; Ben Husin and Port, 2021). They thus emphasized the necessity to apply EPNs on the above-ground part of the tomato plant to ensure effective control of this pest using the most suitable isolates against a particular pest in a given environment (Abbas *et al.*, 2021; Mansour and Biondi, 2021).

The ability of EPNs to reach and kill *T. absoluta* larvae in leaf galleries was also reported (Batalla-Carrera *et al.*, 2010; Van Damme *et al.*, 2016; Kamali *et al.*, 2018). The local EPN isolates were able to kill *T. absoluta* and had even been found to be effective against white grubs in Rwanda (Kajuga *et al.*, 2018; Ndereyimana *et al.*, 2019), while it was not easy to find EPNs which can kill them (Laznik *et al.*, 2015). Although EPNs live naturally in soil, different researchers found that they can be used on above-ground parts of the plant to control effectively the pests living in cryptic habitats like in leaf galleries (Batalla-Carrera *et al.*, 2010; Garcia-del-Pino *et al.*, 2013; Lankin *et al.*, 2021), which concurs with the results of the present study under laboratory conditions.

The IJs multiply quickly and kill the host 24 – 72 h after infection (Gozel and Kasap 2015; Van Damme *et al.*, 2016; Kasi *et al.*, 2021). This was verified in the present study where all local EPN isolates caused between 53.3 and 96.7 % mortality just within the first 24 h after inoculation, while in 72 h, they all had caused between 96.3 and 100 % mortality. This is not the case of other entomopathogens like entomopathogenic fungi, which require 3–5 days or even more time to kill their host (Reda and Hatem 2012; Abbas *et al.*, 2021).

Different pathogenicity levels displayed by the studied EPNs agree with other studies, using different EPN isolates (Gozel and Kasap 2015; Van Damme *et al.*, 2016; Han *et al.*, 2019). This underlines the necessity of EPNs screening and selection as emphasized (Sharma *et al.*, 2011; Biondi *et al.*, 2018), in a view to boost their efficacy of EPNs. The highest efficacy of local EPN isolates than the exotics could be explained by the fact that these EPNs (Yan *et al.*, 2016; Kasi *et al.*, 2021; Mansour and Biondi, 2021) and they might be more adapted to the local conditions than the exotics, which were isolated in a completely different environment. These results agree with the earlier findings where locally isolated biological control agents, including EPNs, performed better than exotics (Lima *et al.*, 2017; Nough, 2021). The larvae of *T. absoluta* present susceptibility to EPNs, unlike their pupae, which present tolerance and thus a low mortality rate of less than 20% caused by EPNs (Batalla-Carrera *et al.*, 2010; Garcia-del-Pino *et al.*, 2013; Ben Husin and Port, 2021; Kasi *et al.*, 2021).

The results of this study revealed that local EPN isolates were able to find and kill *T. absoluta* larvae stages inside the leaf galleries under laboratory conditions and their efficacy increased with exposure time. The efficacy of local indigenous EPN isolates was significantly superior to that of the exotic species. This is the first study carried out in Rajghar on the good biocontrol potential of indigenous strains (HR1 and HR2) EPNs against *T. absoluta*. The results of this study form the basis for further research. High EPN efficacy obtained under laboratory conditions cannot easily be extrapolated to field efficacy. Therefore, future field experiments on tomato crops are justified to fully determine the potential of local EPN isolates against *T. absoluta* in Himachal Pradesh conditions.

List Of Abbreviations

EPN: Entomopathogenic nematodes; IJs: Infective juveniles; OPSTAT: Operational Status

Declarations

Credit authorship contribution statement

All authors jointly designed the experiment. KI conducted the laboratory bioassays, performed data analysis and drafted the manuscript with inputs from all authors. MS, MW and PS collaborated closely with KI in the whole process especially during data analysis. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements.

We would like to acknowledge the Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India-173230 for support of this research. And Indian council of Agricultural Research (ICAR) for providing necessary facilities and financial support, respectively We are also grateful to Department of entomology, Nematology laboratory used in this study.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding authors on reasonable request.

Consent for publication

All authors agree to the publication of the submitted manuscript.

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Figures

Figure 1

Efficacy of *S. feltiae* and *H. bacteriophora* against 1st instar of *T. absoluta*

Figure 2

Efficacy of *S. feltiae* and *H. bacteriophora* against 2nd instar of *T. absoluta*

Figure 3

Efficacy of *S. feltiae* and *H. bacteriophora* against 3rd instar of *T. absoluta*

Figure 4

Efficacy of *S. feltiae* and *H. bacteriophora* against 4th instar of *T. absoluta*

Figure 5

Efficacy of *S. feltiae* and *H. bacteriophora* against pupae of *T. absoluta*

Figure 6

1st instar Log Dose response Curve

Figure 7

2nd instar Log Dose response Curve

Figure 8

3rd instar Log Dose response Curve

Figure 9

4th instar Log Dose response Curve

Figure 10

Pupae log Dose response Curve