



Evaluation of Indigenous Strains of Entomopathogenic Nematodes, in Combination with Low-Toxicity Insecticides at Low and High Dosages South American Tomato Pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae)

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ABSTRACT

The laboratories experiments were conducted during September, 2021 to test the pathogenicity of two EPNs species *S. feltiae* and *H. bacteriophora* at different (IJs cm⁻²) concentrations against *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae). The experimental location in Department of Entomology, Nematology Laboratory, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India. The native isolate was obtained from soil samples, collected from survey was carried out in Mid-hills of the North Himalayas, India, to investigate the diversity and frequency of entomopathogenic nematodes (EPNs). Our objective was to evaluate the efficacy of *S. feltiae* (HR1) and *H. bacteriophora* (HR2) in combination with low-toxicity insecticides at low and high dosages to control final-instar larvae in bioassays. The use of *S. feltiae*+Spinosad or chlorantraniliprole caused larvae mortality of over 100% at 96 h and second bioagent *H. bacteriophora*+Spinosad and chlorantraniliprole mortality of over 97.50% at 96 h at the high dose and should be included as a least toxic strategy to control *T. absoluta*. Our results showed that *T. absoluta* were suitable hosts for local indigenous strains performed good potential for biological control against invasive species. In combination with low-toxicity insecticides at low and high dosages to control final-instar larvae mortality of over 100% at 96 h at the high dose and should be included as a least toxic strategy to control tomato pinworm.

KEYWORDS: Dosages, efficacy, larvae mortality, synergistic effect, *Tuta absoluta*

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Invasive species are key threats to agronomic and natural ecosystems. The tomato leafminer (TLM), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is an invasive pest which has spread its geographic range to many parts of the world in recent years. Features such as high reproductive potential, relatively short life cycle, and the invasive feeding behavior of this insect have caused difficulties in the successful control of this pest and have facilitated the development of pesticide resistance (Batalla-Carrera et al., 2010, Barati et al., 2018, Waiba et al., 2021a). The larvae of *T. absoluta* can penetrate and feed on all aerial parts of host plants such as; stems, leaves, buds, and fruits (Desneux et al., 2010). Since its recognition as an economic pest, chemical control has been the major strategy for controlling this pest (Lietti et al., 2005). In India, *T. absoluta* was first-time detected at the central-west (Maharashtra) (Sridhar et al., 2014) in October 2014. The new invasive pest, tomato pinworm/leaf minor, *T. absoluta* was recorded first in Pune in tomato plant which grown in field and poly-house in 2014 and south-west India (Karnataka state) (Anonymous, 2015, Vysali et al., 2021), and to our knowledge, there are no reports of this pest in the east and north-eastern India. Subsequently, the pest has been reported in the farmer's fields in major tomato growing states including Himachal Pradesh.

Entomopathogenic nematodes (EPNs) from the families Heterorhabditidae and Steinernematidae are soil-inhabiting organisms that are obligate insect parasites in nature (Kaya and Gaugler, 1993, Kasi et al., 2020). These nematodes have evolved a mutualistic association with bacteria in the genera *Photorhabdus* is associated with *Heterorhabditis* spp., is carried in the intestine of infective juveniles (IJs) (Bird and Akhurst, 1983, Arthurs et al., 2004, Silva et al., 2002). *Xenorhabdus* is connected with *Steinernema* spp. and confined to a specific vesicle within the intestine of the IJs. Nematodes locate their potential host by following insect cues (Lewis et al., 2006). After IJs locate a host, they infect it through an orifice such as the mouth, anus, or spiracles or by penetrating the cuticle (particularly in *Heterorhabditis* spp.). Once IJs enter the host, they shed their outer cuticle (Sicard et al., 2004) and begin ingesting hemolymph, which triggers the release of symbionts by defecation (in *Steinernema* spp.) or regurgitation (in *Heterorhabditis* spp.) (Martens et al., 2004, Grewal et al., 2005, Waiba et al., 2021b). The nematode-bacteria complex kills the host within 24 - 48h through septicemia or toxemia (Dowds and Peters, 1971, Forst and Clarke, 2002). Bacteria recolonize the nematodes, which emerge as IJs from the depleted insect cadaver in search of new hosts (Poinar 1990, Kasi et al., 2021b).

More than 100 species of EPNs have been identified globally (approximately 80% are steinernematid) and at least 13 of

these species have been commercialized (Shapiro-Ilan et al., 2014, Sharma et al., 2022). Commonly, the innate virulence against different pest species varies among EPN species. Moreover, differences among EPN species in host-seeking strategy and tolerance to environmental conditions like temperature and desiccation can determine the field efficacy of EPNs (Martens et al., 2004). EPNs have been broadly used in the biological control of a variety of economically important pests occupying different habitats (Grewal et al., 2005). However, EPN formulation to retard desiccation or the addition of adjuvants to increase leaf coverage and persistence of the IJs has enhanced the use of EPNs against foliar pests (Arthurs et al., 2002, Head et al., 2004, Kasi et al., 2021a, Kasi et al., 2021b). Our objective The evaluation of local isolated Strains of entomopathogenic nematodes, use to new Combination with Low-Toxicity Insecticides at Low and High Dosages South American Tomato Pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae) as a biocontrol.

2. MATERIALS AND METHODS

The laboratories experiments were conducted during September, 2021 to test the pathogenicity of two EPNs species *S. feltiae* and *H. bacteriophora* at different (IJs cm⁻²) concentrations against *Tuta absoluta* (Meyrick) (Lepidoptera, Gelechiidae), in Department of Entomology, Nematology Laboratory, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh, India.

2.1. Insects

T. absoluta colony was maintained on tomato plants under greenhouse conditions. The colony had been established from larvae collected in September 2021 from the vegetable science departmental form tomato greenhouse in Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Solan, HP, India, that used local strains *S. feltiae* (HR1) and *H. bacteriophora* (HR2) indigenous strains (Poinar) for the pest's management.

2.2. Entomopathogenic nematodes

Two isolates of *S. feltiae* and *H. bacteriophora* were used in this study. They used directly in the experiments without culturing. The native isolate was obtained from soil samples, collected from Rajgarh, Himachal Pradesh, India using *G. mellonella* larvae as nematode traps. This isolate was cultured based on the method (Kaya and Gaugler, 1993) at 21±1°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. The nematodes were acclimatized at room temperature for about 30 m before being used in the experiments.

2.3. Rearing of tomato pinworm, *Tuta absoluta*

The larvae and pupae of TLM were collected from

an infested greenhouse. *T. absoluta* was reared in the greenhouse, at 26±2°C, 60±10% RH, and an L:D 8:16 photoperiod. The insects were reared in wooden framed cages covered with 80 mesh organdy cloth on tomato plants (*S. lycopersicum* L.) (Figure 1). The adults were fed by 10% sugar solution in the oviposition cage.



Figure 1: Invasive pest of tomato pinworm *Tuta absoluta* rearing

2.4. Methodology

Ten larvae were placed in an 8 cm petri dish containing tomato leaf's natural diet. Low and high registered label dosages (converted to lab dosages) of 2 biological agents, 3 synthetic insecticides, and the biological agent+low-toxicity insecticides were used (Table 1). 10 larvae were treated

topically with 200 µl of insecticide solution dosage⁻¹. The control was treated only with distilled water. Treated Petri dishes were held in a CRD design with 4 replications (total n=40 larvae dosage⁻¹) in the lab at 18–20°C and photoperiod 12:12 h (L:D). Larval mortality was evaluated between 24 and 96 h after application.

2.5. Statistical analysis of data

Abbott's formula, (Abbott, 1925) was used to correct the data for control larval mortality in the bioassays and PROBIT analysis was conducted. Also, LSD ($p < 0.05$) values were calculated to differentiate means among treatments.

3. RESULTS AND DISCUSSION

3.1. Bioassays with *S. feltiae*+*T. absoluta*+Insecticides

In general, a higher mean % mortality was noted at higher dosages ($F=52.99$; $df=12$; $p < 0.05$) (Table 2). However, *S. feltiae* and Spinosad caused 80–97% of larvae mortality with low and high dosages at 96 h. The use of chlorantraniliprole and Spinosad resulted in % of mortality up to 97.50% at 96 h at the higher dosage. These results are different from those reported (Belay et al., 2012), where Spinosad and chlorantraniliprole caused larvae mortality over 80% at the same period. The LD₅₀ was 1600 ppm for chlorantraniliprole and 400 ppm for Spinosad at 96 h in this study (Figure 2). Differences might be related to (1) the prolonged use of these active ingredients caused some level of resistance, (2) instar stage, third vs fifth used in this research, or

Table 1: Active ingredients and laboratory dosages to evaluate the efficacy of 2 biological agent, 3 synthetic insecticides, and least toxic combinations of insecticides to control *Tuta absoluta* larvae

Insecticides	Commercial name* Active ingredient and %	Low dosage		High dosage	
		g or ml l ⁻¹	PPM	g or ml l ⁻¹	PPM
Biological agent (nematode)					
Steinernema feltiae (Sf)+rape seed oil 85%	Local strain+Addit	1.1 g+2.5 ml	1134+2500	2.2 g+2.5 ml	2268+2500
H. bacteriophora (Hb)+rape seed oil 85%	Local strain+Addit	1.1 g+2.5 ml	1134+2500	2.2 g+2.5 ml	2268+2500
Toxicity insecticides					
Chlorantraniliprole 18.4%	Coragen®	0.8 ml	800	0.16 ml	1600
Indoxacarb 14.5%	Syngenta	0.12 ml	120	0.24 ml	240
Spinosad 2.5%	MH Dow Success®	0.2 ml	200	0.4 ml	400
Combinations					
Chlorantraniliprole+SF+oil	---	0.8+1.1+2.5	---	0.16+2.2+2.5	---
Indoxacarb+SF+oil	---	0.12+1.1+2.5	---	0.24+2.2+2.5	---
Spinosad+SF+oil	---	0.2+1.1+2.5	---	0.4+2.2+2.5	---

Manufacturers: DuPont (Coragen), Bayer (Desis), Manufacturer Technical Supplier Exporter India (Syngenta), Corteva (MH Dow Success)

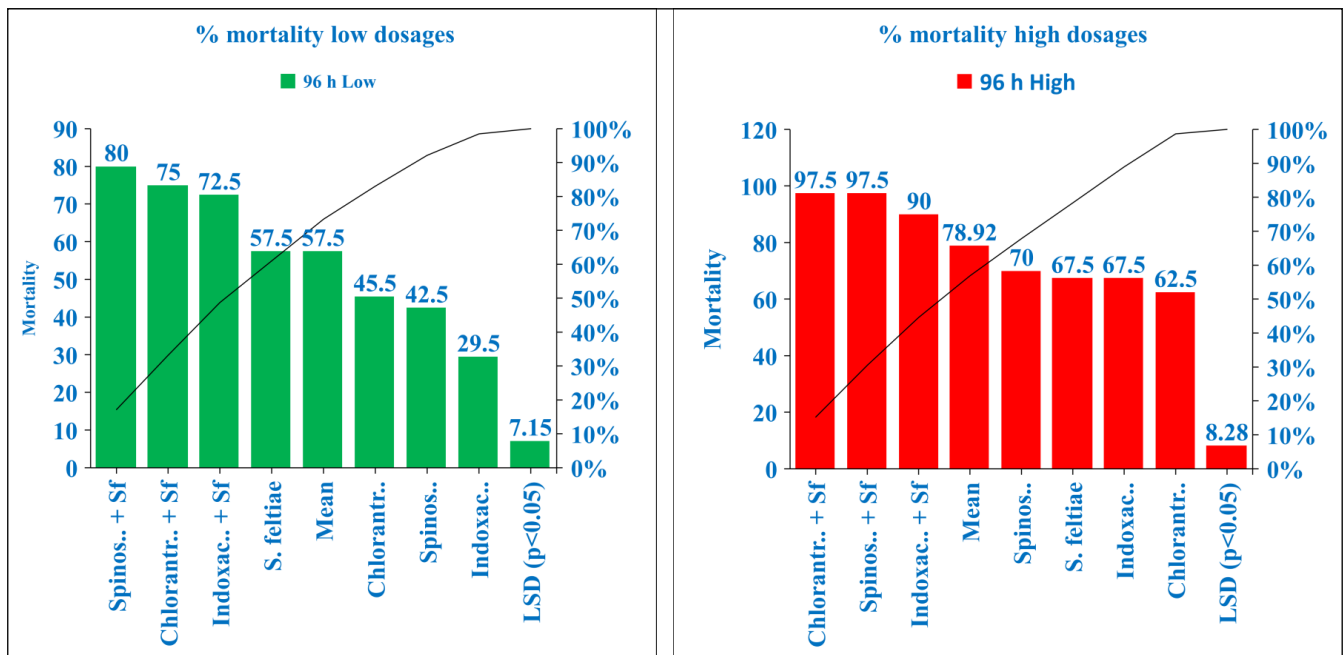


Figure 2: Mortality of *S. feltiae* IJs exposed for 96 h to different concentrations of Spinosad, Chlorantraniliprole, Indoxacarb. Means with different letter are significantly different ($p < 0.05$)

(3) differences among fall armyworm populations on the island (Viteri et al., 2018). In contrast, indoxacarb caused 72.50 and 90% of larvae mortality in low and high doses, respectively, at 96 h (Figure 5 and 6).

The use of *S. feltiae* in combination with Spinosad and chlorantraniliprole produced higher % of mortality at 24 h

compared with the use of these insecticides alone regardless of the dosage used (Table 2). Furthermore, the highest % of larval mortality (over 95%) were noted with high dosages at 72 h. Likewise, the combination of Spinosad+*S. feltiae* was effective (70.00% of dead larvae) with the high dose at 96 h compared with the lowest mortality caused

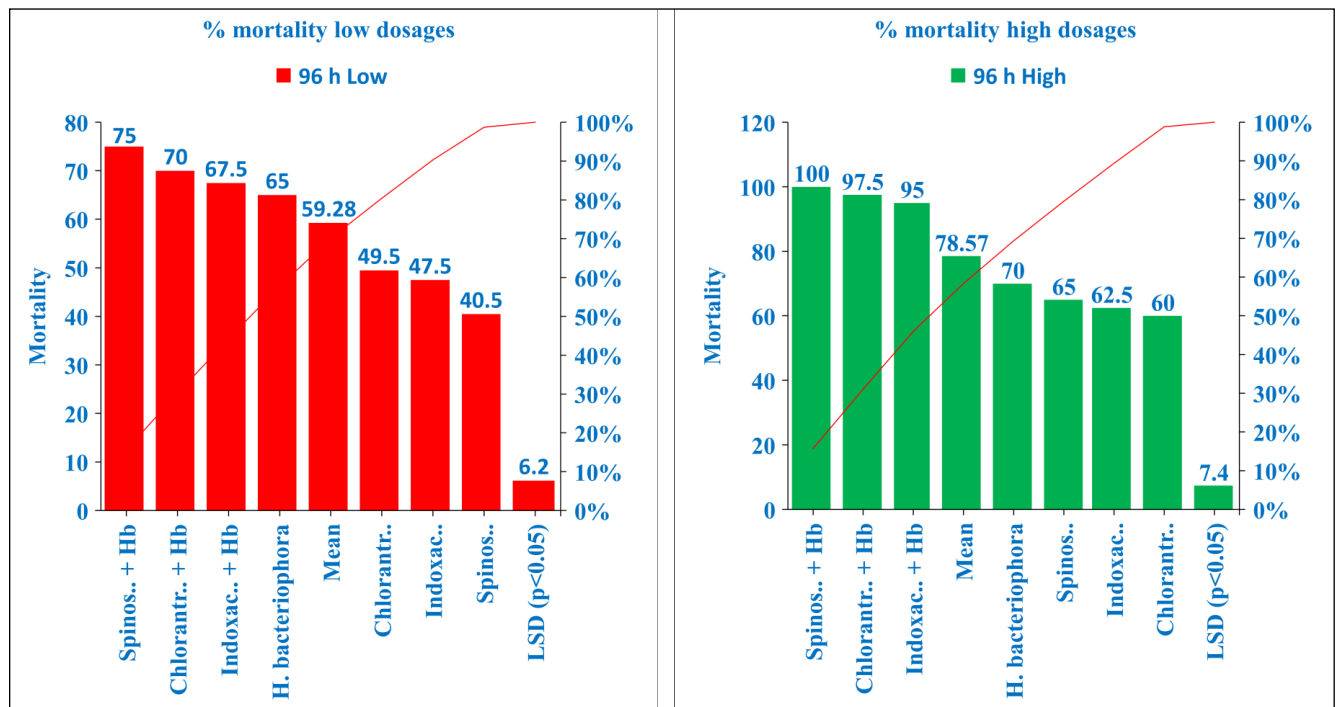


Figure 3: Mortality of *H. bacteriophora* IJs exposed for 96 h to different concentrations of Spinosad, Chlorantraniliprole, Indoxacarb. Means with the different letters are significantly different ($p < 0.05$)

Table 2: Mortality percentage of tomato pinworm [*Tuta absoluta* M.] larvae in the final-instar at low and high dosages of 3 insecticides, and least toxic combinations among them evaluated from 24–96 h

Insecticides	24 h		48 h		72 h		96 h		Interaction
	Low	High	Low	High	Low	High	Low	High	
Bio agent (nematode)									
<i>Steinernema feltiae</i> (Sf)	10.00	15.00	30.00	32.50	45.00	55.00	57.50	67.50	(+)
Toxicity insecticides									
Chlorantraniliprole	9.50	12.50	10.00	17.50	20.50	30.00	45.50	62.50	(-)
Indoxacarb	4.50	7.50	8.50	12.50	19.50	25.00	29.50	67.50	(-)
Spinosad	5.00	10.00	9.00	15.00	20.50	27.50	42.50	70.00	(-)
Combinations									
Chlorantraniliprole+ Sf	22.50	27.50	40.00	42.50	52.50	57.50	75.00	97.50	Synergistic
Indoxacarb+Sf	20.00	30.00	30.00	37.50	40.00	57.50	72.50	90.00	Synergistic
Spinosad+Sf	25.00	27.50	37.50	45.00	52.50	70.00	80.00	97.50	Synergistic
Mean	13.78	18.57	23.57	28.92	35.78	46.07	57.50	78.92	
LSD ($p<0.05$)	4.82	5.21	5.12	5.83	4.90	5.47	7.15	8.28	



Figure 4: Bio-efficacy of EPNs against *Tuta absoluta* (M.)

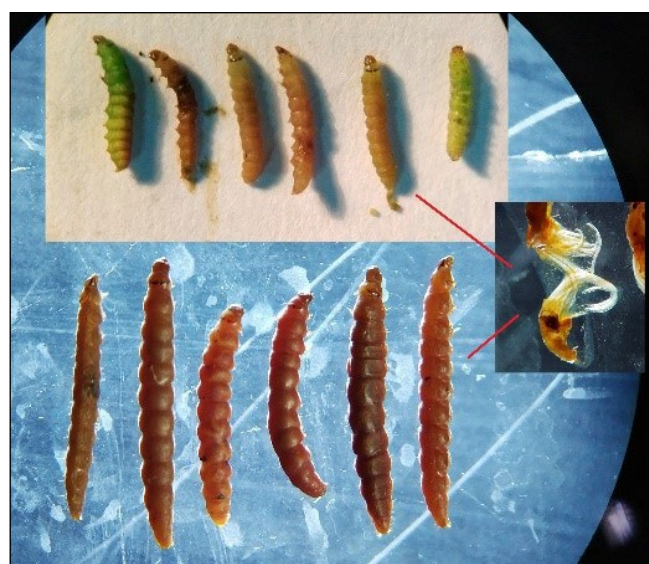


Figure 5: After treatment tomato pinworm *Tuta absoluta* by Spinosad (20.50%), or *S. feltiae* (45.00%) applied alone (Viteri et al., 2018). Larvae exposed to 2 different modes of action [septicemia (*S. feltiae*)+lysed midgut epithelial cells (Spinosad), impaired regulation of muscles (chlorantraniliprole), or Indoxacarb neural transmission (spinetoram)] (Viteri et al., 2018) at the same time, caused their higher mortality. In fall armyworm populations from Florida (Yu, 1991). However, further research is required to corroborate this synergistic effect (Figure 4).

3.2. Bioassays with *H. bacteriophora*+*T. absoluta*+Insecticides

In general, a higher mean % mortality was noted at higher dosages ($F=72.98$; $df=12$; $p<0.05$) (Table 3). However, *H. bacteriophora* and Spinosad caused 75.00–100% of larvae

Table 3: percentage of mortality of tomato pinworm [*Tuta absoluta* M.] larvae in the fifth-instar at low and high dosages of 3 insecticides, and least toxic combinations among them evaluated from 24–96 h

Insecticides	24 h		48 h		72 h		96 h		Interaction
	Low	High	Low	High	Low	High	Low	High	
Bio agent (nematode)									
<i>H. bacteriophora</i> (Hb)	22.50	27.50	45.00	47.50	57.50	62.50	65.00	70.00	(+)
Toxicity insecticides									
Chlorantraniliprole	22.50	35.00	31.50	47.50	41.50	55.00	49.50	60.00	(-)
Indoxacarb	17.50	32.50	25.50	47.50	43.50	60.00	47.50	62.50	(-)
Spinosad	20.00	40.00	28.50	55.00	35.50	62.50	40.50	65.00	(-)
Combinations									
Chlorantraniliprole+Hb	47.50	62.50	57.50	77.50	65.00	87.50	70.00	97.50	Synergistic
Indoxacarb+Hb	45.00	60.00	57.50	75.00	62.50	85.00	67.50	95.00	Synergistic
Spinosad+Hb	52.50	65.00	65.00	90.00	70.00	95.00	75.00	100.0	Synergistic
Mean	32.50	46.07	44.35	62.85	53.64	72.50	59.28	78.57	
LSD ($p < 0.05$)	5.12	6.31	7.15	8.05	5.29	6.59	6.20	7.40	



Figure 6: After nematode infected dead tomato pinworm larvae dissected under microscopic condition and conformation Synergistic activity

mortality with low and high dosages at 96 h. The use of chlorantraniliprole and Spinosad resulted in % of mortality up to 97.50% at 96 h at the higher dosage. These results are different from those reported (Belay et al., 2012), where Spinosad and chlorantraniliprole caused larvae mortality over 95% at the same period. The LD₅₀ was 400 ppm for Spinosad and 1600 ppm for chlorantraniliprole and at 96 h in this study (Figure 3). Differences might be related to (1) the prolonged use of these active ingredients caused some level of resistance, (2) instar stage, third vs fifth used in this research, or (3) differences among fall armyworm populations on the island (Viteri et al., 2018, Martens and Goodrich-Blair 2005). In contrast, indoxacarb caused 67.50 and 95.00% of larvae mortality in low and high doses,

respectively, at 96 h (Figure 5 and 6).

The use of *H. bacteriophora* in combination with Spinosad and chlorantraniliprole produced higher percentage of mortality at 24 h compared with the use of these insecticides alone regardless of the dosage used (Table 3). Furthermore, the highest percentage of larval mortality (over 95.00%) were noted with high dosages at 72 h. Likewise, the combination of Hb+Spinosad was effective (100% of dead larvae) with the high dose at 96 h compared with the lowest mortality caused by Spinosad (40.50%), or Hb (65.00%) (Figure 4) applied alone (Viteri et al., 2018). Larvae exposed to 2 different modes of action [septicemia (*H. bacteriophora*)+lysed midgut epithelial cells (Hb), impaired regulation of muscles (Spinosad), or abnormal neural transmission (spinetoram)] (Viteri et al., 2018) at the same time, caused their higher mortality. In fall armyworm populations (Yu, 1991). However, further research is required to corroborate this synergistic effect.

4. CONCLUSION

High EPN efficacy obtained under laboratory conditions cannot easily be extrapolated to field efficacy. Therefore, were field experiments on tomato crops are justified to fully determine the potential of local EPN isolates against *T. absoluta* in Himachal Pradesh conditions. In combination with low-toxicity insecticides at low and high dosages to control fifth-instar larvae in bioassays.

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