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Virulence of native entomopathogenic nematodes to manage cotton insect pests *Helicoverpa armigera*, *Earias vittella* and *Spodoptera litura*

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ABSTRACT: The virulence of 27 native entomopathogenic nematodes (EPN) comprising of 16 *Steinernema carpocapsae* strains, 3 *Steinernema siamkayai* strains, 1 *Steinernema monticolum* strain and 7 *Heterorhabditis bacteriophora* strains on cotton pests *viz.*, American bollworm *Helicoverpa armigera*, spotted bollworm *Earias vittella* and cotton leafworm *Spodoptera litura* was tested by *in vitro* lethal concentration and lethal time assays. *H. bacteriophora* strains KKMH1, TRYH1 and *S. carpocapsae* strain APKS2 were found to be more virulent to *H. armigera* with LC_{50} values of 28, 33, 42 infective juveniles (IJs)/ml respectively; and with LT_{50} values of 11.72, 11.72 and 22.99 h, respectively. The S. *carpocapsae* strains ASDS1, ASDS2 and APKS1 were less virulent to *H. armigera*. For the control of *Earias vittella,* the same EPN strains KKMH1, TRYH1 and APKS2 recorded significantly lesser LC_{50} values of 28, 35, and 38 IJs/ml respectively; and significantly lesser LT_{50} values of 11.67, 13.05 and 11.71 h, respectively. The EPN strains SCS1, SCS2, ASDS1, ASDS2 and APKS1 were least virulent to *Earias vittella*. For the control of *S. litura,* strains KKMH1, TRYH1 and APKS2 were more virulent where the strains SCS2, ASDS1 and ASDS2, APKS1 were least virulent. The LC_{50} values of KKMH1, TRYH1 and APKS2 were 56, 65 and 71 IJs/ml, respectively and LT_{50} values were 11.60, 13.73 and 11.50 h, respectively. The results of this study suggest that the *H. bacteriophora* strains KKMH1, TRYH1 and *S. carpocapsae* strain APKS2 are promising against *H. armigera*, *E. vittella* and *S. litura* and could be developed as potential bio-pesticide after testing their field performance.

Keywords : Biological control, cotton, bollworm complex, eco-friendly, *Heterorhabditis bacteriophora, Steinernema carpocapsae*, *Steinernema monticolum, Steinernema siamkayai*

Cotton (*Gossypium hirsutum*: Malvaceae), popularly known as **'White Gold'**, is an important cash crop of India. One of the prime challenges to attain high cotton production is damage caused by insect pests. The nature of cotton plant such as its succulent leaves, nectarines and fruit abundance is more attractive to many injurious species of insects. The pest spectrum of cotton is wide and as many as 200 species of insects have been described to feed on cotton throughout its life span from germination to harvest (Dhaliwal *et al.,* 2010). Among them, bollworm complex consisting of American bollworm *Helicoverpa armigera* Hub. (Lepidoptera: Noctuidae), spotted bollworm *Earias vittella* Fab. (Lepidoptera: Noctuidae) and cotton leafworm *Spodoptera litura* (Fab) are most important pests affecting the cotton plants and they can cause

yield loss of 20 – 80% (Dhaliwal *et al.*, 2010).

The synthetic pyrethroids and organophosphate group of insecticides have been used to control bollworms during the last two decades. However, the chemical control of cotton bollworms became failure due to the development of resistance against many insecticide groups. The chemical control also caused problems of human health risk, devastation of natural enemies, resurgence of minor pests and environment pollution (Seenivasan and Murugan, 2011). The threats posed by them demanded an eco-friendly alternative method and the biological control of bollworms has become a good option. In recent years, the focus has been towards the use of genetically modified *Bt* (*Bacillus thuringiensis*) cotton for bollworm management and it has proved to be effective. However, resistance to the insecticidal crystal proteins of *B. thuringiensis* in cotton bollworms has now been documented and the sustainable use of *Bt* cotton for bollworm management has become highly questionable. In this situation, introduction of an alternate bio-control agent for cotton bollworm management will be a promising approach.

In this situation, exploitation of naturally occurring entomopathogenic nematodes (EPN) from two families *viz,* Heterorhabditidae and Steinernematidae to develop biopesticide for the control of cotton bollworms is an ecologically sound novel approach. These nematodes are characterized by their ability to carry specific pathogenic bacteria, *Photorhabdus* with Heterorhabditidae and *Xenorhabdus* with Steinernematidae, which are released into the insect haemocoel after penetration of the insect hosts by the infective stage of the nematodes. Most biocontrol agents require days or weeks to kill the pest, but entomopathogenic nematodes with their symbiotic bacteria kill insects in 24-48 hr. As soil is the natural habitat for EPN, they have been widely used to manage insect pest having life cycle in soils during last decade. However, foliar application of EPN against brinjal shoot and fruit borer, *Leucinodes orbonalis* Guenee and pegionpea pod borer *Helicoverpa armigera* were successfully demonstrated. Cryptic foliar habitats are considered most favorable, enhancing the infectivity, survival, and persistence of EPN because these environments minimize nematode death from ultraviolet radiation and desiccation (Seenivasan and Sivakumar, 2012). As cotton bollworms survive mostly in cryptic habitats such as inside squares, flowers and bolls, EPN can be exploited to contain them.

Exploring indigenous EPN in cotton fields of Tamil Nadu state in India, Seenivasan *et al.* (2012) recovered 27 strains belonging to 16 *Steinernema carpocapsae*, 3 *Steinernema siamkayai,* 1 *Steinernema monticolum* and 7 *Heterorhabditis bacteriophora* from cotton ecosystem. Later, Seenivasan and Sivakumar (2012) established that all the 27 EPN strains were suitable against cotton bollworms *H. armigera*, *E. vittella* and *S. litura*. Various species and isolates of EPN differ in infectivity and pathogenicity with respect to insect hosts (Seenivasan and Sivakumar, 2014). Screening of biocontrol agents in the laboratory is a routine practice before a new biopesticide is developed and used in the field. Less number of infective juveniles (IJs) to cause high mortality and less time to cause insect death are the important criteria for selection of potential EPN for field

application. Hence, this study was conducted with the objective to select most virulent EPN strains with low lethal concentration and short lethal time potential on *H. armigera*, *E. vittella* and *S. litura* as part of a strategy to develop EPN for cotton bollworm management.

MATERIALS AND METHODS

EPN and test insects: Twenty seven strains of EPN namely, *S. carpocapsae* (KKMS1, KKMS2, SCS1, SCS2, ASDS1, ASDS2, APKS1, APKS2, MDUS1, MDUS2, OCMS1, ALRS1, ALRS2, ALRS3, MTPS1, BSRS1), *S. siamkayai* (CBES1, PKMS1, PKMS2) and *S. monticolum* (OCMS2) and *H. bacteriophora* (TRYH1, KKMH1, KKMH2, KKMH3, APKH1, PKMH1, OCMH1), earlier isolated by baiting of soil samples collected from different cotton fields of Tamil Nadu, India were taken from the Department of Nematology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. They were cultured in the laboratory using last instar larvae of the rice grain moth, *Carcyra cephalonica* (Lepidoptera: Pyralidae). The infective juveniles (IJs) releasing from *C. cephalonica* larval cadavers were collected in sterile distilled water using a modified White's trap, sterilized in formalin (0.05%) solution and maintained on aerated sterile water in plastic tissue-culture flasks at 15°C. The nematodes were allowed to acclimatize at room temperature for at least 6 h prior to subsequent testing in bioassays. The test insect larvae (*H. armigera*, *E. vittella* and *S. litura*) were collected from a standing cotton crop on TNAU farm and from the farmer's fields at Thondamuthur village in Coimbatore district of Tamil Nadu. They were sorted out and the fourth instar larvae of uniform size were used in the experiment.

Assay for lethal concentration fifty (LC_{50}): The LC_{50} assay was conducted in 6-cm-diam petri dishes lined with moist filter paper disc. Three separate assays were performed for each test insects *H. armigera*, *E. vittella* and *S. litura*. Five forth instar larvae of *H. armigera*, *E. vittella* and *S. litura* were put in petri dishes lined with filter paper. Then 1 ml of appropriate EPN species/strain suspension containing 25, 50, 100, 200, 400 and 800 IJs were applied to the petri dishes. Control plates received only 1 ml distilled water. The dishes were sealed with parafilm, arranged in a completely randomized design (CRD) and incubated at room temperature. Each treatment consisted of five replicates (One Petri dish = one replicate). After 4 days, larval mortality was recorded. The dead insects were dissected in Ringers solution to confirm the death by EPN. LC_{50} values were calculated for each nematode species/strain using a probit analysis. The experiments were repeated once.

Assay for lethal time fifty (LT_{50}): To determine the time needed for EPN species/ strains to kill 50 per cent of *H. armigera*, *E. vittella* and *S. litura* larvae, 1000 IJs were suspended in 1 ml of distilled water and uniformed distributed on to 6 cm dia petri dishes lined with moist filter paper disc. Then the test insect larva was introduced at 5 / petri dish. This assay used the same 27 EPN strains as in the previous assay and three separate assay for each test insect was conducted. For each assay, 130 petri dishes were set up and 5 additional

dishes served as a untreated control, which received distilled water only. Each petri dish was sealed with parafilm and kept at room temperature. The assay was set up in CRD with five replication for each strains (One Petri dish = one replicate). Insect larval mortality was recorded after 12, 24, 36, 48, 60, 72, 84 and 96 h. The dead insects were dissected in Ringers solution to confirm the death by EPN. LT_{50} values were calculated for each nematode species/ strain using a probit analysis. Each assay was repeated one more time.

RESULTS AND DISCUSSION

The LC_{50} and LT_{50} values calculated for the 27 EPN strains to kill fourth instar larvae of *H. armigera*, *E. vittella* and *S. litura* are presented in Table 1. For the control of *H. armigera*, lethal concentration required to kill 50 per cent of the insects by the tested EPN varied from 28 to 381 IJs. The LC_{50} values of *S. carpocapsae* strains was 42 – 381 IJs; *S. siamkayai* was 194 – 238 IJs; *S. moticolum* was 163 IJs; and *H. bacteriophora* was 28 – 300 IJs. Over all, *H. bacteriophora* KKMH1, TRYH1 and *S. carpocapsae* strain APKS2 got significantly lesser LC_{50} values of 28 – 42 IJs. There was no significant difference among them based on the fiducial limit values overlapping. All other EPN strains got higher LC_{50} values of 144 – 381 IJs. For the control of *H. armigera*, the *H. bacteriophora* strains KKMH1 and TRYH1 had the significantly lesser LT_{50} values of 11.53 and 11.72 h. However, the other strains of *H. bacteriophora* caused 50 per cent mortality of *H. armigera* in 22.99 – 45.96 h. The *S. carpocapsae* strains have taken 22.98 – 57.57 h to kill 50% *H. armigera* whereas *S. siamkayai* strains 34.37 – 34.53 h and *S. monticolum* 34.45 h. The result indicated that *H. bacteriophora* strains KKMH1 and TRYH1 were more virulent on *H. armigera*. Among *Steinernema* spp. the *S. carpocapsae* strain APKS2 was considered more virulent on *H. armigera* as it recorded significantly least LC_{50} value though it require 22.99 h to kill 50 per cent larvae. The S. *carpocapsae* strains ASDS1, ASDS2 and APKS1 were the least virulent to *H. armigera* as they were not able to generate LC_{50} and LT_{50} values. The pathogenicity of EPN species *S. carpocapsae* (Hussain *et al.,* 2014), *S. siamkayai*, *S. monticolum* and *H. bacteriophora* (Kary *et al.,* 2012) against *H. armigera* was reported earlier. This study confirmed the earlier findings and established the lethal effect of different strains of these EPN species against *H. armigera*. The lethal concentration of *S. carpocapsae, S. moticolum* and *H. bacteriophora* to kill 50 per cent of H. *armigera* was not reported earlier and this study established that LC_{50} for *S. carpocapsae* strains vary from 42 – 381 IJs, for *S. moticolum* 163 IJs and for *H. bacteriophora* strains 28 – 300 IJs. The time to kill 50 per cent of *H. armigera* larvae by EPN was reported as 49.38 – 63.97 h by *H. indica*, 50.44 - 88.34 h by *S. glaseri*, 89.9 h by *S. siamkayai*, 22 - 48 h by *S. carpocapsae*, 39.8 h by *S. riobrave* and 48 h by *S. feltiae*. The lethal time reported for *S. carpocapsae* in this study was in agreement with who reported the LT_{50} of 36 – 48 h for *S. carpocapsae* IJs to kill 50 per cent *H. armigera* larvae.

For the control of *E. vittella,* the LC_{50} values of *H. bacteriophora* strains KKMH1 (25 IJs) and TRYH1 (28 IJs); and *S. carpocapsae* strains KKMS1 (35 IJs) and APKS2 (38 IJs) were lower than those of the other EPN strains. The *S.*

carpocapsae strains SCS1, SCS2, ASDS1, ASDS2 and APKS1 did not have LC_{50} values as their virulence to kill *E. vittella* was less than 50 per cent. All other EPN strains had significantly higher LC_{50} values *viz.*, 113 – 338 IJs for other *S. carpocapsae* strains, 158 – 319 IJs for *S. siamkayai* strains, 147 IJs for *S. monticolum* strain and 116 – 234 IJs for other *H. bacteriophora* strains. The average lethal time, across all EPN strains against *E. vittella*, was 27.23 h. The *S. carpocapsae* strains SCS1, SCS2, ASDS1, ASDS2 and APKS2 did not have LT_{50} values as their virulence to kill *E. vittella* was less than 50 per cent even after 96 h. The *H. bacteriophora* strains KKMH1, TRYH1 and *S. carpocapsae* strain APKS2 produced shortest LT_{50} values (11.67 – 13.05 h), and all other EPN strains had longest LT_{50} (18.86 – 46.10 h). The LT_{50} values for KKMH1, TRYH1 and APKS2 differed significantly from all other strains but there was no significant difference among three of the strains, according to the fiducial limits (95%), which overlapped. The host status of various species of EPN such as *S. siamkayai* (Adiroubane *et al.,* 2010), *H. indica* (Pal and Prasad, 2012), *Steinernema pakistanense*, *Steinernema asiaticum*, *S. feltiae* (Shahina *et al.,* 2014) was reported on *Earias vittella.* This study established that *S. carpocapsae, S. monticolum* and *H. bacteriophora* were also lethal to *Earias vittella.* The LT_{50} value of *S. siamkayai* for *E. vittella* was ranged from 19.95 – 47.02 h (Adiroubane *et al.,* 2010) which is in accordance with our result.

For the control of *S. litura,* the *S. carpocapsae* strains SCS2, ASDS1 and ASDS2, APKS1 did not have LC_{50} values. All other EPN strains screened had LC_{50} values smaller than 403 IJs/ml, demonstrating significant lethality against *S. litura.* Among 27 EPN strains, *H. bacteriophora* strains KKMH1, TRYH1 and *S. carpocapsae* strain APKS2 showed stronger virulence against *S. litura*. The LC_{50} values of these three strains were 56, 65 and 71 IJs/ml, respectively. The remaining *S. carpocapsae, H. bacteriophora, S. siamkayai* and *S. monticolum* showed a $LC_{50} > 147$ IJs/ml against fourth-instar *S. litura* larvae in 96 h and were considered not promising. LT_{50} values for EPN strains against *S. litura* varied from 11.50 – 45.83 h, with an average of 29.28 h. Among *S. carpocapsae* strains, APKS2 demonstrated the shortest LT_{50} based on non overlap of 95 per cent fiducial limits and was significantly faster acting than other *S. carpocapsae* strains. The *S. siamkayai* and *S. monticolum* had the longest LT_{50} at 25.16 - 34.68 h. Among *H. bacteriophora* strains, KKMH1 and TRYH1 had the shortest LT_{50} of 11.36 – 13.73 h. This was significantly shorter than LT_{50} values for strains KKMH2, APKH1, PKMH1 and OCMH1. The lethal effect of *S. glaseri, S. riobravis*, *S. carpocapsae, Steinernema kushidai*, S. *feltiae*, *Steinernema tami, Steinernema abbasi, H. indica and H. bacteriophora*. This study extended the host status of *S. siamkayai* and *S. monticolum* on *S. litura*. The LC_{50} values of EPN species reported were 300 IJs of *Heterorhabtitis indica* (Divya *et al.,* 2010)*,* 22.5 IJs of *S. siamkayai*, 1.2 IJs of *S. riobrave* against *S. litura*; 1.2 - 250 IJs of *S. carpocapsae* against *Spodoptera littoralis* and 50 IJs of *S. feltiae* against *Spodoptera exigua*. The LT_{50} values of EPN species reported were 60 h for *H. indica* (Divya *et al.,* 2010)*,* 89.8 h for S. siamkayai, 22 – 36.5 h for *S. carpocapsae* 39.8 h for *S. riobrave*; 23.5 h for *H. bacteriophora* against *S. litura* and 24 h for *H. indica* against *Spodoptera littoralis*. The LC_{50} and LT_{50} values of *S.*

Table 1. Levels of lethal concentrations fifty (LC_{50}) and lethal time fifty (LT50) of the entomopathogenic nematode strains/species on *Helicoverpa armigera*, *Earias vittella* and *Spodoptera llitura*

Species/Strains	*Helicoverpa armigera*		*Earias vittella*		*Spodoptera llitura*	
	LC_{50} (95% Fiducial limits)	LT_{50} (95% Fiducial limits)	LC_{50} (95% Fiducial limits)	LT_{50} (95% Fiducial limits)	LC_{50} (95% Fiducial limits)	LT_{50} (95% Fiducial limits)
S. carpocapsae						
KKMS1	144 a (86 – 268)	22.98 a (17.23 – 35.68)	35 b (18 -64)	20.71 a (18.56 -28.83)	147 a (108 -274)	23.15 a (19.65 – 36.75)
KKMS2	238 a (164 – 508)	37.88 a (24.72 - 54.52)	204 a (122 – 296)	34.57 a (24.14 – 55.20)	240 a (152 – 454)	34.42 a (19.32 – 58 .29)
SCS1	246 a (180 – 517)	57.37 a (34.68 - 78.26)	-	-	291 a (146 – 464)	45.83 a (27.35 – 80.24)
SCS2	303 a (192 – 548)	47.17 a (34.71 - 68.20)	-	-	-	-
ASDS1	-	-	-	-	-	-
ASDS2	-	-	-	-	-	-
APKS1	-	-	-	-	-	c
APKS2	42 a (18 – 69)	22.99 a (20.25 - 28.14)	38 b (18 – 64)	11.71 b (6.38 – 13.31)	71 b (56 – 89)	11.50 d (6.35 – 17.46)
MDUS1	181 a (116 – 326)	34.49 a (29.82 - 51.73)	150 a (85 – 268)	34.43 a (20.28 – 38.52)	162 a (108 – 306)	34.54 a (22.43 – 58.97)
MDUS2	381 a (192 – 628)	47.66 a (30.63 - 68.74)	338 a (169 – 542)	46.10 a (26.24 – 84.27)	403 a (218 – 725)	36.71 a (20.54 – 66.46)
OCMS1	236 a (105 – 472)	45.85 a (32.32 - 64.61)	159 a (91 – 272)	34.33 a (26.58 – 62.94)	212 a (128 – 362)	34.68 a (26.76 – 63.73)
ALRS1	185 a (86 – 368)	45.94 a (33.82 - 67.16)	-	-	165 a (107 – 302)	36.59 a (27.43 – 69.86)
ALRS2	-	-	203 a (90 – 302)	34.71 a (21.26 – 60.81)	229 a (146 – 396)	45.81 a (24.52 – 82.94)
ALRS3	181 a(81 – 393)	34.47 a (24.15 - 48.73)	113 a (73 – 192)	34.38 a (20.16 – 66.42)	224 a (152 – 364)	34.50 a (26.47 -65.52)
MTPS1	187 a (94 – 358)	34.41 a (23.61 - 49.18)	115 a (86 – 236)	23.14 a (19.64 – 39.26)	158 a (114 – 262)	26.32 a (20.84 – 46.85)
BSRS1	180 a (104 – 349)	34.50 a (22.71 - 487.34)	119 a (76 – 218)	23.24 a (18.43 – 36.28)	159 a (97 – 284)	23.10 a (19.75 – 39.95)
S. siamkayai						
CBES1	194 a (88 – 337)	34.37 a (20.62 - 48.27)	319 a (184 – 582)	34.43 a (24.56 – 67.45)	400 a (257 – 714)	34.61 a (23.36 – 59.73)
PKMS1	238 a (142 – 421)	34.41 a (23.53 - 49.18)	275 a (164 – 486)	45.81 a (26.84 - 74.41)	291 a (152 – 495)	34.50 a (24.62 – 61.58)
PKMS2	194 a (94 – 382)	34.53 a (22.45 - 47.26)	158 a (96 – 261_	23.05 a (18.62 – 31.64)	164 a (115 – 302)	25.16 a (19.25 – 46.32)
S. monticolum						
OCMS2	163 a (78 – 294)	34.45 a (21.28 - 51.23)	147 a (83 – 242)	34.48 a (22.42 – 58.81)	168 a (75 – 268)	34.68 a (25.26 – 60.15)
H. bacteriophora						
TRYH1	33 b (15 – 59)	11.72 b (7.16 - 14.46)	28 b (13 – 46)	13.05 b (8.83 – 16.62)	65 b (28 – 78)	13.73 b (7.84 – 18.45)
KKMH1	28 b (14 – 56)	11.53 b (6.48 - 14.64)	25 b (11 – 43)	11.67 b (7.14 – 15.35)	56 b (26 – 68)	11.60 b (6.24 – 16.76)
KKMH2	181 a (86 – 382)	22.99 a (18.82 - 34.81)	116 a (72 – 192)	18.86 a (17.26 – 36.41)	149 a (109 – 276)	23.16 a (19.65 – 36.84)
KKMH3	193 a (98 – 384)	23.03 a (18.37 - 36.24)	119 a (79 – 185)	23.05 a (18.65 – 36.24)	153 a (96 – 279)	25.06 a (19.76 – 45.87)
APKH1	194 a (76 – 362)	34.48 a (25.62 - 49.25)	153 a (89 – 283)	23.14 a (19.62 -38.82)	215 a (116 – 349)	23.08 a (19.65 – 41.56)
PKMH1	233 a (94 – 427)	34.36 a (24.81 - 47.27)	200 a (124 – 369)	22.95 a (18.64 – 36.81)	224 a (104 – 369)	26.31a (20.27-47.76)
OCMH1	300 a (164 – 562)	45.96 a (32.45 - 60.24)	234 a (138 – 402)	34.62 a (19.76 – 63.62)	297 a (139 – 438)	34.49a (27.56 – 71.23)

LC_{50} or LT50 values within a column followed by the same letter are not significantly different based on overlap of 95% fiducial limits.

- LC_{50} or LT50 could not be calculated due to cumulative control value was less than 50%.

carpocapsae, H. bacteriophora, S. siamkayai and *S. monticolum* reported in this study are close with the earlier reports.

EPN species and strains of the same species tested in this study differed greatly in their virulence against *H. armigera*, *E. vittella* and *S. litura* larvae. Significantly lower level of LC_{50} and LT_{50} occurred in *H. bacteriophora and S. carpocapsae* than in *S. siamkayai* and *S. monticolum*. Differences on virulence among EPN species when tested on same insect, and also the existence of variability among strains of same EPN species was demonstrated earlier. Fallon proved that invasion by different EPN species varied quantitatively and higher number of penetration caused higher insect mortality. They also suggest that the differences in efficacy among different strains of same EPN species were the result of differences in level of nematode infectivity. The variation in the virulence between EPN species and strains might also be due to the differences in the time of establishment of the symbiotic bacteria in the host insect larva. Saunders and Webster showed the strong correlation between times of insect death by EPN species or strains with the presence of number of *Xenorhabdus* or *Photorhabdus* symbiotic bacterial cells. In this current study, the low virulence of *S. siamkayai* and *S. monticolum* is attributed to its symbiont to establish on *H. armigera*, *E. vittella* and *S. litura*. The improved virulence of *H. bacteriophora* might be due to the presence of tooth like structure in mouth to enable more penetration of test insect larvae and their cruiser type of foraging strategy. The ambusher type of foraging behavior and the ability to attack motile host insect larvae by jumping may be the reason for more virulence of *S. carpocapsae* reported in this study (Kamali *et al.,* 2013).

In conclusion, the *S. carpocapsae* strain APKS2 and *H. bacteriophora* strains KKMH1 and TRYH1 show promise for biological control of the cotton pests *H. armigera*, *E. vittella* and *S. litura*. Additional bioassays using different larval instars of *H. armigera*, *E. vittella* and *S. litura* including pre-pupal larvae can strengthen the potential of these EPN strains. Future research will test these nematodes in the field.

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