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Efficacy of Entomopathogenic Nematode, *Steinernema abbasi* PN-1 against *Helicoverpa armigera* Hubner

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Abstract

The experiment was conducted during May 2022 at College of Agriculture, G. B. Pant University of Agriculture & Technology, U. S. Nagar, Pantnagar, Uttarakhand, India. The *Steinernema abbasi* PN-1 is a local isolate of entomopathogenic nematode isolated from the soil collected from Uttarakhand, India. Under the present study, virulenceof *Steinernema abbasi* PN-1 against different stages of *Helicoverpa armigera* Hubner were tested. Virulence studies of *S. abbasi* PN-1 against *H. armigera* proved that all larval stages and pupae of *H. armigera* were found susceptible to the IJs of *S. abbasi* PN-1. There was a positive correlation between insect mortality and the nematode concentration. The *S. abbasi* PN-1 caused 100% larval mortality at 48–60 h of post treatment in all tested doses in laboratory. Among the larval instars, 4th instar larvae of *H. armigera* were more susceptible with a median lethal concentration (LC_{50}) value of 24.37 IJs $larva^{-1}$ and the median lethal time (LT_{50}) values of 25.63 hours. The 2nd instar larvae was least susceptible with an LC_{50} value of 78.96 IJs $larva^{-1}$ and LT_{50} values of 41.33 hours. The pupal stage was less susceptible than the larval stage with the LC_{50} value of 98.3 IJs $larva^{-1}$. Our results showed that *S. abbasi* PN-1 can be used as efficient biological control agents against *H. armigera* with further field studies.

Keywords: *Helicoverpa armigera*, Infective Juveniles, *Steinernema abbasi* PN-1

1. Introduction

The American bollworm *Helicoverpa armigera* is a highly polyphagouspest and cosmopolitan in distributionthat infects more than 200 host plant species from various families, including many economically significant crops including chickpea, cotton, sorghum, maize, sunflower, soybean, pigeonpea, fruits, and vegetables (Riaz et al., 2021). The larvae are the most destructive life stage and primarily feed on reproductive structures, particularly from the third instar onwards (Das et al., 2022). The Chemical control of *H. armigera* has often proved to be ineffective due to the development of resistance to several insecticides (Upendhar et al., 2017, Wang, 2021). It is necessary to take specific action to resolve these resistance problems. Thus, it is imperative to look for novel biological methods of controlling this pest. The entomopathogenic nematodes (EPNs) are soil inhabiting, delicate or soft bodied, non-segmented roundworms that are obligate or facultative parasites of insect pests. The EPNs belonging to Heterorhabditidae and Steinernematidae families are more effective and have widely been used as biological control agents for insect pests (Kasi et al., 2022, Devi, 2023) due to special qualities like wide host range, wide distribution and quick action. The genera Steinernema and Neosteinernema belong to the Steinernematidae family, while Heterorhabditidae is represented by the genus Heterorhabditis (Askary and Abd-Elgawad, 2021). Nematodes of the genera *Heterorhabditis* and *Steinernema* are found Worldwide, infecting about 250 distinct insect species belonging to ten orders (Poinar, 2018). The EPNs have simple life cycle, viz., egg, four juvenile stages (separated by moults) and adult stages. The infective stage is J3 (third juvenile stage), and it is known as infective juveniles (IJs). The IJs are non-feeding, free-living active stage capable of withstanding adverse environmental circumstances and the non-availability of host for an extended time period. The IJs enters the host through the natural openings or by direct cuticle penetration. After penetrating the EPN discharges bacteria which grow rapidly in the host hemolymph, causing septicemia and resulting in the host's death. Later, the nematode and bacterium feed on the host tissues, reproduce and multiplies until the host tissues deplete and emerge as IJs, which seek a new host (Chaubey

and Aasha2021). The mutual symbiotic relationship with entomopathogenic bacteria, *Photorhabdus* and *Xenorhabdus* genera for *Heterorhabditis* and *Steinernema*, respectively boosts their efficacy (Tarasco et al., 2023). However, their effectiveness is mostly dependent on the presence of particular conditions like as high relative humidity, low temperature and sunlight (UV radiation).

The *Stienernema abbasi* was first isolated by Hubeisfrom the soilof alfalfa fields in the Sultanate of Oman and characterized by Elawad (Elawad et al., 1997). The *Xenorhabdus indica* is a symbiotic bacteria associated with *S. abbasi* (Tailliez et al., 2006). The present EPN *S. abbasi* PN-1 was isolated from the potato rhizosphere of Pantnagar, Uttarkhand (Mehra 2021). The effectiveness of EPNs against insect pests such as *H. armigera* (Gokte-Narkhedkar et al., 2019, Nagach and Rabose, 2022) has been successfully demonstrated in India but least studies are carried out on pupal bioassay. EPNs can also infect the pupae of several insect pests (Patil, 2020). Infectivity of EPNs against pupal stage of lepidopteran insect also widens its scope to be used effectively against management of different stage of insect pests. Nowadays EPNs are mostly utilized in environments where chemical substances fail, such as galleries of boring insects, soil, or situations where pesticide resistance has developed (Koppenhöfer et al., 2020). Therefore, present investigation was done to test efficacy of entomopathogenic nematode *S. abbasi* PN-1 against different developmental stages of *H. armigera*.

2. Materials and Methods

2.1. Nematode culture

The experiments were carried out during 2022 (March-May). The entomopathogenic nematode (EPN) *S. abbasi* PN-1 was collected from the Department of Entomology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttrakhand, India. The EPN was cultured on late instar larvae of the greater wax moth, *Galleria mellonella* Linnaeus (Woodrin and Kaya, 1988). The wax moth larvae are treated with 200 IJs in a Petri dish lined with Whatman filter paper which was further incubated at 25±2°C. After 2 days, the dead larvae were collected and washed in distilled water for two times. The washed cadavers were placed on a white trap and incubated at 25±2°C. The new IJs started emerging from the cadaver after 4–5 days and were collected in a beaker. The 2 days old Fresh IJs solution was used for all the experiments.

2.2. Rearing of H. armigera

The larvae of *H. armigera* were collected from chickpea fields of CRC, Pantnagar, Uttarakhand, India and were reared singly in a sterilized rearing cagebox (to avoid cannibalism) at 25±2°C and 75±5% RH in BOD under laboratory conditions. The larva was fed with a chickpea flour modified diet as suggested by Chittibabu et al. (2014). The rearing boxes were sterilized daily by cleaning with ethanol (70%) and uneaten food was changed. The larvae which reached the pupal stage were collected and stored in a Petri dish lined with moist filter paper. A running culture of *H. armigera* was maintained and used for experiments in the laboratory. The larvae and pupae of the same days were used for the bioassay study.

2.3. Virulence of S. abbasi PN-1 against H. armigera

Larval bioassays were carried out in Petri plates (50×10 mm^2), which were lined with filter paper. A single larva was released into each plate and ten plates were maintained for each treatment. The virulence of *S. abbasi* PN-1 to larvae of *H. armigera* was determined by pipetting 500 µl of distilled water containing 25, 50, 100, 200 and 400 IJs onto the filter paper of each plate. In control, larvae were treated with 500 µl of distilled water alone. Treatment plates were then placed in incubator at 27±2°C. The treatments were replicated three times. Larval mortality was assessed at different time intervals. In order to verify nematode infection, the dead larvae were put on white traps.

Pupal bioassay was conducted by using 200 ml plastic boxes and the boxes were filled with 150 cm^3 of sterilized soil. A single two-day-old pupa was released into each container. The virulence of *S. abbasi* to *H. armigera* pupae were determined by adding 50, 100, 200, 400, 600 and 800 IJs 3 ml^{-1} onto the soil surface of each box. Control was treated with 3 ml of distilled water without EPN. Then boxes were placed in the dark at room temperature (25±2°C). The treatments were replicated 10 times and the experiment was repeated thrice. Pupal mortality was recorded at different time intervals. The dead pupa was kept in a white trap to confirm the death by nematodes.

2.4. Stastical analysis

Using SPSS 10.0 for Windows software, the median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were estimated using probit analysis (Finney, 1962). Abbott's formula was used for calculating corrected mortality (Abbott, 1925). By dividing the lowest LD_{95} by the LD_{95} for each instar or stage and multiplying the result by 100, relative toxicity (RT) was determined (Cherry and Dusky, 1983).

3. Results and Discussion

3.1. Virulence of S. abbasi PN-1 against different stages of H. armigera

The mortality of 2^{nd}, 3^{rd} and 4^{th}instar larvae and pupae (Table 1 and Plate 1) of *H. armigera* were recorded in all the concentrations of *S. abbasi* PN-1 and a positive correlation between the concentration of IJs and the insect mortality was observed.

At 24 hours after treatment, the mean mortality rate of *H. armigera* larvae exposed to different concentrations was significantly different. The maximum mortality of 40% was recorded in 4^{th} instar larvae at 400 IJs $larva^{-1}$. In 25 IJs, 50 IJs $larva^{-1}$ and control treatments, no mortality was recordedin 2^{nd} instar larvae. At 36 hours after treatment, the mean

Table 1: The % mortality of *H. armigera* larvae and pupae at different concentrations of *S. abbasi* PN-1

Treatment:	(IJs 500 µl^{-1})	T_1:25	T_2:50	T_3:100	T_4:200	T_5:400	T_6: Control	F value	*p* value
Mean % Mortality of 2nd instar	24 HAT	0^{a}	0^{a}	3.3^{a}	16.6^{b}	30^{c}	0^{a}	41.7	0
	36 HAT	26.6^{b}	40^{c}	53.3^{d}	70^{e}	83.3^{f}	0^{a}	163.7	0
	48 HAT	76.6^{b}	86.6^{bc}	96.6^{cd}	100^{d}	100^{d}	0^{a}	268.8	0
	60 HAT	100^{b}	100b	100^{b}	100^{b}	100^{b}	0^{a}	-	-
Treatment:	(IJs 500 µl^{-1})	T_1:25	T_2:50	T_3:100	T_4:200	T_5:400	T_6: Control	F value	*p* value
Mean % Mortality of 3rd instar	24 HAT	0^{a}	6.6^{ab}	16.6^{c}	26.6^{cd}	33.3^{d}	0^{a}	26.65	0
	36 HAT	46.6^{b}	66.6^{c}	83.3^{d}	96.6^{e}	100^{e}	0^{a}	192.4	0
	48 HAT	100^{b}	100^{b}	100^{b}	100^{b}	100^{b}	0^{a}	-	-
Treatment:	(IJs 500 µl^{-1})	T_1:25	T_2:50	T3:100	T_4:200	T_5:400	T_6: Control	F value	*p* value
Mean % Mortality of 4th instar	24 HAT	0	16.6^{b}	26.6^{bc}	33.3^{cd}	40^{d}	0^{a}	51.3	0
	36 HAT	53.3^{b}	73.3^{c}	93.3^{d}	100^{d}	100^{d}	0^{a}	271.2	0
	48 HAT	100^{b}	100^{b}	100^{b}	100^{b}	100^{b}	0^{a}	-	-
Treatment: (IJs 3 ml^{-1})	T_1: 50	T_2:100	T3:200	T4:400	T_5:600	T_6:800	T_7: Control	F value	*p* value
Mean % mortality of pupae	36.66b	53.33^{c}	63.33^{cd}	70de	80^{ef}	86.66^{f}	3.33^{a}	103.33	0
Corrected mortality	34.48	50	62.06	68.96	79.31	86.2	-	-	-

Mean followed by the same letters in the column do not differ by Tukey's test (p<0.05); HAT=Hours aftertreatments

Plate 1: Virulence of *Steinernema abbasi* PN-1 against *Helicoverpa armigera* (A) Infected 2nd instar larvae (B) Infected 3rd instar larvae (C) Infected 4th instar larvae (D) White trap for dead Pupae

mortality rate (%) of *H. armigera* larvae exposed to different concentrations was significantly different. The maximum mortality of 100% was recorded at 400 IJs larva^{-1} in 3rd and 4th instar larvae. The least mortality of 26.6% was recorded at 25 IJs larva^{-1} in 2nd instar larvae. At 48 hours after treatment, the mean % mortality rate of *H. armigera* larvae exposed to different concentrations was significantly different. The maximum mortality of 100% was recorded in 3rd and 4th instar larvae at all the concentrations. The least mortality of 33.3% of 2nd instar larvae was recorded at 25 IJs larva^{-1}. In the pupal bioassay, maximum corrected mortality (86.2%) was recorded at 800 IJs 3 ml^{-1} concentration which was significantly higher over all other treatments and minimum corrected mortality of 34.4% recorded in 50 IJs 3 ml^{-1} concentration while 3.3% mortality was observed in control. Vashisth et al. (2019) reported that the efficacy of *Heterorhabditis* spp against the larvae of pod borer, *H. armigera* which showed 73.3% mortality of insect larvae in 96 h exposure time against third instar larvae and observed the positive correlation between the concentration of Us and larval mortality. Rana et al. (2021) reported that *S. abbasi* CS38 was highly virulent against the, *G. mellonella*, *H. armigera* and *Spodoptera litura* and caused (100%) mortality within 48 h under laboratory conditions.Banu et al. (2007) tested the virulence of *H. indica* and *S. glaseri* against different stages of *H. armigera* and concluded that the pupal stage the was least susceptible stage compared to the larval stage. The pupa was least susceptible because of fewer natural openings, only spiracles are the way for entry to EPNs.

3.2. Median lethal concentration

The median lethal time (LT_{50}) of *S. abbasi* PN-1 against different stages of *H. armigera* are presented in Table 2. The median lethal time (LT_{50}) of *S. abbasi* PN-1 against 2nd instars of *H. armigera* were reported to be 27.68 h at 400 IJs larva^{-1}, 31.22 h at 200 IJs larva^{-1}, 34.44 h at 100 IJs larva^{-1}, 37.98 h at 50 IJs larva^{-1} and 41.33 h at 25 IJs larva^{-1}. The LT_{50} of *S. abbasi* PN-1 against 3rd instars of *H. armigera* were 26.27 h at 400 IJs larva^{-1}, 27.6 h at 200 IJs larva^{-1}, 29.39 h at 100 IJs larva^{-1}, 32.89 h at 50 IJs larva^{-1} and 36.86 h at 25 IJs larva^{-1}. LT_{50} of *S. abbasi* PN-1 against 4th instars of *H. armigera* were 25.63 h at 400 IJs

Table 2: Dose-mortality response of *Steinernema abbasi* PN-1 against different stages of *Helicoverpa armigera*

Stages of *H. armigera*	LC 50 IJ insect	95% CI (Lower-Upper)	LC 95 IJinsect	95% CI (Lower-Upper)	Slope (±SE)	χ^2	RT*
2nd instar	78.29	41.08-149.19	1396.77	732.95-2661.82	1.13(0.14)	0.99	8.74
3rd instar	29.70	18.07-48.83	183.97	111.9-302.45	2.089(0.11)	0.98	66.35
4th instar	24.37	15.02-39.56	122.08	75.21-198.15	2.354(0.10)	0.87	100
Pupae	98.3	48.23-200.54	3127.42	1533.73-6377.4	1.09(0.15)	1	3.90

CI: confidence interval; Relative toxicity (RT) was calculated by dividing the lowest LD 95 by the LD 95 for each instar and multiplying by 100

larva^{-1}, 26.27 h at 200 IJs larva^{-1}, 27.03 h at 100 IJs larva^{-1}, 30.71 h at 50 IJs larva^{-1} and 34.74 h at 25 IJs larva^{-1}. It was revealed that the higher concentration of infective juveniles took lesser time for causing 50% mortality of all the tested larval instars of *H. armigera* and the LT_{50} was negatively correlated with IJs concentration (as the concentration of infective juveniles increased the values of LT_{50} decreased). Lower LT_{50} values were observed in fourth instar larvae as compared to second and third instar at different concentrations. The study conducted by Waret al. (2024) revealed that *Heterorhabditis casmirica* SKUAST-K 104 produced good results against *H. armigera* with a LC_{50} values calculated at 24 hours for 2nd, 3rd, 4th and 5th instar larvae was 256.88, 277.24, 326.25 and 384.25, respectively, whereas at 120 hours it was 126.11, 160.22, 184.36 and 219.14, respectively. Similarly, LT_{50} values calculated at inoculum level of 50 IJs 2nd, 3rd, 4th and 5th instar larvae^{-1} were 105.0, 113, 122 and 131 hours.

3.3. Median lethal concentration

The median lethal concentrations (LC_{50}) of *S. abbasi* PN-1 against different stages of *H. armigera* are presented in Table 3. The LC_{50} value ranged from 24.37 to 98.3 IJs larva^{-1}. The 4th instar larvae were highly susceptible with a LC_{50} value of 24.37 IJs larvae^{-1} and the pupae were least susceptible with LC_{50} value of 98.3 IJ pupae^{-1}. As the larval instars advanced, a decrease in LC_{50} was observed. The relative toxicity varied from 3.9–100%. The relative toxicity was 100, 66.35, 8.74 and 3.9% for 4th instar, 3rd instar, 2nd instar and pupae, respectively. The findings were similar to studies conducted by Srivastava et al. (2022) who reported that *S. abbasi* isolate CS-39 at 100 IJs and 200 IJs larva showed 100% mortality at 60 h and 36 h respectively against *H. armigera*. The LC_{50}=83.21 IJs of the *S. abbasi* isolate CS-39 was quite sufficient to achieve 100% mortality after 24 h of exposure. Acharya et al. (2020) tested the virulence of seven EPNs against larval and pupal stages of *S. frugiperda* and reported that in pot and soil column

Table 3: Comparison of median lethal times (LT_{50}) for *Steinernema abbasi* PN-1 against different stages of *Helicoverpa armigera*

Larval stage	No of IJs	LT50 (h)	95% CI (Lower-Upper)	LT95 (h)	95% CI (Lower-Upper)	R2	SD
2nd instar	25	41.33	36.63-46.13	58.35	51.99-65.49	0.997	0.1
	50	37.98	33.91-42.75	53.72	47.72-60.48	1	0.092
	100	34.44	30.59-38.77	47.14	41.88-53.07	0.083	0.995
	200	31.22	26.97-36.13	48.82	42.18-56.50	1	0.118
	400	27.68	23.69-32.34	43.28	37.04-50.56	0.996	0.09
3rd instar	25	36.86	33.46-40.6	53.12	46.43-59.63	0.999	0.061
	50	32.89	28.96-37.75	46.45	40.89-52.76	1	0.091
	100	29.39	25.76-33.54	41.49	36.36-47.34	1	0.091
	200	27.6	23.59-30.37	34.89	30.95-39.35	1	0.072
	400	26.27	23.56-29.28	33.28	28.04-38.56	0.979	0.075
4th instar	25	34.74	30.75-39.25	48.14	42.88-54.07	0.997	0.092
	50	30.71	26.58-35.49	46.51	40.53-53.98	1	0.111
	100	27.03	23.75-30.77	37	32.51-42.12	1	0.083
	200	26.27	23.56-29.28	33.28	28.04-38.56	0.979	0.075
	400	25.63	22.79-28.82	31.84	26.72-41.27	0.973	0.084

CI: confidence interval

assays *S. carpocapsae*, *H. indica* and *S. longicaudum* were more virulent on late larval and pupal stages in contrast with the other EPN species.

4. Conclusion

Virulence studies of *S. abbasi* PN-1 proved that the all-larval stages and pupae of *H. armigera* were found susceptible to the IJs. The above finding concluded that *S. abbasi* PN-1 can be utilized as a potential bio control agent against *H. armigera*. There is a great scope of these entomopathogenic nematodes for managing insect pests of different insect orders and can be used as a component of Integrated Pest Management with further field studies.

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