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Susceptibility of Different Developmental Stages of the Tobacco Cutworm, *Spodoptera litura* (Fabricius) to Entomopathogenic Nematode, *Steinernema abbasi* PN-1

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The tobacco cutworm, *Spodoptera litura* Fabricius is a widely distributed polyphagous pest that is causing economic damage to different crop plants. Many pesticides have been used extensively to control *S. litura*, but they are currently ineffective because of the development of pesticide resistance and their detrimental effects on the environment and human health. Although entomopathogenic nematodes (EPNs) have been employed as biological control agents against *S. litura*, little is known about the pathogenicity of these EPNs. Here we studied the virulence of *Steinernema abbasi* PN-1 against different stages of *S. litura*. The results reveled that all tested

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larval stages and pupae of *S. litura* were found susceptible to *S. abbasi.* There was a positive correlation between insect mortality and the nematode concentration. The *S. abbasi* caused 100 per cent larval mortality at 48-60 h of post treatment in all tested doses in laboratory. There was a positive correlation between insect mortality and the nematode concentration while the time to cause complete mortality was negatively correlated with the increase in larval days and nematode concentration. However, further field studies are required use *S. abbasi* as efficient biological control agents against *S. litura*.

Keywords: Steinernema abbasi PN-1; Spodoptera litura; Entomopathogenic nematodes (EPNs); pathogenicity.

1. INTRODUCTION

Spodoptera litura (Fabricius), the tobacco cutworm, is a defoliating, chewing pest that is distributed around the world and feeds on over a hundred host plants [1]. All year long, *S. litura* causes significant economic loss by damaging broad leaf plants including legumes, brassicas, and other economically significant crops [2]. First- to second-instar larvae gather in the rear of the leaf, where they consume the mesophyll and leave behind the vein pattern of the leaf on the plant. As they grow, caterpillars eat entire leaves, as well as fruits and flowers, causing serious harm [3]. The larva is death feigning and stays away from light. When the larvae reach the third instar, they move out to feed at night and hide beneath the ground during the day. Pupation occurs in the soil close to the plant base [4]. The larvae of *S. litura* older than the third instar exhibit a strong tolerance to a wide range of chemical pesticides [5]. Chemical pesticide usage in excess leads to major environmental and resistant issues. Because of pesticide resistance, *S. litura* outbreaks have become more frequent in Asian locations in recent years [6]. 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.

Entomopathogenic nematodes (EPNs) are biological agents that can be used for controlling various pest species, including *S. litura* [7]. Heterorhabditidae and Steinernematidae are two EPN families that are widely spread in a variety of soil types [8]. EPNs kill insect hosts due to mutualistic bacteria present in nematode gut. Infective juveniles (IJs) pierce the host and then enter the hemocoel, releasing symbiotic bacteria that proliferate and ultimately kill the host due to toxemia or septicemia. After 7–14 days of infection, nematodes moult and finish their life cycle inside the host, releasing new IJs [9]. To increase the effectiveness of pest control in integrated pest management, EPNs can be used with various chemical and biopesticides [10,11]. Additionally, the EPNs are linked to pathogenicity in a variety of host species. Moreover, the developmental stage of the host insect species affects the effectiveness of Entomopathogenic nematodes in pest management. EPNs have the potential to act as biological control agents against a variety of lepidopteran pests, including *S. litura*, according to earlier research [12-17]. Few research, meanwhile, have examined the effectiveness of EPNs in controlling *S. litura* at its various stages of growth. In the present study we evaluated the effect of *S. abbasi* PN-1 against different developmental stages of *S. litura*.

2. MATERIALS AND METHODS

2.1 Insect and Nematode Culture

The egg masses of *S. litura* were collected from castor and guava trees in CRC, Pantnagar, Uttarkhand. After hatching of the egg young larvae were provided with fresh and well-sterilized soft castor leaves in the rearing boxes. The rearing boxes are cleaned and sterilized daily and provided with fresh leaves. A running culture of *S. litura* was maintained and used for experiments in the laboratory. The different larval stages and pupae of approximately the same size and weight were used in the bioassay study.

The EPN *S. abbasi* PN-1 was collected from the Department of Entomology, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttrakhand. The EPN was cultured on late instar larvae of the greater wax moth, *Galleria mellonella* Linnaeus [18]**.** The 2 days old Fresh IJs solution was used for all the experiments.

2.2 Effect of *S. litura* Larval Developmental Stage and Exposure Time on *S. abbasi* PN-1 Virulence

Larval bioassays were carried out in Petri plates (50 × 10 mm), which are lined with filter paper. A single larva was released into each plate and ten plates are maintained for each treatment. The virulence of *S. abbasi* PN-1 to larvae of *S. litura* was determined by pipetting 500 µl of distilled water containing 25, 50, 100, 200 and 400 IJs onto the filter paper of each plate. Control treatments were treated with 500 µl of distilled water alone. Then plates were placed in incubator at 27 ± 2°C (Acharya *et al*. 2020a). The treatments were replicated three times. Larval mortality was assessed at 24, 36, 48 and 60 HAT. 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 *S. litura* pupae were determined by adding 50, 100, 200, 400, 600 and 800 IJs/3ml 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 12 days after treatment. The dead pupa was kept in a white trap to confirm the death by nematodes.

2.3 Statistical Analysis

The mortality data of *S. litura* were transformed into mean per cent mortality and a one-way analysis of variance was used for statistical analysis of these data. The median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were estimated using probit analysis [19]. Abbott's formula was used for calculating corrected mortality [20]. 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 [21].

3. RESULTS AND DISCUSSION

The mortality of 5, 8 and 11 days old larvae and pupae (Table 1) of *S. litura* 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 per cent mortality rate of *S. litura* larvae exposed to different concentrations was significantly different. The maximum mortality of 33.3 per cent was recorded at 400 IJs/larvae in 11 days old larvae. In 25IJs, 50IJs/larvae and control treatments no mortality was recorded. At 36 hours after treatment the mean per cent mortality rate of *S. litura* larvae exposed to different concentrations was significantly different. The maximum mortality of 100 per cent was recorded at 400 IJs/larvae in 11 days old larvae. The least mortality of 6.6 per cent was recorded at 25 IJs/larvae in 5 days old larvae. At 48 hours after treatment the mean per cent mortality rate of *S. litura* larvae exposed to different concentrations was significantly different. The maximum mortality of 100 per cent was recorded in 8 and 11 days old larvae at all the concentrations. The least mortality of 33.3 per cent of 5 days old larvae was recorded at 25 IJs/larvae.

The mean per cent mortality of pupae at different concentrations was significantly different ($F = 54.46$; $P = 0.00$). The maximum mortality of 63.3 per cent was recorded at 800 IJs/3ml conc. which was significantly higher than all other treatments followed by 600, 400, 200, 100 and 50 IJs/3ml conc. where 63.3, 53.3, 43.3, 36.6, 30 and 23.3 per cent mortality was recorded respectively while 3.3 per cent mortality was observed in control.

The median lethal concentrations (LC_{50}) of *S. abbasi* PN-1 against various stages of *S. litura* is presented in Table 2. The range of the LC_{50}value was 56.38 to 450 IJs/larvae. With an LC_{50}value of 56.38 IJs/larvae, the fourth instar larvae were very vulnerable, and the pupae, with an LC_{50} value of 450.11 IJs/pupae, were the least susceptible. There was a drop in LC_{50} as the larval instars developed. There was a range of relative toxicity from 2.83 to 100 per cent. The fourth instar, third instar, second instar, and pupae had relative toxicity values of 100, 92.78, 32.04, and 2.83 per cent, respectively.

Table 3 displays the median lethal time (LT_{50}) of *S. abbasi* PN-1 against various stages of *S. litura*. The LT_{50} of *S. abbasi* PN-1 against 2nd instars of *S. litura* were to be 34.73 h at 400 IJs/larva, 40.56 h at 200 IJs/larva, 45.1 h at 100 IJs/larva, 48 h at 50 IJs/larva and 53.89 h at 25 IJs/larva. The LT_{50} of *S. abbasi* PN-1 against 3rd

Table 1. Per cent mortality of *S. litura* larvae and pupae at different concentrations of *S. abbasi* PN-1

Treatment	(IJs/500µl)	T1:25	T2:50	T3:100	T4:200	T5:400	T6: Control	F value	P value
Mean per cent Mortality of 5 days larvae	24HAT	0^{a}	0^{a}	3.3^{ab}	10^{bc}	16.6^{bc}	0^{a}	12.9	0
	36HAT	6.6^{a}	13.3^{abc}	16.6^{bc}	26.6^{bc}	43.3^{d}	0^{a}	25.7	0
	48HAT	33.3^{b}	50^{c}	63.3^{d}	73.3^{d}	86.6^{e}	0^{a}	130.6	0
	60HAT	100^{b}	100^{b}	100^{b}	100^{b}	100^{b}	0^{a}	-	-
Mean per cent Mortality of 8 days larvae	24HAT	0^{a}	0^{a}	6.6^{ab}	16.6^{bc}	23.3^{c}	0^{a}	18.1	0
	36HAT	26.6^{b}	36.6^{bc}	46.6^{c}	63.3^{d}	86.6^{e}	0^{a}	96.9	0
	48HAT	100^{b}	100^{b}	100^{b}	100^{b}	100^{b}	0^{a}	-	-
Mean per cent Mortality of 11 days larvae	24HAT	0^{a}	0^{a}	16.6^{b}	26.6^{bc}	33.3^{c}	0^{a}	40.3	0
	36HAT	36.6^{b}	43.3^{b}	60^{c}	76.6^{d}	100^{e}	0^{a}	215.3	0
	48HAT	100^{b}	100^{b}	100^{b}	100^{b}	100^{b}	0^{a}	-	-
Treatment: (IJs/3ml)	T1:50	T2:100	T3:200	T4:400	T5:600	T6:800	T7: Control	F value	P value
Mean per cent mortality of pupae	23.33^{b}	30^{bc}	36.66^{bc}	43.3^{cd}	53.33^{de}	63.33^{e}	0^{a}	54.46	0

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

Table 2. Dose-mortality response of *Steinernema abbasi* PN-1 against different stages of *S. litura*.

Stages of *S. litura*	LC_{50} IJ/ insect	95% CI (Lower-Upper)	LC_{95} IJ/insect	95% CI (Lower-Upper)	Slope (±SE)	χ 2	RT*
5 days larvae	217.66	111.12-426.34	4112.47	2099.54-8055.3	1.29(0.15)	0.98	32.04
8 days larvae	87.76	47.34-162.69	1420.4	766.16-2633.31	1.37(0.13)	0.95	92.78
11 days larvae	56.38	27.85-114.14	1317.86	650.93-2668.14	1.20(0.15)	0.88	100
Pupae	450.11	179.47-1128.85	46478.62	18532.45-116566.49	0.81(0.2)	0.99	2.83

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.

Table 3. Comparison of median lethal times (LT_{50}) for *Steinernema abbasi* PN-1 against the various developmental stages of *S. litura* larvae

Larval stage	No of IJs	LT_{50} (h)	95% CI (Lower-Upper)	R^2	SD
5 days larvae	25	53.89	45.11-64.38	1	0.11
	50	48	41.1-56.05	1	0.11
	100	45.1	37.8-53.82	0.95	0.14
	200	40.56	33.57-48.99	0.92	0.16
	400	34.73	29.23-41.28	0.94	0.14
8 days larvae	25	38.72	35.56-42.17	1	0.05
	50	35.41	31.43-39.89	0.95	0.08
	100	34.06	30.01-38.66	0.96	0.09
	200	32.39	27.55-38.09	1	0.13
	400	28.18	24.55-32.34	1	0.09
11 days larvae	25	37.66	34.33-41.31	1	0.05
	50	36.88	33.42-40.7	1	0.06
	100	33.09	27.93-39.22	1	0.14
	200	28.93	24.56-34.09	0.97	0.13
	400	25.92	22.84-29.42	1	0.07

CI: confidence interval

instars of *S. litura* were to be 28.18 h at 400 IJs/larva, 32.39 h at 200 IJs/larva, 34.06 h at 100 IJs/larva, 35.41 h at 50 IJs/larva and 38.72 h at 25 IJs/larva. The LT_{50} of *S. abbasi* PN-1 against 4th instars of *S. litura* were to be 25.92 h at 400 IJs/larva, 28.93 h at 200 IJs/larva, 33.09 h at 100 IJs/larva, 36.88 h at 50 IJs/larva and 37.66 h at 25 IJs/larva. It was discovered that the LT_{50} was negatively linked with the concentration of infective juveniles (IJs) and that the higher concentration of infective juveniles required less time to cause 50% mortality of all studied larval instars of *S. litura*. At various doses, fourth-instar larvae showed lower LT_{50} values than second- and third-instar larvae. There was a positive correlation between insect mortality and the nematode concentration while the time to cause complete mortality was negatively correlated with the increase in larval days and nematode concentration.

3.1 Discussion (No Need to Explain Separate Discussion Section)

The *S. abbasi* was evaluated against different *S. litura* larval instar at different densities using bioassay method. The data showed that *S. litura* larval mortality was affected by nematode density, as well as larval development stage.

The findings of the virulence of *S. abbasi* PN-1 against larvae and pupae of *S. litura* were similar to studies conducted by Yan [22] as they reported that *S. arenarium, Steinernema* sp. 24-3 were more virulent against third and fourth-instar larvae of *S. litura* than against second-instar larvae. Differences in susceptibility of different development stages to EPN are common. Banu [23] tested the virulence of *H. indica* and *S. glaseri* against different stages of *H. armigera* and concluded that the pupal stage was the least susceptible compared to the larval stage. The pupa was least susceptible because of fewer natural openings, only spiracles are the way for entry to EPNs. Kondo [24] reported that the infectivity of *S. carpocapase* was greatly affected by pupal sclerotization of *S. litura*.

Many studies reported that lepidopteran pupae are also susceptible to EPNs, like Acharya [25] tested the virulence of seven EPNs against larval and pupal stages of *S. frugiperda* and reported that in pot and soil column assays *S*. *carpocapsae*,*H*. *indica* and *S*. *longicaudum* we re more virulent on late larval and pupal stages in contrast with the other EPN species. Henneberry [26] tested the pathogenicity of *S. riobravis* and *S. carpocapsae* against larvae and pupae of *P. gossypiella* and reported that the pupal stage is also more effective to treat in contrast with the larval stage. To validate the present results, the control efficacy of *S. abbasi* against *S. litura* should be evaluated in a field study.

4. CONCLUSION

In conclusion, All the tested concentrations of the *S. abbasi* PN-1 were found effective to kill the

different day's old larvae and pupae of *S. litura* at different periods. There was a positive correlation between insect mortality and the nematode concentration while the time to cause complete mortality was negatively correlated with the increase in larval days and nematode concentration. The older larvae of *S. litura* were more susceptible than younger larvae but the larval stage is more susceptible than the pupal stage. The *S. abbasi* PN-1 could potentially be used as biological control agent to sustainably manage the overlapping generations of *S. litura* in the environment.

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DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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