The Pharma Innovation Journal 2021; 10(12): 1884-1888

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(12): 1884-1888

www.thepharmajournal.com
Received: 28-10-2021
Accepted: 30-11-2021

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Virulence of entomopathogenic nematode isolate *Steinernema bicornutum* against diamondback moth (*Plutella xylostella* L.)

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DOI: https://doi.org/10.22271/tpi.2021.v10.i12aa.9710

Abstract
Plutella xylostella L., commonly known as the diamondback moth, is a significant pest of cole crops. It has established resistance to a range of pesticides, including chemical insecticides and Bt toxins. The use of appropriate bio-agents to manage the diamondback moth is a viable alternative to chemical pesticides. The current study was carried out with the goal of using entomopathogenic nematode (EPN) isolate *Steinernema bicornutum* to manage diamondback moths. Laboratory studies were carried out to know the virulence and reproductive potential of an indigenous population of *Steinernema bicornutum* at five doses i.e., 2IJs, 5 IJs, 8 IJs, 10 IJs and 12 IJs per third instar larvae of diamondback moth. Percent mortalities were recorded after 24h, 48h and 72h of post inoculation. Maximum percent mortality (81.25) was recorded after 72h with the highest dose i.e., 12 IJs per larva and the LC_{50} value was 7.99 IJs/100µl. The result of reproduction and recovery of isolates of entomopathogenic nematodes revealed that the highest IJs was yielded (2255.7) from dose of 12 IJs/larva. The overall results strongly suggested that *P. xylostella* is more susceptible to the entomopathogenic nematode and can be the host for multiplication of entomopathogenic nematodes under laboratory conditions.

Keywords: diamondback moth, entomopathogenic nematode, *Steinernema bicornutum*, virulence, reproductive potential, LC_{50}

Introduction
In India's intensive farming system, vegetables are a significant source of nutritional security, economic viability, and a source of remunerative revenue and employment for many small and marginal farmers. More than 60 different types of vegetables are grown in the country's tropical, subtropical, and temperate agro-climates. India is the world's greatest vegetable producer, ranking first in okra output and second in potato, onion, cabbage, and cauliflower production. Cole crop is high in Vitamin C, β -carotene, lutein, DL-α -tocopherol and phenolics, all of which help to prevent cancer. In India, cauliflower was grown on 459 thousand hectares, yielding 8800 thousand tonnes with a productivity of 19.17 tonnes per hectare. India generates approximately 32.5 percent of the world's total cauliflower crop. Individually cauliflower accounts for 5.06% of vegetable production of the country. Cauliflower accounts for 5.06 percent of total vegetable production in the United States. Cauliflower is produced in excess of 20 million tonnes per year worldwide, with China and India among the main producers. Cole crop production is plagued by common pests like diamondback moth (DBM) (*Plutella xylostella*) and cabbage looper (*Trichoplusia ni*). The diamondback moth (*Plutella xylostella* L.) is a global pest. It's a defoliating caterpillar that makes it more difficult to grow cabbage successfully over the world. In India it was first recorded (Flethcer, 1914) [7] on cruciferous vegetables. Now the pest has been noticed all over India on all the crops grown belonging to the family Brasicaceae (Devi *et al*., 2004) [5]. Several cultural and biological control measures have been practiced with varying degrees of success targeting the DBM larvae. However, due to the cryptic behaviour of DBM hiding inside the spongy leaf tissue during its early instar stage, it is a challenge for the farmers to use contact pesticides to kill the larvae effectively. Entomopathogenic nematodes (EPNs) are the beneficial nematodes which parasitize and kill the insects and are being effectively used as a bio pesticide against a wide variety of insect pests. The most commonly found entomopathogenic nematode species belong to the families Allantonematidae, Mermithidae, Steinernematidae, and Heterorhabditidae.

Of all the nematodes studied for biological control of insects, the *Steinernematids* and *Heterorhabditids* have received more attention because they are found lethal to insects and possess many of the attributes of effective biological control agents (Kaya and Gaugler, 1993) [9]. The current study was carried out with the goal of using *Steinernema bicornutum* to control diamondback moths. A laboratory investigation was carried out in order to come to a more accurate conclusion about the involvement of EPNs in the management of DBM.

Materials and Methods

The study was carried out under laboratory conditions in Jan 2021, to evaluate the efficacy of the *S. bicornutum* against diamondback moth in the Entomology Section, College of Agriculture, Nagpur Maharashtra.

Collection of test insect

Larvae of diamondback moths were collected from nearby fields of Nagpur.

Virulence test

The *Plutella xylostella* were reared in the laboratory conditions on cabbage and cauliflower leaves up to F_1 generation and 3rd instar larvae were used for the pathogenicity test. Infective juveniles (IJs) of the EPN isolate were taken into the beakers. The serial dilution as per the treatments were prepared in separate beakers. The count of the infective juveniles was taken for 100 µl and the count was repeated five times. Twenty third instar larvae of DBM were added in petri dish lined with two layers of 9cm filter paper. A volume of 100µl (per larva) of 2IJs(T_1), 5 IJs(T_2), 8 IJs(T_3), 10 IJs(T_4) and 12 IJs(T_5) suspension were added on the filter paper by pipette. Sterilized water (100 µl) instead of IJ suspension was used as control (T_6). Three replicates were set up for each treatment. After that, the petri dishes were sealed with parafilm and kept in the incubator at 25 °C and 60–70% RH. Insect mortality was recorded after 24h, 48h and 72h of inoculation. The LC_{50} values were calculated as per Finney (1971) [6] using probit analysis with the help of online software available on Hissar Agricultural University, Hissar, after computation of corrected percentage mortalities as per Abbott (1925) [1]. Dead DBM were placed on white trap in the petri dishes and water was added. Then the petri dishes were kept under the same condition for their emergence from the body (White, 1927) [13] and dissected 1 week later to examine the invasion rate of the EPN. Larvae were collected daily for up to a period of 20 days till the emergence of IJs will stop from insect cadavers and total number of IJs/larva were determined. The nematodes emerged from cadavers moved into surrounding water in the petri dish and this water containing infective juveniles was taken out in a beaker. The suspension taken out was subjected to check for nematode population count, which was taken by observing 100 µl suspension under stereo zoom binocular microscope for number of IJs in the droplet. Total count of nematode suspension taken out from petri plate was noted and total population count was calculated. These observations for population count were taken for all treatments and replications separately.

Statistical analysis

The data, thus, obtained were statistically analysed with the help of online software (OPSTAT) available on Hissar Agricultural University, Hissar and depicted in tables under respective subheads.

Result and Discussion

The results representing the biocontrol efficiency of *S. bicornutum* isolates against the insect pest diamondback moth, *Plutella xylostella* and were presented in the Table 1 and Figure 1 which clearly indicated that as the entomopathogenic nematode inoculum's level and exposure time increased, there was significant increase in mortality of larvae. All the treatments showed significantly high mortality over control. As far as effect of different doses of IJs is concerned, high mortality of 81.25% was observed after 72h of exposure period, at the maximum dosage of 12 IJs/larva followed by 63.75% at 10 IJs/larva and 45% at 8 IJs/larva. Whereas, lowest mortality was recorded at dose of 2 IJs/larva i.e., 28.75% after 72h of inoculation. Results depicted in Fig 2 on median lethal concentration of *S. bicornutum* required for 50 per cent mortality of 3rd instar larvae of *P. xylostella* indicated that the LC_{50} value was 7.99 IJs/100µl recorded after 72h. The effectiveness of nematode was increased with increase of dosage, which was in confirmation with findings of Atwa and Hassan (2014) who reported that insect mortality was high (60-90%) and low (<45%) at higher and lower nematode concentrations, respectively. Vyas *et al*. (2002) reported that the infective juveniles of *S. carpocapsae* provided a possible control of DBM by 27.8% at 400 IJs/pot. NanGong *et al*. (2016) [11] reported that *Xenorhabdus nematophila* HB310 has high insecticidal activity when fed to DBM. Nyasani *et al*. (2008) [12] conducted laboratory bioassays and recorded 86.7% mortality of DBM larvae at 200IJs/mL of *S. weiseri* within 72 hrs. Ganguly *et al*. (2004) [8] recorded 100% mortality of DBM within 48 hrs of infection, with *Steinernema thermophilum*. Nyasani *et al*. (2008) [12] used three EPN isolates, *Steinernema sp.*, *S. weiseri* and *H. indica* in a field experiment to test their ability to reduce DBM populations and damage to kale and the results had shown that all the three nematodes caused significant reductions in populations of DBM and DBM damage, with the population reductions being similar to those caused by application of *Bacillus thuringiensis ssp. kurstaki*. Adiroubane *et al*. (2010) [2] reported that the dosage and time mortality relationship of *S. siamkayai* against the 3rd, 5th larval instars and prepupal stage of *S. litura*, *P. xylostella*, *L. orbonalis*, *E. vitella*, and *C. medinalis* indicated that as the dosage and exposure time increased the susceptibility also increased. The results regarding reproduction and recovery of nematode infective juveniles of *S. bicornutum* from the cadavers of *Pluttella xylostella* presented in Table 2 and Fig 3 revealed that the maximum number of infective juveniles obtained from 3rd instar larva were 2257.7 at 12IJs/larva followed by 2170.7 at 10 IJs/larva.

Table 1: Efficacy of *S. bicornutum* isolates against the insect pest diamondback moth, *Plutella xylostella*

Sr.no.	Treatments	Mortality % *Pluttella xylostella*		
		24hrs	48 hrs	72hrs
1	2IJs/larva	6.25 (12.44)	16.25 (23.57)	28.75 (32.14)
2	5IJs/larva	10.00 (18.13)	20.00 (26.46)	36.25 (36.95)
3	8IJs/larva	12.50 (20.60)	28.75 (32.35)	45.00 (42.10)
4	10IJs/larva	17.50 (24.43)	35.00 (36.12)	63.75 (53.07)
5	12IJs/larva	36.25 (36.89)	61.25 (51.63)	81.25 (64.72)
6	Control	2.50 (6.45)	6.25 (14.29)	13.75 (21.54)
SE(m) ±		2.92	2.92	2.45
SE(d) ±		4.13	4.13	3.46
CD		8.76	6.85	7.33

(Figures in the bracket are arcsine transformed values; **F test highly significant at 1% level of significance)
(Larval mortality (%) = (No. of larvae died/ Total no. of larvae) x 100)

Table 2: Multiplication of *S. bicornutum* on *Pluttella xylostella.*

Sr.no	Treatments	Number of infective juveniles emerged from single larvae
1	2IJs/larva	1331.2 (36.36)
2	5IJs/larva	1942.5 (44.04)
3	8IJs/larva	2150.2 (46.28)
4	10IJs/larva	2170.7 (46.44)
5	12IJs/larva	2255.7 (47.43)
6	Control	0 (1)
	SE(m) ±	1.56
	SE(d) ±	2.21
	CD	4.69

(Figures in the bracket are square root transformed values.)

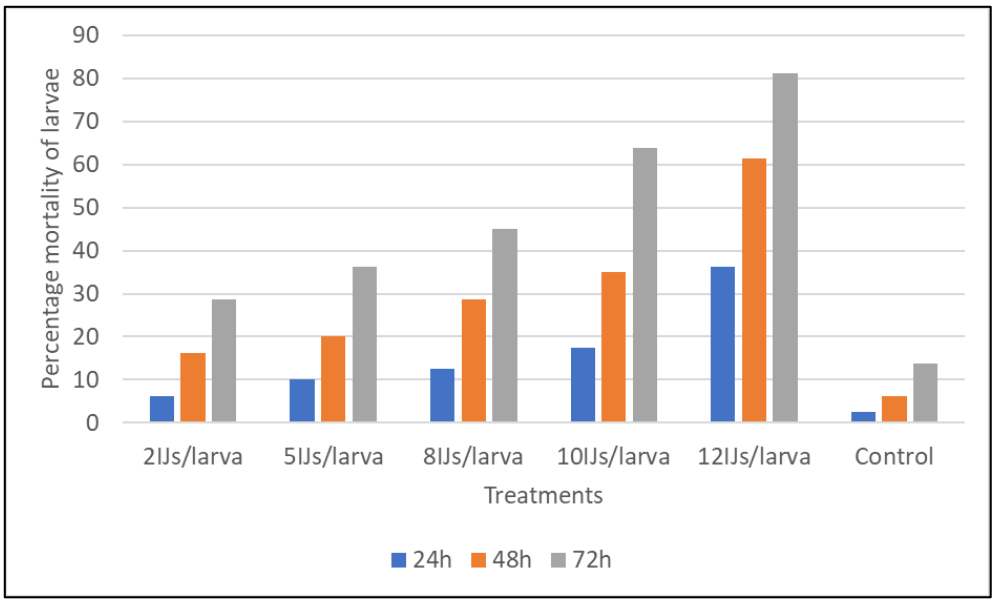

Fig 1: Pathogenicity of EPN isolate *S. bicornutum* against 3rd instar larvae of DBM

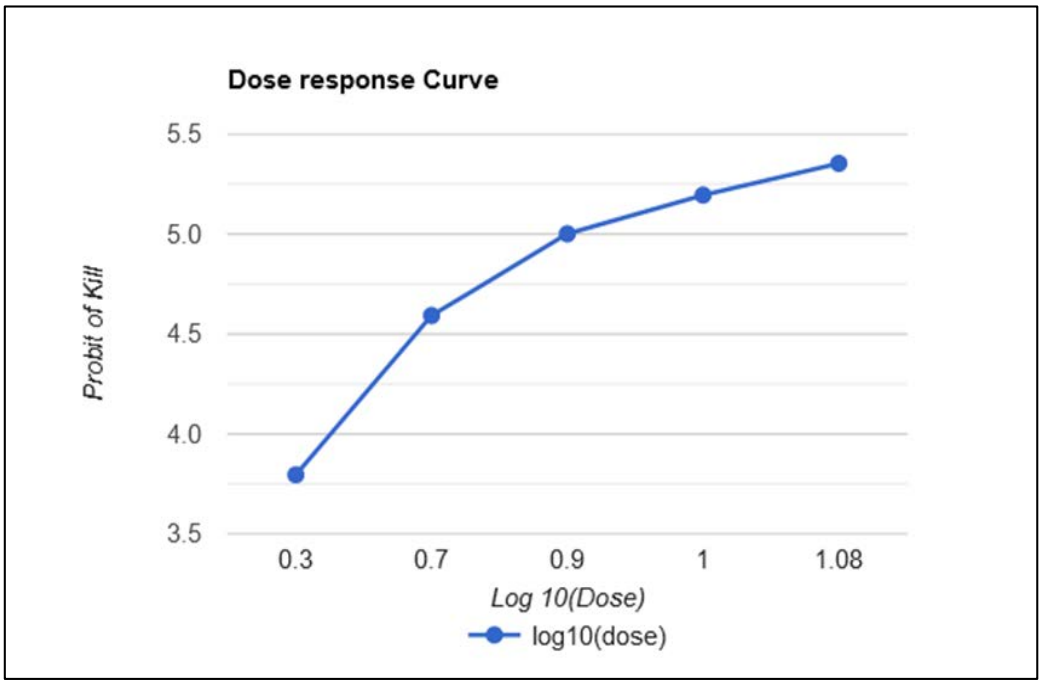

Fig 2: Dose response curve for median lethal concentration value

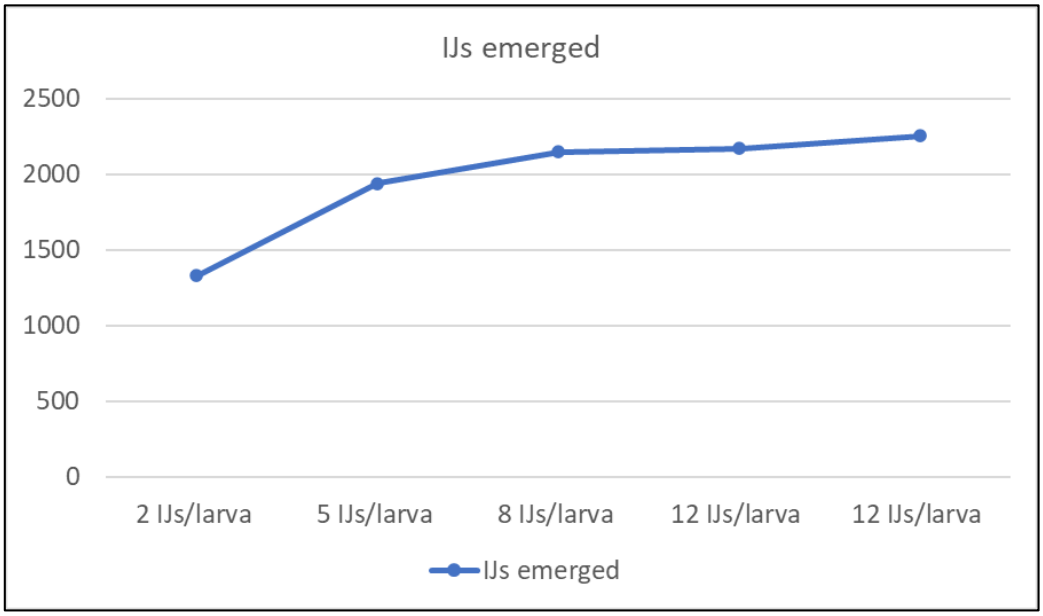

Fig 3: Multiplication of *S. bicornutum* on *Pluttella xylostella*

Conclusion

The present investigation provides basic knowledge regarding the efficacy of entomopathogenic nematode *S. bicornutum* against *Pluttella xylostella*. The results strongly suggested that the diamondback moth, *Plutella xylostella* is susceptible to the entomopathogenic nematode. A thorough investigation at field level on this aspect to develop *S. bicornutum* as a pest control agent, is necessary.

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