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Biocontrol Potential of Isolated Native Strain of EPNs from Southern Rajasthan against *Plutella xylostella* (Linnaeus) (Lepidoptera:Plutellidae)

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ABSTRACT: The diamondback moth, *Plutella xylostella* L., is a serious pest of cole crops. It has developed resistance to several pesticides, including as Bt toxins and chemical insecticides. An effective substitute for chemical pesticides in the management of the diamondback moth is the use of suitable bio-agents. The purpose of the current study was to control diamondback moths using an entomopathogenic nematode (EPN) strain from southern Rajasthan. To determine the virulence and reproductive capacity of an indigenous population, laboratory investigations were conducted using five dosages of the diamondback moth—50, 100, 200, 400, and 1000 IJs per third instar. The percentage of fatalities was noted 24 and 48 hours following the vaccination. With the highest dose, 600 IJs per larva, the maximum percent mortality (66%) was observed after 48 hours, and the LC50 value was 735 IJs/larva. Overall, the data clearly indicated that *P. xylostella* is more vulnerable to entomopathogenic nematodes and may serve as a host for their growth in laboratory settings.

Keywords: *S. siamkayai, Plutella xylostella, Heterorhabditis, Steinernematidae.*

INTRODUCTION

Vegetables are an important source of nutritional security, economic sustainability, and a source of remunerative revenue and employment for many small and marginal farmers in India's intensive agricultural system. In the tropical, subtropical, and temperate agroclimates of the nation, more than sixty varieties of vegetables are cultivated. India leads the globe in the production of vegetables, coming in first for okra and second for potatoes, onions, cabbage, and cauliflower. Vitamin C, β-carotene, lutein, DL-α-tocopherol, and phenolics are abundant in cole crop and contribute to cancer prevention. Approximately 32.5% of the global cauliflower crop is produced in India. The leafy vegetable identified as cabbage (*Brassica oleracea* var. capitat L.) is grown for its edible head. It is an excellent supply of vitamins and other minerals. In the temperate parts of the world, such as Bangladesh, India, China, Nepal, Bhutan, Tibbat, etc., cabbage is abundantly farmed. The states of Madhya Pradesh, West Bengal, Orissa, and Gujarat are collectively referred to for growing the most cabbage. There are small areas where cabbage grows in Andhra and Karnataka, as well as in the southern regions of Kerala. According to Pal *et al*. (2023), India is estimated to have harvested 9.95 million metric tons of cabbage in the fiscal year 2023. While in Rajasthan, the productivity of the cabbage crop was good in 2021–2022, averaging 9.74 metric tons per ha (Noopur *et al*., 2023).

Due to the risks to the environment and human health that come to employ chemicals more frequently and the residue that chemicals leave after in the soil which influences soil health, there is at present a need to develop substitutes to chemical pesticides. Entomopathogenic nematodes (EPNs) are helpful "bio-predators" or biocontrol agents that have been efficiently employed globally to manage a variety of insect pests that live in soil (Bhat *et al*., 2020). Since EPNs are naturally occurring enemies that are extremely virulent and have the ability of quickly eliminating their hosts and reducing the population of a variety of insect pests, they have played a crucial role in the development of integrated pest management (IPM). Toxins produced by symbiotic bacteria (*Photorhabdus luminescens* and

Xenorhabdu snematophilus) associated with entomopathogenic nematodes (EPNs) *indica*te another potential option for addressing this issue. Toxins are often referred to as toxic complexes, and or Tc toxin *S.* Gram-negative bacteria *Photorhabdus luminescens* and *Xenorhabdus* spp. create a symbiotic complex with nematodes that are entomopathogenic, *Heterorhabditis* spp. and *Steinernema* spp., respectively. Because of their tremendous virulence towards insect pests, these nematode-bacteria complexes are regarded as one of the greatest non-chemical pest control alternatives (Adithya *et al*., 2020). In pest biological control, the families Heterorhabditiae and *Steinernematidae* have been employed more frequently and with greater success (Bal *et al*., 2017).

Targeting the DBM larvae, a number of biological and cultural control techniques have been used with variable degrees of success. However, farmers find it difficult to employ contact insecticides to efficiently kill the larvae because of the cryptic nature of DBM, which involves hiding inside the spongy leaf tissue during its early instar year. Entomopathogenic nematodes (EPNs) are helpful nematodes that kill and parasitize insects. They are a useful biopesticide that works against a range of insect pests. The families Allantonematidae, Mermithidae, Steinernematidae, and Heterorhabditidae contain the majority of entomopathogenic nematode species that are widely seen. The objective of the current study was to manage diamondback moths using *Steinernema siamkayai*. For assessing with greater accuracy the extent to which EPNs are involved in DBM management, a lab study was conducted.

MATERIALS AND METHODS

The studies were carried out at the Maharana Pratap University of Agriculture, Rajasthan College of Agriculture, Rajasthan, India during the year 2022-23. The details of the materials used and the method employed are as follows:

A. Collection of soil samples

A survey is going to be done in different areas of Udaipur and Southern Rajasthan surrounding regions (KHERWADA, Badgaon) covering undisturbed and disturbed forest areas, organic farm, Fruit orchards, Grassland areas, and various horticultural and agricultural crop ecosystems (100 samples). From each collection site, a total of 400–500 g of soil sample collected from a depth of 10–15 cm. The collected soil samples transferred to the laboratory and immediately bait with the last instar larvae of *G. mellonella* in a container and incubate at room temperature. For 10 consecutive days, larval mortality checked every 24 h of time and dead larvae are transferred to a modified white trap as per Kaya and Stock (1997).

B. Rearing of Greater wax moth, Galleria mellonella (L.)

Nematodes that are pathogenic are created *in vivo* or *in vitro* (solid and liquid culture) using a variety of ways (Shapiro-Ilan & Dolinski 2012; Mouniga *et al*., 2022, Lal *et al*., 2023). *In vivo* production is a straightforward approach of producing EPNs in live insect hosts that requires little technology and involves the use of a surrogate host such as larvae of the greater wax moth, *Galleria mellonella* L., maintained in a laboratory under standard artificial diet.

C. Collection of DBM

Larvae of Diamondback moth are going to be collected from infested cabbage field and reared in the laboratory using their natural diet at 26±2 °C. 3rd and 5th larval instars and pupal stages was selected for the evaluation of their susceptibility against the locally isolated EPN species.

D. Larval mortality assay

Insecticidal activity of the EPNs were tested using 3rd and 5th instar larvae of *P. xylostella* that placed individually in plastic cups (10 cm diameter and 6 cm deep) fill with 100 gm. sterile wet sandy soil. Different concentrations of EPN was prepared (50, 100, 200, 400, and 600 IJs/larvae), and mixed with the soil then the larvae placed in the cups which cover with plastic lids. For each concentration, 5 replications has used. The control treatment carried out using distilled water. The cups incubated in a dark growth chamber at 26+2 °C. The mortality rate recorded after 48 h post-treatment

E. Statistical analysis

The data, thus, obtained were statistically analysed by Tukey's test with the help of online software minitab available on Rajasthan College of Agriculture, MPUAT, Udaipur.

RESULTS AND DISCUSSION

Native Strains of Entomopathogenic Nematodes (EPNs) Collected from Southern Rajasthan and their Biocontrol Potential against *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae).

The study determined that the highest mortality rate of third-instar larvae of *Plutella xylostella* was observed in *S. siamkayai* (66.00%), at 4 days following treatment. This was achieved by administering a dose of 600IJs/larva, as *indicated* in (Table 1) followed by 40.00% mortality, at 4 DAT. Upon analysing the mortality observed after the bioassay, significant differences in virulence were found among the EPN species *S. siamkayai* (third-instar: $F = 45.75$, $df = 5$, $P < 0.0001$). When assessing the susceptibility of *Plutella xylostella* larvae to EPN species (based on the absence of overlap in the 95% confidence intervals for LC50 and LC90 values), *S. siamkayai* (LC50 = 735.08/larva, LC90 = 1263 IJs/larva for third instar.

Table 1: Mortality (mean % ± SD) of third-instar larvae of Diamondback Moth, *Plutella xylostella,* at 2 and 4 days after treatment (DAT) at different concentrations of infective juveniles of each EPNs in laboratory conditions.

Treatment	*S. siamkayai*	Treatment
	2 DAT	**4 DAT**
50	16.00±8.94^{d}	24.00±8.94^{c}
100	22.00±4.47^{cd}	30.00±10.00^{c}
200	30.00±7.07^{bc}	38.00±8.37^{bc}
400	40±7.07^{b}	50.00±10.00^{b}
600	52.00.±4.47^{a}	66.00±5.48^{a}
Control	0.56^{E}	0.56^{d}
F value	**45.75**	**40.51**
P value	0.00	0.00

Different letters on the superscript of the means *indica*te statistically different values for different treatments at ($P < 0.05$) using Tukey's test.

Table 2: Mean number of infective juveniles required to cause 50% (LC50) and 90% (LC90) mortality in third of Diamondback Moth, *Plutella xylostella* after treatment in the laboratory conditions

Treatment Third instar	LC50	95%FL		LC90	95% FL		Slope ± SE	Pearson's χ^2	*P*^{a}
		Lower	Upper		Lower	Upper			
S. siamkayai	735.08	492.79	977.37	1763.21	1277.99	3091.97	0.0015±0.0038	10.71	0.030

P value for the χ^2 value. A nonsignificant χ^2 *indica*tes a good fit of the line to the data.

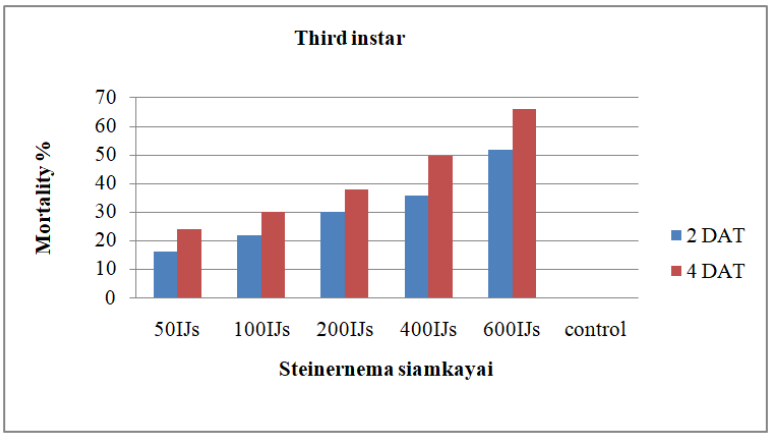

Fig. 1. Mortality (mean % ± S.D) in Third instar larvae of *P. xylostella* at different concentrations of *S. siamkayai.*

Gowda *et al.* (2020) isolated *S. siamkayai* (IIVR JNC01 strain) from the Bundelkhand Region of Uttar Pradesh. The strain demonstrated a good potential for biocontrol against lepidopteran and coleopteran pests. The third instar larvae of *Spodoptera litura* Fabricius, *Spilosoma obliqua* Walker, and *Spoladeare curvalis* Fabricius suffered mortality rates of 100, 100, and 85%, respectively, due to the *S. siamkayai* strain. Koul *et al.* (2008) found that indigenous strains of *Heterorhabditis indica*, *Steinernema asiaticum*, and *S. siamkayai* caused 80, 10, and 40% mortality in *Helicoverpa armigera* (after 48 h) in vitro and that *S. siamkayai* caused 100% mortality after 72 h at all temperature. These findings were made in relation to *Pieris brassicae* and *Agrotis ipsilon*. Three EPN strains—*Steinernema siamkayai*, *S. carpocapsae*, and *Heterorhabditis indica*—were assessed by Javed *et al.* (2020) against different stages of *P. sinuata* to combat serious radish pests in China. The findings of the study demonstrated that all three EPN species were capable of eliminating third-instar larvae, pupae, and adults of *P. sinuata* in a laboratory environment.

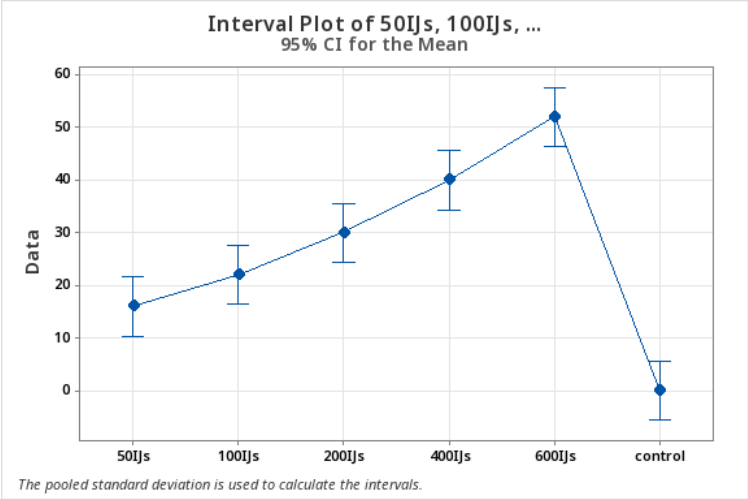

Fig. 2. Tukey Simultaneous Tests for Differences of Means.

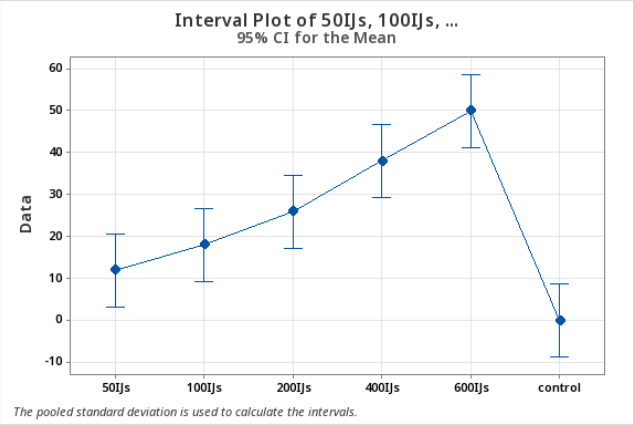

Fig. 3. Tukey Simultaneous Tests for Differences of Means.

CONCLUSIONS

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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