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Isolation and Characterization of *Steinernema seemae* Entomopathogens Against *Helicoverpa armigera* (Hubner) from North-Eastern Uttar Pradesh, India

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ABSTRACT

Background and Objective: In India, the application of natural Entomopathogenic Nematodes (EPNs) are considered potential biological control agents against soil-borne insect pests last 4-5 decades. Local isolates of EPNs have strong potential and are usually more effective for controlling indigenous insect pests as they are adapted to the local environmental conditions and the insect pest species. The present study aims to isolate indigenous EPN from the eastern part of Uttar Pradesh, India, to test the occurrence and their use as a biological control agent in IPM. **Materials and Methods:** The distribution, occurrence and diversity of *Steinernema seemae* entomopathogenic nematode community were investigated and isolation of *S. seemae* was carried out by using the galleria wax baiting technique and morphological identification methods which reveal that it belongs to the genus Steinernema. Further study was done to examine *S. Seemae* virulence's impact on soil temperature and soil moisture. **Results:** The effect of temperature, 12, 15, 20, 25 30 and 37°C and relative humidity, 35, 55, 85 and 100% on *S. seemae* was observed and they were listed against the *Helicoverpa armigera* larvae. The results reveal that, at 25°C temperature and 85% relative humidity, *S. seemae* is a more effective bio-control agent in terms of penetration and multiplication against host *H. armigera* larvae under controlled conditions. **Conclusion:** The EPNs isolated from the soil samples increase the information of *S. seemae* species showing bio-control activities, which was available for developing suitable bio-control strategies as well as enabling us the recognition of bio-control EPN. EPN species would serve as an alternative to chemical pesticides and fit well in integrated pest management.

KEYWORDS

Biocontrol, diversity, relative humidity, temperature, entomopathogenic nematodes, *Helicoverpa armigera*, *Steinernema seemae*

INTRODUCTION

India is an agro-based country more than two-thirds of the Indian population depends on agriculture for their livelihood[1,2]. It has always been the country's most important economic sector and constitutes one

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of the important Gross Domestic Product (GDP) contributors[3]. Food has always scared a problem for human beings in context with its fast-growing human population[2]. To meet this end, the production of food has to be boosted through various multiple ways, such as, by enhancing food grain production, increasing crop intensity, agricultural land reforms, and protecting crop damage against pests in the fields as well as during storage in the godowns etc[2]. High yield production can be achieved by using hybrid high vigour seeds implementation via biotechnological tools[4,5] (green revolution phase II). Land reforms and crop intensity increases can be yielded through government policies and agricultural practices, respectively.

Pest control leads to major destruction of the crop at both stages i.e., during agricultural practices as well as at storage time[2,6] and will cause huge damages to crops which create an overburden to the economy (GDP). Entomopathogenic Nematodes were second to *Bacillus thuringiensis* used as bio-insecticides around the world[7].

Pest control can be achieved in many ways i.e., chemical control includes, the use of chemicals known as herbicides, insecticides, pesticides etc[8]. Initially, it was thought to be a great success and the people sighted a relief, but soon that they create more problems than were supposed to solve, or in other words, the insecticidal crisis proved to be a multifaceted one affecting society and the environment in diverse ways[9]. Secondly, biological control includes the utilization of natural organisms against harmful pathogens[10]. They seem to be environmentally friendly and don't seem to disturb the environment in any way i.e., environmental pollution such as soil, water, and air pollution is negligible[11]. Recent reports revealed that biological control of the pest also enhances crop yield via helping them through various means such as making plant nutrients available to plants[12,13], secretion of plant hormones, chelation of essential micro and macro elements etc[14]. Thus the effort shifted from chemical control towards biological control, which refers to direct or indirect manipulation by man, of living natural control agents to increase their attack on pest species[15]. Further, indiscriminate and injudicious use of chemical pesticides made conditions more severe as well as awareness of public concern for safe food and a healthy environment has catalyzed the search for more environmentally friendly procedures.

With due emphasis on the safety of human beings and the environment including benefits, the search for suitable potential biological control agents in the control of *H. armigera* pest against the chickpea crop is an utmost urge of researchers. The objective of the present study is to isolate indigenous entomopathogenic nematodes from the eastern part of Uttar Pradesh, India, a biodiversity hotspot region, to record the occurrence and their further use as a biological control agent.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Zoology, D.D.U. Gorakhpur, U.P. Gorakhpur, from 2015 to 2017.

Geographical location: Soil samples were collected from geographical locations in triplicates from the different North Eastern divisions of Uttar Pradesh, India. The sampling location and sample type were tabulated. The study site includes the Gorakhpur division with four districts (Gorakhpur, Kushinagar, Deoria and Maharajganj). The four districts had coordinates 26°75'88"N 83°36'97"E (Gorakhpur), 26°74'1"N 83°88'8"E (Kushinagar), 26°30'36"N 83°46'48"E (Deoria) and 27°07'48"N 83°34'12"E (Maharajganj), respectively.

Soil characterization: Soil texture estimation was carried out by wet sieving methodology while, soil moisture was determined through the direct gravimetric method. Soil pH values of the suspension were determined with the help of a laboratory pH meter (Cyber scan pH 1500, Eutech Instruments, Singapore).

Organic content was determined according to the Walkley-Black method[16]. Percentage C and organic matter were calculated by using the following method:

Easily oxidizable organic C (%):

$$C\left(\%\right)=\frac{(B-S)\times M\text{ of }Fe^{2+}\times 12\times 100}{g\text{ of soil}\times 4000}$$

where:

B	=	mL of Fe^{2+} solution used to titrate blank
S	=	mL of Fe^{2+} solution used to titrate sample
12/4000	=	Milliequivalent weight of C in g

To convert easily oxidizable organic C into total C, divide it by 0.77 (or multiply by 1.30). To convert total organic C to organic matter use the following equation:

Organic matter (%):

$$OM\left(\%\right)=\frac{\text{Total C}\times 1.72}{0.58}$$

All analytical grade chemicals were procured from Merk India, Bangalore, Karnataka, India.

EPN isolation and culture: The isolation of indigenous entomopathogenic soil nematode will be carried out via the *Helicoverpa* pupae/larvae bait method[17] in the laboratory. The surface-sterilized *Helicoverpa* pupae/larvae of different age groups will be placed below the surface of collected soil samples in a sterilized beaker (250 mL) and will be incubated for 12-18 days (25±2°C, 75±5% RH). Almost 3-4 pupae/larvae of different age groups will be taken for each soil sample. After 12-18 days of the incubation period, the treated pupae will be taken out for isolation and identification.

Evaluation of the factors affecting infectivity of *S. seemae*: Evaluation of the effects of abiotic stress such as temperature and relative humidity on the infectivity of the *S. seemae* will be tested at six different constant temperatures (12, 15, 20, 25 (control), 30, 35 and 37°C) and four relative humidity (35, 55, 85 and 100%) in the laboratory.

RESULTS AND DISCUSSION

The occurrence and distribution of entomopathogenic nematodes were assessed throughout an extensive soil survey in the Gorakhpur, Deoria, Kushinagar and Maharajganj regions of the Gorakhpur division. A total of 11826.4 km^2 sampling area covered i.e., 2873.5 km^2 (Gorakhpur), 2,873.5 km^2 (Kushinagar), 2,535 km^2 (Deoria), 2,934.1 km^2 (Maharajganj) regions. Gorakhpur division comes under a humid subtropical climate. Annual temperatures of all districts were reported as 26°C. Soil characterization was also examined (Table 1) and results revealed that soil texture varies from sandy loam to clay loam while minimum to maximum pH, organic matter and soil humidity (w/w) were recorded to be 7.0-7.8, 0.13-0.36 and 20-24, respectively. Entomopathogenic nematode *S. seemae* isolation results revealed that the distribution of *S. seemae* varies as they were procured from only cultivated soils from Gorakhpur, Deoria, Kushinagar and Maharajganj districts respectively. For long decades, many researchers reported entomopathogenic nematodes from all over the world[18,19]. The distribution of entomopathogenic nematodes in our results showed a positive correlation with soil physiological properties such as soil

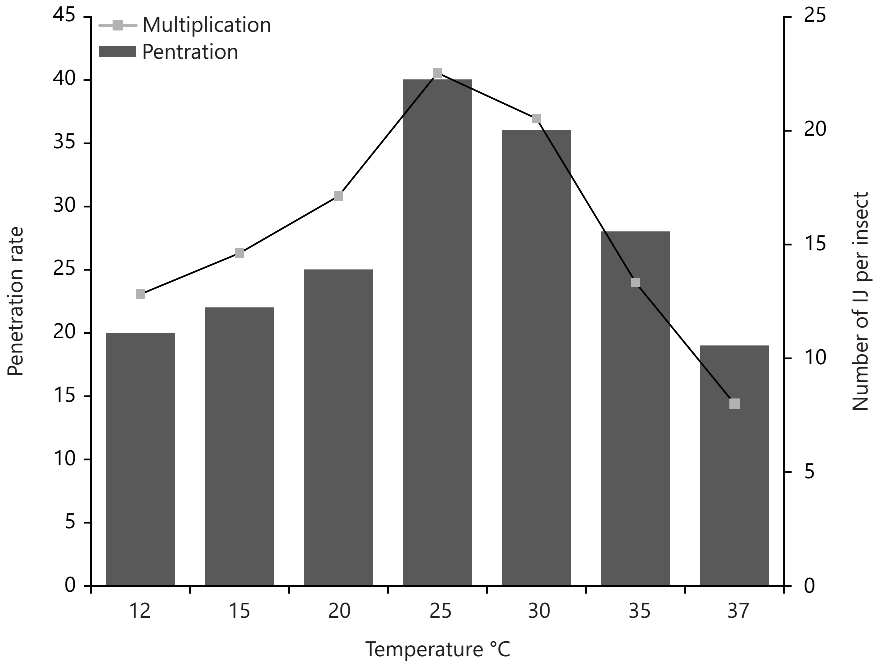

Fig. 1: Evaluation of penetration rate and multiplication rate of *S. seemae* on *H.armigera* larvae at different temperatures

Table 1: Sampling location and characterization of soil samples isolated from Gorakhpur division of North-Eastern Uttar Pradesh, India

Sampling site (Districts with Coordinates)	Sampling types	Soil texture	pH	Organic matter (%)	Soil humidity (w/w)
Gorakhpur	Cultivated	Sandy loam, sandy clay loam, clay loam	7.1 -7.6	0.29	23
(26°75'88"N83°36'97"E)	Uncultivated	Sandy loam		0.13	22
	Garden/Forest	Sandy loam, sandy clay loam, clay loam		0.34	24
Deoria	Cultivated	Sandy loam, sandy clay loam, clay loam	7.0-7.6	0.26	23
(26°30'36"N 83°46'48"E)	Uncultivated	Sandy, sandy clay loam, clay loam		0.12	22
	Garden/Forest	Sandy loam, sandy clay loam, clay loam		0.28	24
Kushinagar	Cultivated	Loam	7.1-7.8	0.32	22
(26°74'1"N 83°88'8"E)	Uncultivated	Sandy loam		0.36	21
	Garden/Forest	Sandy, sandy clay loam, clay loam		0.35	24
Maharajganj	Cultivated	Loam	7.0-7.5	0.30	20
(27°07'48"N 83°34'12"E)	Uncultivated	Loam		0.16	20
	Garden/Forest	Sandy loam		0.33	22

temperature, soil porosity as well as relative humidity. Other researchers also reported the effect of soil physiological parameters such as soil texture, soil temperature, pH and relative humidity limits the distribution of EPN in soils[19,20].

For control of particular insect pest selection of entomopathogenic nematodes based on several factors like nematode's host range, host finding, tolerance of environmental factors and their effects on survival and efficacy (temperature, moisture, soil type, exposure to ultraviolet light, salinity and organic content of the soil, means of application, agrochemicals etc). Continued prospection for new EPN species or races that are infective for *H. armigera* is highly warranted[20-22]. The foremost critical factors include moisture, temperature, pathogenicity for the targeted insect etc[10].

Experimental evaluations of several species for biological control including *H. bacteriophora, H. megidis, Heterorhabditis* spp., *Steinernema kushidai* and *Steinernema* spp. were recently carried out[23,24], However, virulence habits of *S. seemae* were on *H. armigera* have not been reported at all. In this effort, we tried to

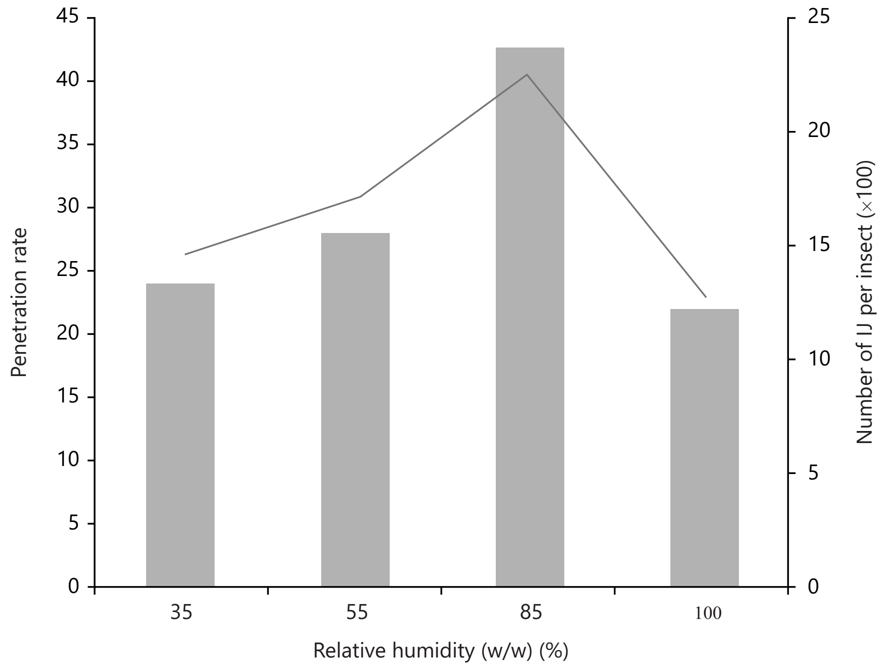

Fig. 2: Evaluation of penetration rate and multiplication rate of *S. seemae* in *H. armigera* larvae at different relative humidity (w/w) (%)

evaluate the relative infectivity of *S. seemae* on *H. armigera* pest and our results showed that maximum penetration and multiplication rate of *S. seemae* in *H. armigera* at 25°C and 85% relative humidity respectively. Temperature effect on penetration and multiplication rate increasing with the increase of temperature up to 25°C followed by decreases upto 37°C (Fig. 1). Similarly, relative humidity effect on penetration and multiplication rate of *S. seemae* was also observed and results revealed that the penetration and multiplication rate increases relative humidity from 35% up to 85%. However, at 100% relative humidity, the penetration and multiplication rate decrease drastically (Fig. 2). Thus our experimental results revealed that at 25°C and 85% (w/w) temperature and relative humidity *S. seemae* showed effective biocontrol agents against *H. armigera* larvae pest, respectively. The temperature range for survival and infectivity will depend on the species of EPN and its native habitat and centre of origin[25-27]. Our result was also confirmed by other researchers, they recently evaluated two commercially produced species, *S. riobrave* and *H. indica* as effective biocontrol agents at temperatures (27±2°C) in coarse sandy soils[28].

Therefore, in this investigation, we find that the isolation of *S. seemae* entomopathogenic nematode in North-Eastern Uttar Pradesh, India of Gorakhpur division has contributed to our knowledge of the distribution and population diversity of *S. seemae* which further helps us to understand its ecological distribution as well as physiological variation in a particular niche. Further biocontrol study results revealed that *S. seemae* showed best biocontrol activity against *H. armigera* at temperature and humidity of 25°C and 85% (w/w) at controlled conditions respectively.

CONCLUSION

It was concluded that the EPNs are of great importance to use in biological control. It is possible to rely on the results of this research in the control of *H. armigera* for use in the field application of pests. EPNs species would serve as an alternative to chemical pesticides and fit well in integrated pest management programs against larvae as well as pupae and adults of many economic insect pests which inhabit the soil.

SIGNIFICANCE STATEMENT

This study discovers the biocontrol agent in the form of EPN that have great importance to use in biological control. This study will help the researcher to increase the information to the researcher that entomopathogenic nematodes show biocontrol activities, which was available for developing suitable biocontrol strategies as well as enable us the recognition of biocontrol EPN from the North-Eastern region of India in place of chemical control.

REFERENCES

1. Reddy, T.K. and M. Dutta, 2018. Impact of agricultural inputs on agricultural GDP in Indian economy. Theor. Econ. Lett., 8: 1840-1853.
2. Popp, J., K. Pető and J. Nagy, 2013. Pesticide productivity and food security. A review. Agron. Sustainable Dev., 33: 243-255.
3. Vardhan, S., A.K. Yadav, A.K. Pandey and D.K. Arora, 2013. Diversity analysis of biocontrol *Bacillus* isolated from rhizospheric soil of rice-wheat (*Oryza sativa*-*Triticum aestivum* L.) at India. J. Antibiot., 66: 485-490.
4. Malviya, N., M.S. Yandigeri, A.K. Yadav, M.K. Solanki and D.K. Arora, 2014. Isolation and characterization of novel alkali-halophilic actinomycetes from the Chilika brackish water lake, India. Ann. Microbiol., 64: 1829-1838.
5. Whitford, R., D. Fleury, J.C. Reif, M. Garcia, T. Okada, V. Korzun and P. Langridge, 2013. Hybrid breeding in wheat: Technologies to improve hybrid wheat seed production. J. Exp. Bot., 64: 5411-5428.
6. Damalas, C.A. and I.G. Eleftherohorinos, 2011. Pesticide exposure, safety issues and risk assessment indicators. Int. J. Environ. Res. Public Health, 8: 1402-1419.
7. Divya, K. and M. Sankar, 2009. Entomopathogenic nematodes in pest management. Indian J. Sci. Technol., 2: 53-60.
8. Nicolopoulou-Stamati, P., S. Maipas, C. Kotampasi, P. Stamatis and L. Hens, 2016. Chemical pesticides and human health: The urgent need for a new concept in agriculture. Front. Public Health, Vol. 4. 10.3389/fpubh.2016.00148.
9. Humboldt-Dachroeden, S. and A. Mantovani, 2021. Assessing environmental factors within the one health approach. Medicina, Vol. 57. 10.3390/medicina57030240.
10. Pandey, A.K. and C.P.M. Tripathi, 2008. Effect of temperature on the development, fecundity, progeny sex ratio and life-table of *Campoletis chlorideae*, an endolarval parasitoid of the pod borer, *Helicoverpa armigera*. BioControl, 53: 461-471.
11. Bardin, M., S. Ajouz, M. Comby, M. Lopez-Ferber, B. Graillot, M. Siegwart and P.C. Nicot, 2015. Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? Front. Plant Sci., Vol. 6. 10.3389/fpls.2015.00566.
12. Bale, J.S., J.C. van Lenteren and F. Bigler, 2008. Biological control and sustainable food production. Philos. Trans. R. Soc. B: Biol. Sci., 363: 761-776.
13. Kumar, S., A. Chandra and K.C. Pandey, 2008. *Bacillus thuringiensis* (Bt) transgenic crop: An environment friendly insect-pest management strategy. J. Environ. Biol., 29: 641-653.
14. Seenivasagan, R. and O.O. Babalola, 2021. Utilization of microbial consortia as biofertilizers and biopesticides for the production of feasible agricultural product. Biology, Vol. 10. 10.3390/biology10111111.
15. Graham, J.H. and S.L. Strauss, 2021. Biological Control of Soilborne Plant Pathogens and Nematodes. In: Principles and Applications of Soil Microbiology, Gentry, T.J., J.J. Fuhrmann and D.A. Zuberer (Eds.), Elsevier, Amsterdam, Netherlands, ISBN: 9780128202029, Pages: 633-654.
16. Walkley, A. and I.A. Black, 1934. An examination of degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37: 29-38.
17. Orozco, R.A., M.M. Lee and S.P. Stock, 2014. Soil sampling and isolation of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae). J. Visualized Exp., 10.3791/52083.

18. Grewal, P.S., E.A.B. de Nardo and M.M. Aguillera, 2001. Entomopathogenic nematodes: Potential for exploration and use in South America. Neotrop. Entomol., 30: 191-205.
19. Hazir, S., H.K. Kaya, S.P. Stock and N. Keskin, 2003. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. Turk. J. Biol., 27: 181-202.
20. Cappaert, D.L. and A.M. Koppenhöfer, 2003. *Steinernema scarabaei*, an entomopathogenic nematode for control of the European chafer. Biol. Control., 28: 379-386.
21. Koppenhofer, A.M. and H.K. Kaya, 1998. Synergism of imidacloprid and an entomopathogenic nematode: A novel approach to white grub (Coleoptera: Scarabaeidae) control in turfgrass. J. Econ. Entomol., 91: 618-623.
22. Shapiro-Ilan, D.I., D.H. Gouge and A.M. Koppenhöfer, 2002. Factors Affecting Commercial Success: Case Studies in Cotton, Turf and Citrus. In: Entomopathogenic Nematology, Gaugler, R. (Ed.), CABI Publishing, Wallingford, UK, ISBN: 9780851995670, pp: 333-356.
23. Grewal, P.S., K.T. Power, S.K. Grewal, A. Suggars and S. Haupricht, 2004. Enhanced consistency in biological control of white grubs (Coleoptera: Scarabaeidae) with new strains of entomopathogenic nematodes. Biol. Control, 30: 73-82.
24. Ali, S.S., R. Pervez, M.A. Hussain and R. Ahmad, 2007. Effect of temperature on survival of *Steinernema seemae*, *S. masoodi* and *S. carpocapsae* (Rhabditida: Steinernematidae) and their subsequent infectivity to prepupa of *Helicoverpa armigera* (Hubner). Arch. Phytopathol. Plant Prot., 40: 183-187.
25. Georgis, R., A.M. Koppenhofer, L.A. Lacey, G. Belair and L.W. Duncan *et al*., 2006. Successes and failures in the use of parasitic nematodes for pest control. Biol. Control, 38: 103-123.
26. Lacey, L.A., S.P. Arthurs, T.R. Unruh, H. Headrick and R. Fritts, 2006. Entomopathogenic nematodes for control of codling moth (Lepidoptera: Tortricidae) in apple and pear orchards: Effect of nematode species and seasonal temperatures, adjuvants, application equipment, and post-application irrigation. Biol. Control, 37: 214-223.
27. McCoy, C.W., R.J. Stuart, L.W. Duncan and K. Nguyen, 2002. Field efficacy of two commercial preparations of entomopathogenic nematodes against larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in alfisol type soil. Florida Entomol., 85: 537-544.
28. Stuart, R.J., F.E. El-Borai and L.W. Duncan, 2008. From augmentation to conservation of entomopathogenic nematodes: Trophic cascades, habitat manipulation and enhanced biological control of *Diaprepes abbreviatus* root weevils in Florida citrus groves. J. Nematol., 40: 73-84.