



Evaluation of entomopathogenic nematode (EPN) isolate, *Heterorhabditis indica* of Vidarbha region, against the tobacco cutworm, *Spodoptera litura*

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ABSTRACT

The present study on pathogenicity of entomopathogenic nematodes against *Spodoptera litura* in laboratory conditions was undertaken during 2020-21, with the aim to ascertain the effectiveness of entomopathogenic nematodes, against an obnoxious cosmopolitan pest *S. litura*. Experiments were conducted by using entomopathogenic nematode (EPN) isolate *Heterorhabditis indica* (CICR-Guava), on filter paper, against *Galleria mellonella* and *S. litura* at the treatment dose of 10, 20, 30, 40, 60, 80 and 100 IJs/100µl along with control (Sterile distilled water). The results of our study revealed that, EPN isolate *H. indica* (CICR-Guava) caused 100% mortality at the treatment dose of 40 IJs/100µl within 72 h of infection in 5th instar larvae of *G. mellonella* and in case of *S. litura*, 100% mortality was recorded within 72 h of infection at the treatment dose of 100 IJs/100µl in 3rd instar larvae, which was found more susceptible. The median lethal concentration of *H. indica* (CICR-Guava) for 5th instar larvae was 2.29 IJs/100µl. The result of reproductive potential of isolates of entomopathogenic nematodes revealed that the highest yield was obtained from 5th instar larvae of *G. mellonella* at treatment dose of 100 IJs/100µl 278667 IJs per larva. In case of *S. litura*, the highest yield obtained was 152533 IJs. It could be concluded that, there was a positive correlation between nematode treatment concentration, time of exposure and the insect mortality of the tobacco cut worm and multiplication rate of IJs increased with increase of exposure time and size of larvae. This EPN isolate, *H. indica* (CICR-Guava) can be suggested as biocontrol agents for the control of *S. litura* in the Vidarbha region.

Introduction

Pest management in agriculture is a challenging task in the context of increasing agricultural productivity without disturbing the ecological balance and deteriorating the environment. Agrochemicals in agriculture of course are useful for protecting crops against pests and diseases and

have played a significant role to boost the agricultural production. However, these chemicals are posing enormous problems like environmental pollution, pesticide resistance, pest resurgence, toxicity hazards, secondary pest outbreaks, destruction of biodiversity of useful natural

enemies, residues of harmful chemicals in feeds, foods, soil and water, and some social economic and political problems. Failure of chemical insecticides to control insect pests at recommended dosage and problem associated with the use of pesticides made researchers to concentrate on the safer and effective alternative methods that can well fit into the current concept of integrated pest management. However, thirst on biopesticides is increasing due to increasing demand for organic agriculture. The biopesticides take care of crop losses during seed germination, plant growth in the nursery, fruiting phases, post-harvest storage, transport period and loss of man hours and lives. The annual growth in pesticides use is 1-2% and that of biopesticides is 10-25%.

The tobacco cutworm, *Spodoptera litura* (Fab.), is a defoliating and an obnoxious cosmopolitan pest which feeds on more than hundred host plants (Radhakrishnan and Shanmugam, 2017). It damages broad leaf plants such as legumes, brassicas, and other economically important crops throughout the year (Park *et al.*, 2001) and causes substantial economic loss. Hatched larvae of first to second instar aggregate at the back of the leaf and feed on the mesophyll, leaving the outline of the leaf veins on the plant. As growth continues, caterpillars eat entire leaves, and even flowers and fruits, causes great loss. Larvae older than third instar hide under the surface of the ground in the daytime and move out for feeding at night. They stay 1-3 cm under the soil surface until pupation (Park *et al.*, 2001). Pupation takes place within the soil near the base of the plants. The current research would help to generate some basic information about the pathogenicity of EPN isolate *H. indica* (CICR-Guava) isolated from local areas against *Spodoptera litura* and their dose *i.e.*, at what concentration maximum mortality occurs. It is important to test efficacy of local isolates of nematodes because they are already adapted to specific ecological niches and to some extent, are likely to exert natural biological control to either native or exotic insect pests.

Material and Methods

Collection of nematodes

The entomopathogenic nematode (EPN) isolate *Heterorhabditis indica* (CICR-Guava) was obtained from College of Agriculture, Nagpur, India. This

isolate was reconfirmed on the basis of associated bacterium and symptoms caused by the bacteria inside the insect cadaver. The EPN were cultured and multiplied on larvae of *Galleria mellonella* (Wiesner, 1993). The procedure of *In vivo* production of entomopathogenic nematodes was conducted by following the methods described by Poinar (1979) and summarized by Woodring and Kaya (1988).

Collection and rearing of test insect

Larvae of *Spodoptera litura* were collected from infested fields in Nagpur vicinity and reared on castor and cauliflower leaves. Also, laboratory host *Galleria mellonella* was reared on artificial diet in the laboratory.

Multiplication, culturing and Storage of entomopathogenic nematodes

The individual strain was maintained in the laboratory. Pure cultures of indigenous isolates of entomopathogenic nematodes, were prepared and maintained separately in late instar larvae of *G. mellonella*. These pure cultures were used for preparation of different treatment doses/concentrations for further studies. The infective juveniles of the entomopathogenic nematodes were stored in conical/tissue culture flasks. The double distilled water was used for preparing standard IJs counts. The nematode concentrations were kept in the range of 10,000 IJs/ml of sterile distilled water.

Bioassay against insect pests

In order to know the infectivity and pathogenicity of EPN isolate *Heterorhabditis indica* (CICR-Guava), an experiment was laid down. The EPN isolate was inoculated on different instars of the *G. mellonella* and *S. litura* under similar set of conditions. Infective juveniles (IJs) of the isolate *Heterorhabditis indica* (CICR-Guava), were taken into separate beakers. The serial dilutions were prepared as per the treatments in beakers separately. The infective juveniles count was taken for 100 µl and was repeated for five times. The known IJs were placed in the petri dish lid with the moistened filter paper and in each treatment and replication, five larvae of *Spodoptera litura* of third, fourth, and fifth instar were taken per petri dish. After treatment, the petri dishes were sealed and kept in captivity. After 24 hours the observations for larval mortality in each instar, replication wise and treatment wise were recorded. The observations were taken up to 96 hours at 24

hours interval. Larval mortality data was recorded and larvae were placed on white trap in a petri dish where water was added. In the control larvae were treated with plain distilled water.

Larval mortality was calculated by using the following formula

$$\text{Larval mortality (\%)} = \frac{\text{Number of larvae died}}{\text{Total number of larvae}} \times 100$$

LC₅₀

The LC₅₀ values were calculated as per Finney (1971) using probit analysis with the help of online software (OPSTAT) available on Hissar Agricultural University, Hissar, after computation of corrected percentage mortalities as per Abbott (1925).

Reproduction of EPNs on *Spodoptera litura*

In this experiment, 3rd, 4th and 5th instar larvae of *Spodoptera litura* were exposed to 10, 20, 30, 40, 60, 80 and 100 IJs/100 µl concentration of EPN isolate *Heterorhabditis indica* (CICR-Guava) (Yadav and Lalramliana, 2012) in petri plates. The nematode infected dead larvae were removed from petri plates and transferred individually on to white trap for their emergence from the body of cadaver (White, 1927). Then these petri plates and white traps were observed under stereo zoom binocular microscope for nematode emergence. The nematodes emerged from cadavers moves into surrounding water in the petri dish and this water containing infective juveniles was taken out in a beaker. The suspension taken out was checked for nematode population count, 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 each petri plate was noted and total population count was calculated.

Statistical analysis

The data, thus, obtained were statistically analysed by using one factor analysis (CRD) with the help of online software (OPSTAT) available at Hissar Agricultural University, Hissar and depicted in tables under respective subheads.

Results and Discussion

The results depicted in Table 1. revealed that all the treatment concentrations prepared showed significantly high mortality than control against 5th instar larvae of *Galleria mellonella* in laboratory condition. The maximum mortality was observed at the dosage of 100 IJs/100µl *i.e.*, 38.33% after 24 h. After 96 h 100% mortality was obtained at 30 IJs/100µl and the same trend was continued for next higher doses.

Table 1: Pathogenicity of EPN isolate *H. indica* (CICR-Guava) against *G. mellonella*.

S N	Treatment concentration	Larval mortality (%) of <i>G. mellonella</i>			
		24h	48h	72h	96h
1	10IJs/100µl	15.00 (22.79)	53.33 (46.89)	76.67 (61.12)	93.33 (75.21)
2	20IJs/100µl	16.67 (24.05)	60.00 (50.76)	81.67 (64.66)	96.67 (81.36)
3	30IJs/100µl	21.67 (27.71)	68.33 (55.74)	93.33 (75.21)	100.00 (90.00)
4	40IJs/100µl	23.33 (28.86)	73.33 (58.90)	100.00 (90.00)	100.00 (90.00)
5	60IJs/100µl	31.67 (34.23)	78.33 (62.26)	100.00 (90.00)	100.00 (90.00)
6	80IJs/100µl	36.67 (37.26)	86.67 (68.63)	100.00 (90.00)	100.00 (90.00)
7	100IJs/100µl	38.33 (38.24)	93.33 (75.21)	100.00 (90.00)	100.00 (90.00)
8	Control (distilled sterile water)	0.00 (0.00)	3.33 (8.61)	8.33 (16.59)	13.33 (18.43)
	F Test	sig**	sig**	sig**	sig**
	C.D.@ 5%	3.71	5.97	3.32	5.25
	SE(m)±	1.24	1.97	1.09	1.73
	SE(d)±	1.75	2.79	1.55	2.45
	C.V.(%)	8.66	6.41	2.63	3.83

(Figures in the bracket are arcsine transformation; **F test highly significant at 1% level of significance)

The pathogenicity results of *Heterorhabditis indica* (CICR-Guava) against *Spodoptera litura* demonstrated in Table 2. and clearly indicated that, as the entomopathogenic nematode inoculum's level and time of exposure increased, there was significant increase in mortality of *S. litura*. There was a positive correlation between concentrations and mortality rates. The tested nematode showed the highest mortality at 100 IJ/100µl concentration. The 100% mortality was obtained at 40 IJs/100µl in 3rd instar larvae, whereas, in case of 4th and 5th instar larvae 100% mortality was obtained at 60 IJs/100µl and 80 IJs/100µl respectively.

Table 2: Pathogenicity of EPN isolate *H. indica* (CICR-Guava) against 3rd, 4th and 5th instar larvae of *Spodoptera litura*

Sr. no.	Treatment concentration	Larval mortality (%) of <i>S. litura</i>											
		24h			48h			72h			96h		
		3 rd	4 th	5 th	3 rd	4 th	5 th	3 rd	4 th	5 th	3 rd	4 th	5 th
1	10IJs/100µl	18.33 (25.29)	16.67 (24.03)	13.33 (21.32)	43.33 (41.14)	36.67 (37.24)	31.67 (34.21)	68.33 (55.74)	66.67 (54.72)	63.33 (52.72)	88.33 (70.08)	86.67 (68.63)	83.33 (65.92)
2	20IJs/100µl	21.67 (27.69)	18.33 (25.29)	16.67 (24.03)	48.33 (44.02)	40.00 (39.19)	35.00 (36.22)	73.33 (58.90)	71.67 (57.83)	66.67 (54.72)	93.33 (75.21)	88.33 (70.08)	86.67 (68.63)
3	30IJs/100µl	23.33 (28.84)	21.67 (27.69)	18.33 (25.29)	53.33 (46.89)	45.00 (42.10)	41.67 (40.18)	76.67 (61.12)	75.00 (60.05)	70.00 (56.81)	98.33 (85.68)	93.33 (75.21)	88.33 (70.08)
4	40IJs/100µl	26.67 (31.05)	23.33 (28.84)	21.67 (27.69)	56.67 (48.81)	48.33 (44.02)	45.00 (42.10)	80.00 (63.52)	78.33 (62.26)	73.33 (58.90)	100.00 (90.00)	98.33 (85.68)	93.33 (75.21)
5	60IJs/100µl	33.33 (35.23)	31.67 (34.21)	28.33 (32.12)	65.00 (53.74)	60.00 (50.76)	56.67 (48.81)	88.33 (70.08)	86.67 (68.63)	83.33 (65.92)	100.00 (90.00)	100.00 (90.00)	96.67 (81.36)
6	80IJs/100µl	38.33 (38.22)	36.67 (37.24)	33.33 (35.23)	73.33 (58.90)	71.67 (57.83)	66.67 (54.72)	96.67 (81.36)	93.33 (75.21)	90.00 (71.92)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
7	100IJs/100µl	41.67 (40.18)	38.33 (38.22)	35.00 (36.22)	83.33 (65.92)	78.33 (62.26)	73.33 (58.90)	100.00 (90.00)	98.33 (85.68)	96.67 (81.36)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
8	Control (distilled sterile water)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	10.00 (18.42)	6.67 (14.76)	5.00 (12.91)	11.67 (19.87)	8.33 (16.59)	6.67 (14.75)	13.33 (21.32)	11.67 (19.87)	10.00 (18.43)
	F Test	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**	sig**
	C.D.@ 5%	3.07	3.16	3.67	3.28	4.30	3.52	5.92	6.29	6.60	5.47	5.68	5.63
	SE(m)±	1.01	1.04	1.21	1.08	1.42	1.16	1.95	2.08	2.18	1.81	1.88	1.86
	SE(d)±	1.44	1.48	1.71	1.53	2.01	1.64	2.76	2.94	3.08	2.56	2.66	2.63
	C.V.(%)	6.22	6.73	8.32	3.98	5.66	4.92	5.42	5.99	6.62	4.09	4.42	4.61

(Figures in the bracket are arcsine transformation; **F test highly significant at 1% level of significance)

Table 3: Multiplication of *Heterorhabditis indica* (CICR-Guava).

Sr. no.	Treatment concentration	Number of infective juveniles emerged per larva $\times 10^2$ from 100ml suspension			
		<i>Galleria mellonella</i>	<i>Spodoptera litura</i>		
		5th instar	3rd instar	4th instar	5th instar
1	10 IJs/100 μ l	1103.33 (33.22)	626.76 (25.05)	960.16 (31.00)	1123.56 (33.53)
2	20 IJs/100 μ l	1370 (37.013)	700.76 (26.48)	1037 (32.21)	1175.5 (34.30)
3	30 IJs/100 μ l	1606.67 (40.08)	839.56 (28.99)	1106.2 (33.27)	1263.86 (35.56)
4	40 IJs/100 μ l	1996.67 (44.68)	932.66 (30.55)	1184.76 (34.43)	1332.93 (36.52)
5	60 IJs/100 μ l	2273.33 (47.68)	1010.76 (31.80)	1244.33 (35.28)	1397.7 (37.39)
6	80 IJs/100 μ l	2563.33 (50.63)	1085.8 (32.96)	1304.2 (36.12)	1453.76 (38.14)
7	100 IJs/100 μ l	2786.67 (52.79)	1147.76 (33.89)	1354.06 (36.81)	1525.33 (39.06)
	F Test	sig**	sig**	sig**	sig**
	C.D.@ 5%	1.69	0.54	0.36	0.30
	SE(m) \pm	0.55	0.17	0.11	0.10
	SE(d) \pm	0.78	0.25	0.16	0.14
	C.V.(%)	2.18	1.03	0.60	0.47

(Figures in the bracket are square root transformation; **F test highly significant at 1% level of significance)

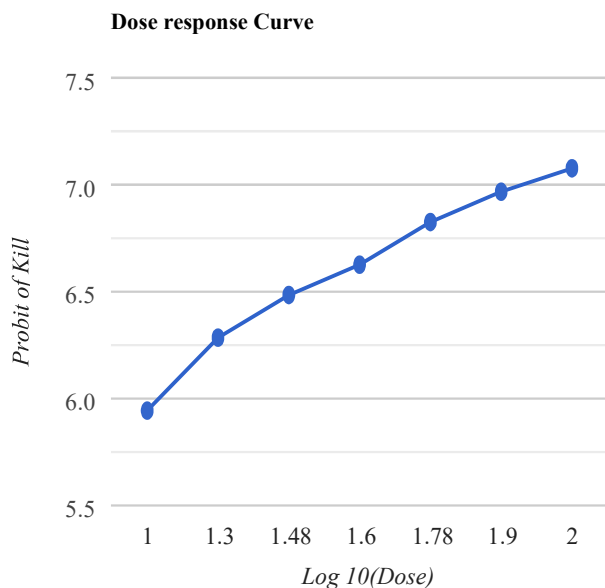


Figure 1: Median lethal concentration (LC50) of *Heterorhabditis indica* (CICR-Guava) to 3rd instar larvae of *Spodoptera litura*.

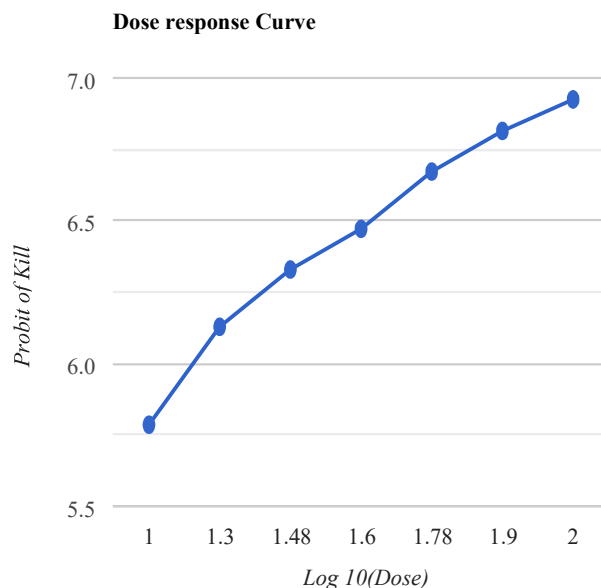


Figure 2: Median lethal concentration (LC50) of *Heterorhabditis indica* (CICR-Guava) to 4th instar larvae of *Spodoptera litura*.

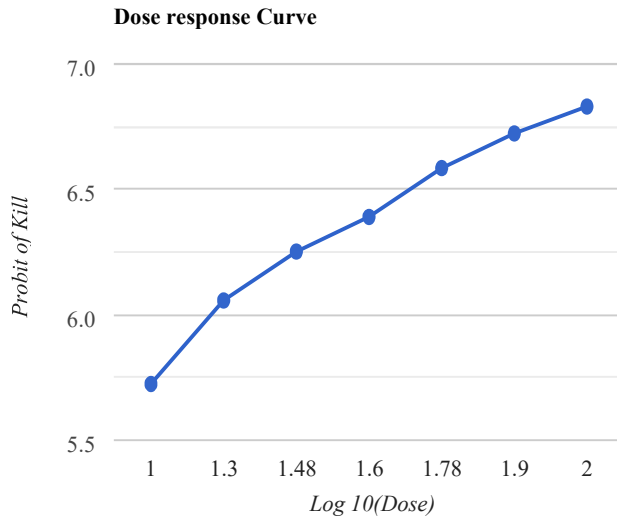


Figure 3: Median lethal concentration (LC50) of *Heterorhabditis indica* (CICR-Guava) to 5th instar larvae of *Spodoptera litura*.

The results were 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. Our results were pertinent with findings of Pal *et al.* (2012) and Kamaliya *et al.* (2019). A more or less similar trend was followed by Ganguly *et al.* (2007), Radhakrishnan and Shanmugam (2017), Yuksel and Canhilal (2018).

The results indicated that the third instar larva are more susceptible to *H. indica* than fourth and fifth instar larva of *S. litura* and 100 per cent mortality was obtained at higher inoculum level (100 IJs/larva) which was in line with the findings of Kim *et al.* (2008) and Yan *et al.* (2019), Acharya *et al.* (2020a) and Acharya *et al.* (2020b). Results of present study revealed that, median lethal concentration of *Heterorhabditis indica* (CICR-Guava) required for 50 per cent mortality of 3rd, 4th and 5th instar larvae of *S. litura* were, 1.47 IJs/100 μ l, 2.04 IJs/100 μ l and 2.21 IJs/100 μ l, respectively (Fig. 1, 2 and 3). The results were in line with findings of Radhakrishnan and Shanmugam (2017). Data in Table 3. clearly indicated that there was significant difference among all treatments with respect to emergence of infective juveniles of entomopathogenic nematode *Heterorhabditis indica* (CICR-Guava) from 5th instar larvae of *Galleria mellonella*. The highest population of infective juveniles 2786.67×10^2 IJs observed from EPN isolate CICR-Guava, when they were infected

with dosage of 100 IJs/100 μ l. Data presented in Table 3. revealed that the number of infective juveniles emerged from cadavers of 3rd, 4th and 5th instar of *Spodoptera litura*. It was observed from the data that the number of infective juveniles emerged increases with the increase of size of larva. The emergence of nematode infective juveniles of *H. indica* from the cadavers of *S. litura* was recorded daily up to the cessation of emergence of infective juveniles. Maximum number of infective juveniles obtained from 3rd instar larva were 1147.76×10^2 IJs at 100 IJs/100 μ l. In case of 4th instar larvae, maximum number of infective juveniles 1354.06×10^2 IJs obtained when they were inoculated at dosage of 100 IJs/100 μ l. Similarly, Maximum number of infective juveniles obtained from 5th instar larva were 1525.33×10^2 IJs at 100 IJs/100 μ l. Similar results were observed by Pal *et al.* (2012), Caccia *et al.* (2014), Holajjer *et al.* (2014) and Dhirta and Khanna (2019).

Conclusion

Based on the results obtained, it can be stated that the tobacco cutworm *S. litura* was found susceptible to the local isolate of entomopathogenic nematodes. As per the data it may be stated that *H. indica* (CICR-Guava) has the ability to kill the insect host within 48-72 h after infection and can be multiplied easily. It could be concluded that the nematode treatment dose, time of exposure and the insect mortality of the tobacco cut worm were positively correlated and multiplication rate of IJs increased with increase of exposure time and size of the larvae. The entomopathogenic nematode isolate *H. indica* (CICR-Guava) can be suggested as biocontrol agent for the control of *S. litura* in the Vidarbha region of Maharashtra.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

- Abbott, W. S. (1925). A method for computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18, 265-267.
- Acharya, R., Hwang, H., Mostafiz, M. M., Yu, Y. S. & Lee, K. Y. (2020b). Susceptibility of various developmental stages of the fall armyworm, *Spodoptera frugiperda*, to entomopathogenic nematodes. *Insects*, 11(868), 1-13.
- Acharya, R., Yu Y. S., Shim J. K. & Lee. K. Y. (2020a). Virulence of four entomopathogenic nematodes against the tobacco cutworm *Spodoptera litura* Fabricius. *Biological Control*, 50, 1-24.
- Atwa, A. & Hassan, S. H. (2014). Bioefficacy of two entomopathogenic nematodes against *Spodoptera littoralis* Boisduval (Lepidoptera) and *Temnorhynchus baal* Reiche (Coleoptera) larvae. *Journal of Biopesticides*, 7(2), 104-109.
- Caccia, M. G., Valle, E. D., Doucet, M. E. & Lax, P. (2014). Susceptibility of *Spodoptera frugiperda* and *Helicoverpa gelatopoeon* (Lepidoptera: Noctuidae) to the entomopathogenic nematode *Steinernema diaprepesi* (Rhabditida: Steinernematidae) under laboratory conditions. *Chilean journal of agricultural research*, 74(1), 123-126.
- Dhirta, B. & Khanna, A. S. (2019). Mass production of *Heterorhabditis bacteriophora* on lepidopteran insect pests. *Journal of Pharmacognosy and Phytochemistry*, 8(5), 1550-1553.
- Ganguly, S. & Somvanshi, V. S. (2007). Efficacy of foliar application of entomopathogenic nematodes against the crucifer diamond back moth *Plutella xylostella*. *Nematologia Mediterranea*, 35, 5-14.
- Holajjer, P., Patil, J. B., Harish, G, Nataraja, M, V., Poonam, J. & Savaliya, S. D. (2014). Evaluation of entomopathogenic nematodes, *Steinernema carpocapsae* and *Heterorhabditis indica* for their virulence against *Spodoptera litura*. *Annals of Plant Protection Sciences*, 22(1), 163-165.
- Kamaliya, R. P., Jethva, D. M., Kachhadiya, N. M., Bhut, J. B. & Ahir, V. R. (2019). Bio-efficacy of entomopathogenic nematode *Heterorhabditis indica* against *Spodoptera litura* (Fabricius). *Journal of Pharmacognosy and Phytochemistry*, 8(2), 1563-1567.
- Kim, H. H., Cho, S. R., Choo, H. Y., Lee, S. M., Jeon, H. Y. & Lee, D.W. (2008). Biological control of tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) by Steinernematid and Heterorhabditid entomopathogenic nematodes. *Korean Journal of Applied Entomology*, 47(4), 447-456.
- Lalramliana, & Yadav, A. K. (2010). Occurrence of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) in Meghalaya, NE India. *Science Vision*, 10(3), 89-100.
- Pal, R., Tiwari, G. N. & Prasad, C. S. (2012). Pathogenicity and mass production of entomopathogenic nematode, *Heterorhabditis indica* on major insects of agricultural importance. *Trends in Biosciences*, 5(1), 38-40.
- Park, S. H., Yu, Y.S., Park, J. S., Choo, H. Y., Bae, S. D. & Nam, M. H. (2001). Biological control of tobacco cutworm, *Spodoptera litura* Fabricius with entomopathogenic nematodes. *Biotechnology Bioprocess Engineering*, 6, 139-143.
- Poinar, G. O. Jr. (1990). *Taxonomy and biology of Steinernematidae and Heterorhabditidae*. In: R. Gaugler and H. K. Kaya, (Eds.). *Entomopathogenic Nematodes in Biological Control*. Boca Raton: CRC Press, pp. 23-61.
- Poinar, G. O. (1979). *Nematodes for biological control of insects*. Boca Raton, CRC Press, FL.
- Radhakrishnan, S. & Shanmugam, S. (2017). Bioefficacy of entomopathogenic nematodes against *Spodoptera litura* (Lepidoptera: Noctuidae) in bhendi. *International Journal of Current Microbiology and Applied Sciences*, 6(7), 2314-2319.
- White, G. F. (1927). A method for obtaining infective nematode larvae from cultures. *Science*, 66(1709), 302-303.
- Wiesner, A. (1993). *Die Induktion der Immunabwehr eines Insekts (Galleria mellonella, Lepidoptera) durch synthetische Materialien und arteigene Haemolymphfaktoren*. Berlin.
- Woodring, J. L. & Kaya, H. K. (1988). *Steinernematid and Heterorhabditid Nematodes: A Handbook of biology and techniques*. Southern Cooperative Series Bulletin 331. Arkansas Agri. Exp. Stat., Fayetteville, Arkansas, USA.
- Yadav, A. K. & Lalramilana. (2012). Efficacy of indigenous entomopathogenic nematodes from Meghalaya, India against the larvae of taro leaf beetle, *Aplosonyxchalybaeus*. *Journal of Parasitic Diseases*, 36(2), 149-154.
- Yan, X., Arain, M. S., Lin, Y., Gu, X., Zhang, L., Li, J., & Han, R. (2019). Efficacy of entomopathogenic nematodes against the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 113(1), 1-9.
- Yuksel, E. & Canhilal, R. (2018). Evaluation of local isolates of entomopathogenic nematodes for the management of black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 28(82), 1-7.

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