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Bio-efficacy of entomopathogenic nematode *Heterorhabditis indica* against *Spodoptera litura* (Fabricius)

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Abstract
The investigations on bio-efficacy of entomopathogenic nematode, *Heterorhabditis indica* against *Spodoptera litura* (Fabricius) was carried out at Biocontrol Research Laboratory, Department of Entomology, Junagadh Agricultural University, Junagadh during 2014-15. Bio-efficacy of *H. indica* against *S. litura* discovered that the larval mortality of *S. litura* directly proportional to dose of infective juvenile and time of exposure. At 120 hrs after application, cent per cent larval mortality was recorded at all the doses (20, 40, 60, 80 and 100 IJs/larva) of *S. litura*.

Keywords: Entomopathogenic nematode, *Heterorhabditis indica*, *Spodoptera litura*, Fabricius

Introduction
Among the different insect pests infesting this crop in Saurashtra region of Gujarat state, *Spodoptera litura* (Fabricius) are considered as a key foliar insect pests infesting this crop in Saurashtra region of Gujarat state.

To overcome these insect pest problems, chemical insecticides are largely in use. Their indiscriminate use has posed several risks and ill effects such as environmental pollution, ecological imbalance, resistance in insects, pests resurgence, destruction of beneficial insects and natural enemies of pests, increased the level of pesticide residues in soil, water, fodder, as well as food crop, health hazards etc. In view of these, today's need is to promote the ecologically and environmentally compatible biocontrol methods in crop protection as alone as well as a major input in overall Integrated Pest Management (IPM) practices/packages. Several bioagents including arthropod parasites, predators and microbes (bacteria, fungi, virus, protozoa and entomophilic nematodes) are now proved very useful in sustainable and safe agriculture.

Entomopathogenic nematodes (EPNs) especially members of genus *Steinernema* (27 species) and *Heterorhabditis* (8 valid species) are innovative bioagents for plant protection scientists of India. These EPNs are having symbiotic bacteria (Genus *Steinernema* – *Xenorhabdus* spp. and Genus *Heterorhabditis* – *Photorhabdus* spp.) which are gram negative, facultative anaerobic rods belonging to enterobacteriaceae having dimorphism nature. These mutually associated bacteria cause quick mortality of target insects having wide host range among class Insecta. They are also found safe to non - target organisms and compatible with many pesticides. Symbiont also produces antifungal and antibacterial metabolites like Xenorhabdin, Xenocoumacins, Xenoxodus, Nematophines (3' indol ethyl 3' methyl-2' oxo) and soluble proteineous compounds which make EPN a broad spectrum bioagents for biological suppression of agricultural pests (Vyas, 2000) [11].

EPNs are naturally found in soil and are extra ordinarily lethal to many important soil insect pests and safe to plants and animals (Smart, 1995). Due to this high degree of safety compared to chemicals, Application of EPN does not require special safety equipments and reduces time. Also they have no residues, avoid ground water contamination, general environmental pollution and are safe to pollinators and arthropod parasites.

In general many biological agents require days to weeks to kill the target, but EPN juveniles (IJs) working with their symbiotic bacteria, kills target insect within 24-72 hrs. Extreme conditions like temperature and moisture will affect moderately to the immature stage of EPN, many EPN species and strains are better adapted to wide range of extreme environmental conditions with long persistence in soil.

In India, *Steinernema* (nr. riobrave) was first time reported from Gujarat state (Ganguly *et al.*, 2002) [1]. Besides these few more species were discovered during last decade in India and many scientists have taken keen interest in entomopathogenic nematodes as an arsenal for soil insect pests in the country.

Entomopathogenic nematode families, *Steinernematidae* and *Heterorhabditidae* have been proved more useful against insect pests. EPNs are now emerged as second most valuable bio insecticide besides *Bacillus thuringiensis* for the effective suppression of insect pests in western countries during last three decades. In India, scientists have tested imported EPN cultures against few important insect pests during last three decades proving them very useful. EPN DD – 136 strain (*S. carpocapsae*) against *S. litura* (Narayan and Gopalkrishna, 1987) [4] DD – 136 strain (*S. glaseri*) against *H. consanguinea* (Vyas and Yadav 1992) [1] and *H. armigera* (Patel and Vyas, 1995) [5].

Materials and methods

Laboratory rearing of *S. litura*

In order to develop the initial culture of *S. litura*, large number of full grown larvae were collected from groundnut fields of Junagadh Agricultural University campus, Junagadh. The field collected larvae were placed in round aluminum cages under laboratory condition for further multiplication. Fresh groundnut leaves were provided as food for larvae and the foods were changed every day in the morning till pupation. The pupae formed from the larvae were transferred to new cage for the emergence of adult and immediately after emergence, male and female moth (1:1) were transferred in wooden cage measuring 45cm × 45cm × 60cm. All sides of cage were covered with white muslin cloth with long sleeves on two lateral side. The bottom of the cage was covered with one cm thick wet urethane foam. Five per cent honey solution was provided as food for the adults. Tender twig of plant was inserted in conical flask containing fresh water to keep it fresh and turgid which facilitates resting and oviposition of adults. The leaves were observed daily for egg masses. As soon as the egg masses were observed on the leaves, the conical flask was kept in separate cage for hatching. The neonate larvae were carefully transferred with the help of wet camel hair brush on groundnut leaves. The food were changed daily in the morning till pupation. The rearing was continued till two consecutive generations on groundnut to acclimatize and thus, the same was used for further laboratory investigations.

Methodology

Bio-efficacy of *H. indica* against 3rd, 4th and 5th instar larvae of *S. litura* was carried out in petri dishes. Different doses (20, 40, 60, 80 and 100 IJs/ larva) of the *H. indica* were prepared in sterile distilled water following serial dilutions. EPN suspension of each dilution was poured using micro pipette into respective petri dishes, simultaneously sterile distilled water was applied in control treatment.

After 10 minute, laboratory reared all the instars of *S. litura* were released individually in each petridish. The tray with petri dish was kept at 27± 2 ºC for 5 days in BOD incubator. Fresh groundnut leaves were provided daily to *S. litura.*

Observation recorded

The dead larvae were recorded at 24 h intervals up to 120 h. *H. indica* induced mortality in the larvae was confirmed by observing under microscope for presence of EPN.

Analysis of data

The data analysis was carried out by appropriate statistical method in Microsoft excel software.

Results and discussion

Investigation on bio-efficacy of *H. indica* against 3rd, 4th and 5th instar larva of *S. litura* were carried out at Biocontrol Research Laboratory. The result of this aspect is presented and discussed here.

Third instar larva

After 24 hrs

The results demonstrated in Table 1 and exemplified in Fig. 1 discovered that the *H. indica* caused 66.69% larval mortality of *S. litura* at 100 IJs/larva during 24 hrs after application. At 80, 60, 40 and 20 IJs/larva caused 53.34%, 46.65%, 33.31% and 26.63% larval mortality, respectively during the same period.

After 48 hrs

The data on bio-efficacy of *H. indica* against 3rd instar larva of *S. litura* is summarized in Table 1 and depicted in Fig. 1. The experimental result indicated that the higher dose (100 IJs/larva) caused 86.29% larval mortality followed by treatments of 80, 60, 40 and 20 IJs/larva which caused 80.16%, 73.37%, 60.13% and 53.34% larval mortality, respectively. In control set, no larval mortality was recorded.

After 72 hrs

At 100 and 80 IJs/larva of *H. indica* (Table 1 and Fig. 1), cent per cent larval mortality was obtained at 24 hrs after the application. In case of 60, 40 and 20 IJs/larva, 92.51%, 86.49% and 73.37% larval mortality, respectively was obtained.

After 96 hrs

The data on bio-efficacy of *H. indica* against *S. litura* is summarized in Table 1 and depicted in Fig. 1. The experimental result indicated that the higher doses (100, 80, 60 and 40 IJs/larva) caused cent per cent larval mortality, whereas, 20 IJs/larva triggered 92.79% larval mortality. No larval mortality was observed in case of control condition.

After 120 hrs

The results (Table 1 and Fig. 1) on bio-efficacy of *H. indica* against third instar after 120 hrs of application showed that all the treatments (100, 80, 60, 40 and 20 IJs/larva) gave cent per cent larval mortality for *S. litura.*

Fourth instar larva

After 24 hrs

The result on bio-efficacy of *H. indica* against 4th instar larva of *S. litura* were precisely framed in Table 2 and portrayed in Fig. 2. It indicated that the higher dose (100 IJs/larva) caused 60% larval mortality followed by treatments of 80, 60, 40 and 20 IJs/larva, which caused 39.96%, 33.29%, 26.59% and 13.89% larval mortality, respectively. In control set, no larval mortality was recorded.

After 48 hrs

The data presented in Table 2 and represented in Fig. 2 discovered that the higher dose (100 IJs/larva) caused 86.29% larval mortality followed by treatments of 80, 60, 40 and 20 IJs/larva which caused 73.41%, 66.85%, 53.34% and 40% larval mortality, respectively. There was no mortality seen in control set.

After 72 hrs

At 100 IJs/larva of *H. indica*, cent per cent larval mortality was obtained at 72 hrs after the application of *H. indica* which is presented in Table 2 and Fig. 2. In case of treatments of 80, 60, 40 and 20 IJs/larva, 92.51%, 86.22%, 79.48% and 66.78%

larval mortality was obtained, respectively. In control set, no larval mortality was recorded.

After 96 hrs

The results (Table 2 and Fig. 2) on mortality of 4th instar larva of *S. litura* at 96 hrs after the application of *H. indica* revealed that the higher dose (100, 80, 60 and 40 IJs/larva) caused cent per cent larval mortality, whereas, treatments of 20 IJs caused 86.49% larval mortality.

After 120 hrs

The results demonstrated in Table 2 and exemplified in Fig. 2 discovered that the all the doses (100, 80, 60, 40 and 20 IJs/larva) caused cent per cent larval mortality of 4th instar larva of *S. litura* at 120 hrs after the application of *H. indica*.

Fifth instar larva

After 24 hrs

The results demonstrated in Table 3 and exemplified in Fig.3 discovered that the higher dose (100 IJs/larva) caused 46.66% larval mortality of 5th instar larva of *S. litura* at 24 hrs after the application of *H. indica* followed by treatments of 80, 60, 40 and 20 IJs/larva, which caused 33.26%, 26.63%, 20% and 7.70% larval mortality, respectively. In control set, no larval mortality was recorded.

After 48 hrs

The data presented in Table 3 and represented in Fig. 3. discovered that the higher dose (100 IJs/larva) caused 73.80% larval mortality followed by treatments of 80, 60, 40 and 20 IJs/larva, which caused 66.69%, 60%, 46.66% and 33.31% larval mortality, respectively. In control set, no larval mortality was recorded.

After 72 hrs

The result on bio-efficacy of *H. indica* against 4th instar larva of *S. litura* were precisely framed in Table 3 and portrayed in Fig. 3. It revealed that 92.51 % larval mortality was obtained at 100 IJs/larva of *S. litura* at 72 hrs after the application of *H. indica*. The treatments of 80, 60, 40 and 20 IJs/larva caused 86.29%, 80.42%, 73.37% and 60% larval mortality, respectively. In control set, no larval mortality was recorded.

After 96 hrs

The data on bio-efficacy of *H. indica* against *S. litura* is summarized in Table 3 and depicted in Fig. 3. The experimental result indicated that the higher dose (100, 80 and 60 IJs/larva) caused cent per cent larval mortality, whereas, treatments of 40 and 20 IJs/larva caused 93.17% and 79.48 % larval mortality, respectively. In control set, no larval mortality was recorded.

After 120 hrs

The results demonstrated in Table 3 and exemplified in Fig. 3 discovered that the all the doses (100, 80, 60, 40 and 20 IJs/larva) caused cent per cent larval mortality of 5th instar larva of *S. litura* at 120 hrs after the application of *H. indica*. In control set, no larval mortality was recorded.

It is concluded that the third instar larva are more susceptible to *H. indica* than fourth and fifth instar larva of *S. litura* and cent per cent mortality was obtained at higher inoculum level (100 IJs/larva) at exposure time of 120 hrs after the time of application.

Our results enunciated that the per cent larval mortality of *S. litura* increased with rise in dosage of IJs and exposure time and it pertinent with findings of Milena *et al*. (2014) [3] who reported 93% and 100% larval mortality at 50 and 100IJs dosage, respectively.

Present findings are in agreement with the results of Richter *et al*. (1988) [7], who concluded that cent per cent mortality in 1st instar larvae and 63.3% in 5th instar larvae at 1 and 13 IJs/ 0.7 ml, respectively at 52 hrs after application.

Similarly, Rishi pal *et al*. (2012) [8] also observed 3.3 to 73.5 % larval mortality of *S. litura* due to *H. indica* at 24 hrs of exposure. Vyas and Yadav (1992) [11] have also reported soil bioassay of *S. glaseri* against *S. litura* and found cent per cent mortality of larvae at 32 IJs/g soil after 72 hrs.

Our results pertinent with findings of Canhilal (2013), who reported 53.6-100, 72-100, 79.8-100 and 92.9-100 % larval mortality of *S. exigua* for nematode species, *H. bacteriophora* Hb and HP88 at the concentrations of 10, 25, 50 and 100 IJs/larva, respectively. Hussaini *et al* (2002) [2] also observed 100 % larval mortality of *Leucinodes orbonalis* within 72 hrs. at 25 IJs/larva.

Raveendranath *et al*. (2007) [6] also reported that *H. indica* was more virulent against pupae of *S. litura* with its lower LC_{50} value (78.3, 83.2, 113.1 and 91.6 IJs/pupa) compared to *S. carpocapsae* in sand, red, black soils at 10 % moisture and black soil at 15 % moisture, respectively.

Table: 1 Bio-efficacy of *H. indica* against third instar larva of *S. litura*

Treatment No.	Dose (IJs / larva)	Percent mortality of third instar larva after				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
T1	20	31.07 (26.63)*	46.91 (53.34)	58.93 (73.37)	74.42 (92.79)	82.73 (100)
T2	40	35.25 (33.31)	50.84 (60.13)	68.43 (86.49)	82.73 (100)	82.73 (100)
T3	60	43.08 (46.65)	58.93 (73.37)	74.12 (92.51)	82.73 (100)	82.73 (100)
T4	80	46.91 (53.34)	63.55 (80.16)	82.73 (100)	82.73 (100)	82.73 (100)
T5	100	54.75 (66.69)	68.26 (86.29)	82.73 (100)	82.73 (100)	82.73 (100)
T6	control	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)
S. Em. ±		1.32	1.76	1.51	1.30	0.57
C. V. %		6.32	6.19	4.19	3.29	1.41
C.D. at 5%		4.09	5.43	4.65	4.02	1.76

*Arc sin transformed values

Figures in parenthesis are retransformed values.

Table 2: Bio-efficacy of *H. indica* against fourth instar larva of *S. litura*

Treatment No.	Dose (IJs / larva)	Percent mortality of fourth instar larva after				
		24 hrs.	4 hrs.	7 hrs.	9 hrs.	12 hrs.
T1	20	21.28 (13.89)*	39.23 (40.00)	54.80 (66.78)	68.43 (86.49)	82.73 (100)
T2	40	31.04 (26.59)	46.92 (53.34)	63.06 (79.48)	82.73 (100)	82.73 (100)
T3	60	35.24 (33.29)	54.85 (66.85)	68.21 (86.22)	82.73 (100)	82.73 (100)
T4	80	39.21 (39.96)	58.96 (73.41)	74.12 (92.51)	82.73 (100)	82.73 (100)
T5	100	50.77 (60.00)	68.26 (86.29)	82.73 (100)	82.73 (100)	82.73 (100)
T6	control	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)
S. Em. ±		1.13	1.77	1.43	1.13	0.57
C. V. %		6.37	6.69	4.25	2.90	1.41
C.D. at 5%		3.50	5.46	4.41	3.50	1.76

*Arc sin transformed values
Figures in parenthesis are retransformed values.

Table 3: Bio-efficacy of *H. indica* against fifth instar larva of *S. litura*

Treatment No.	Dose (IJs / larva)	Percent mortality of fifth instar larva after				
		24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.
T1	20	16.12 (7.70)*	35.25 (33.31)	50.77 (60.00)	63.06 (79.48)	82.73 (100)
T2	40	26.57 (20.00)	43.09 (46.66)	58.93 (73.37)	74.85 (93.17)	82.73 (100)
T3	60	31.07 (26.63)	50.77 (60.00)	63.74 (80.42)	82.73 (100)	82.73 (100)
T4	80	35.22 (33.26)	54.75 (66.69)	68.26 (86.29)	82.73 (100)	82.73 (100)
T5	100	43.08 (46.66)	59.21 (73.80)	74.12 (92.51)	82.73 (100)	82.73 (100)
T6	control	7.40 (0)	7.40 (0)	7.40 (0)	7.40 (0)	7.40(0)
S. Em. ±		1.21	1.86	1.88	1.73	0.57
C. V. %		7.92	7.73	6.08	4.58	1.41
C.D. at 5%		3.74	5.74	5.82	5.34	1.76

*Arc sin transformed values
Figures in parenthesis are retransformed values.

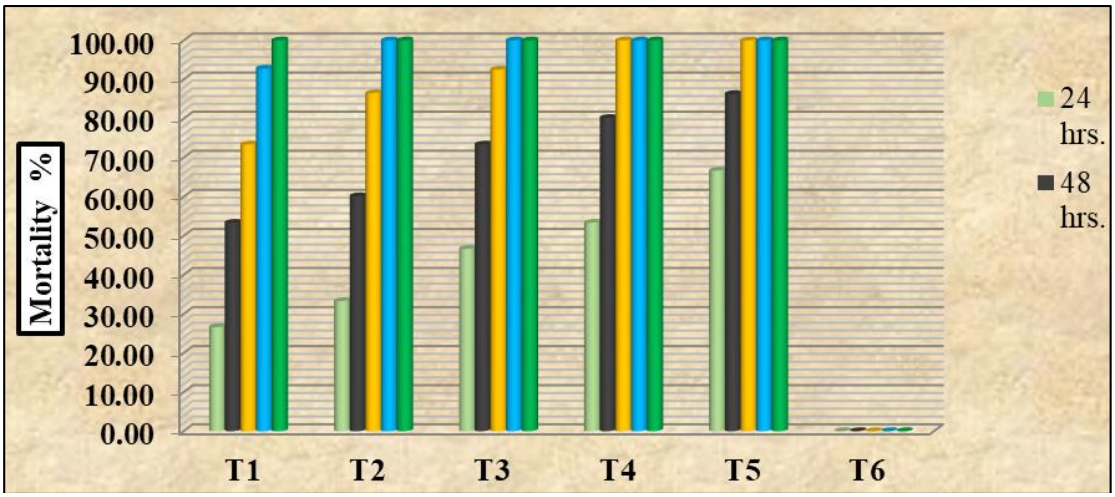

Fig 1: Bio-efficacy of *H. indica* against third instar larva of *S. litura*

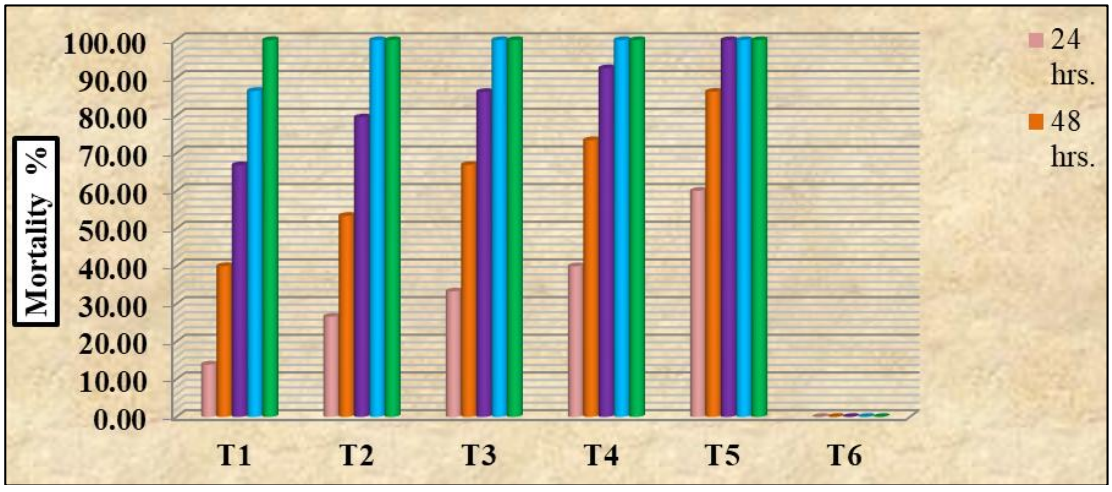

Fig 2: Bio-efficacy of *H. indica* against fourth instar larva of *S. litura*

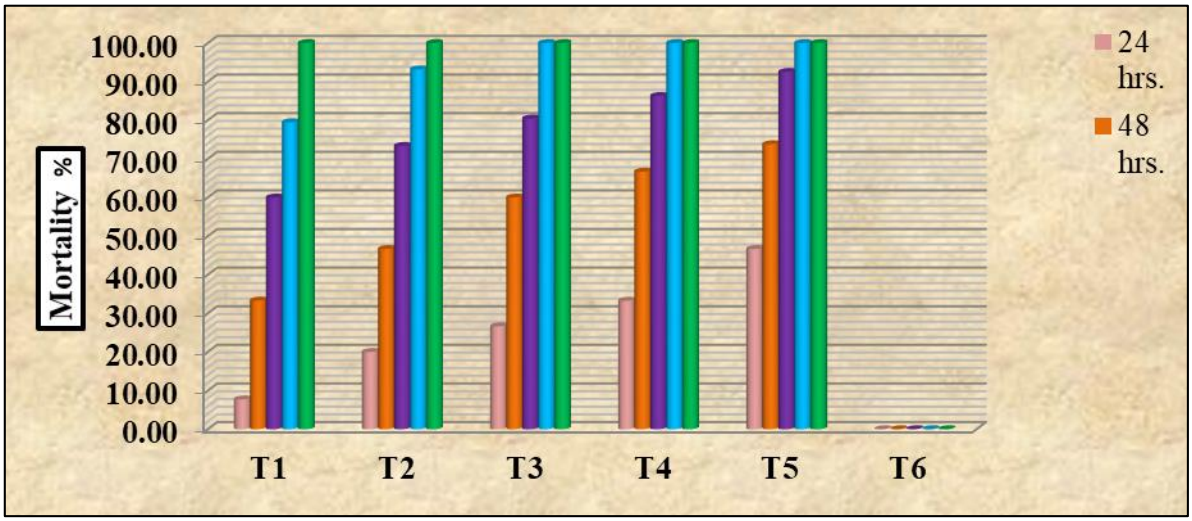

Fig. 3: Bio-efficacy of *H. indica* against fifth instar larva of *S. litura*

Plate I: Different developmental stages of *S. litura*

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