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Research Article

PATHOGENICITY AND MORTALITY BIOASSAY OF *SPODOPTERA LITURA* FABRICIUS (LEPIDOPTERA: NOCTUIDAE) INFECTED WITH FOUR *STEINERNEMA* SP. ISOLATED FROM DIFFERENT AGRO-ECOSYSTEM

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ABSTRACT

The four *Steinernema* sp. isolated from Palladam, Erode, karur and Madathukulam areas were tested for its pathogenicity against 4th instar larvae of *Spodoptera litura* with 100 IJs/ 20 larvae under laboratory conditions. The mortality was observed after 24 hrs of infection with 87%, 88%, 86% and 83% respectively in Palladam, Erode, karur and Madathukulam. In the larval mortality study 0.5 ml inoculam with 1, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 IJ's per twenty larvae of treatment of T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10} and T_{11} respectively for each *Steinernema* sp. isolated from Palladam, Erode, Karur and Madathukulam with five replication for each treatment along with untreated control were identical treatments except with no IJs. Larval mortality was recorded for 48 hrs. The LC50 value to 4th instar larvae of *S. litura* was 4.46, 6.20, 5.56 and 4.21 and LC90 with 13.80, 10.62, 12.40 and 7.74 in Palladam, Erode, karur and Madathukulam respectively. When compared to early studies, the four isolates of *Steinernema* sp. has showed virulence to *S. litura* in low dosage of inoculum in this study. So this isolated *Steinernema* sp. is a potential bio control agent *S. litura.*

Keywords: Entomopathogenic nematode, *Steinernema, Spodoptera litura, Galleria mellonella.*

INTRODUCTION

The greater wax moth or honeycomb moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is the major destructive and economically important pest of wax comb because of their feeding habits and tunneling through the combs (Chandel *et al.*, 2003). The moth is widely distributed throughout the world, causing serious problems in temperate, tropical and subtropical beekeeping regions, where the warm temperature favour the rapid development of the moth (Spangler, 1989). Also, in the recent years, researches have focused on the economic importance of the greater wax moth due to its susceptibility to a wide range of biological control agents, for instance, entomopathogenic nematodes, viruses, fungi along with the natural enemies of predators and parasites (Dindo *et al.*, 2001; Armendariz *et al.*, 2002; Ueno, 2002; Ansari *et al.*, 2003; Parthasarathy and Rabindra, 2003). Mass rearing of the greater wax moth is done on artificial diets, to study various biological parameters *viz*, The present study was designed to investigate the efficacy of *Steinernema* sp. to *Spodoptera litura*. The obtained findings could, subsequently, offer basic information for Integrated Pest Management of this important pest.

MATERIALS AND METHODS

Pathogenicity of *Spodoptera litura* larvae

The infective juveniles of *Steinernema* sp. initially was tested to check their pathogencity to fourth instars larvae of *Spodoptera litura* exposed for a period of time at different inoculum levels. The larval stages were exposed to 1 ml of IJ`s (100 nematodes) sprayed on Whatman's No. 1 filter paper in a petri plate. 20 larvae for each replication with five replicates each with inoculum level of 100 IJs were maintained for each treatment maintaining appropriate untreated control separately. The entire experiment was repeated to confirm the results. After the time of exposure, the treated larva was washed twice in distilled water and transferred onto another fresh petri plate. Observations were made for larval mortality at 12 hours interval till absolute mortality occurred in each treatment. The larval

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death was confirmed by mechanical probing the body, color change of the cadaver due to symbiotic bacteria proliferation.

Larval mortality bioassay

Larval mortality bioassays were carried out in petri dishes lined with layer of Whatman No.1 filter paper, using the methods of Kaya and Stock (1997). Nematodes of 0.5 ml inoculums in deionized water added to the filter paper in concentrations of 1, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 IJ's per twenty larvae as treatment of T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10} and T_{11} respectively. After 30 min, twenty *Spodoptera litura* larvae were placed in each of the petri dish sealed with Parafilm and maintained in laboratory. For each nematode species of Palladam, Erode, Karur and Madathukulam five replications for each treatment from T_1 to T_{11} were maintained along with untreated controls were identical to the treatment except that no IJs were added. Larval mortality was recorded for 48 hours. The larval death was confirmed by observing the larva frequently.

Mortality percentage = control – treated / control × 100.

RESULTS

Isolation of entomopathogenic nematodes and its symbiotic bacteria

An intensive survey was conducted Entomopathogenic nematodes (EPNs) in Palladam, Erode, Karur and Madathukulam in Tamilnadu. Four soil samples were collected randomly from each location and totally 20 samples were collected for the Experiment. 250 g of soil from each location was baited with five *Galleria mellonella* larvae and replicated five times. Entomopathogenic nematodes were recovered from all four samples Palladam, Erode, Karur and Madathukulam. The death resulted in 48 hours. The nematodes were harvested from the cadaver of infected larvae by White's trap method and nematodes were stored in T – flask with 0.01% formalin and kept in a B.O.D incubator at 15˚C. Healthy larvae of *G. mellonella* was taken and infected with each isolate of entomopathogenic namatodes. After 24 hours the haemolymph of infected larva was streaked on the NBTA medium and maintained at 28˚C for 24 hours and plates showed red colour colonies.

Pathogenicity of *Steinernema* sp. to *Spodoptera litura*

Steinernema sp. isolated from Palladam, Erode, Karur and Madathukulam was used to study the pathogenicity on cotton leaf worm *S. litura* under laboratory conditions. In laboratory experiment the 4th instar larvae of *S. litura* was infected with *Steinernema* sp. of all the four areas with inoculum level of 100 IJs/ 20 larvae the death was observed for every one hour. After 24 hours the larval death was recorded in the treatment. Pathogenicity of *Steinernema sp* of Palladam, Erode, Karur and Madathukulam was analyzed in pest of 20 larvae/100IJs with three replication for each treatment. The results were showed 87%, 88%, 86% and 83% mortality respectively.

Larval mortality bioassy

Palladam: The LC_{50} values was observed at 7.464 nematodes and LC_{90} 13.804 nematodes in Palladam species the larval stage of these insects were highly susceptible to *Steinernema sp*. causing 58.4% to 88% mortality at 48 hours exposure for treatments between T_7 - T_{11}, whereas from low susceptibility 2% to 42% mortality percentage was recorded for *S. litura* in treatments T_1 - T_6. The 95% confidential limit of LC_{50} as Lower Confidential Limit (LCL) at 5.680 and Upper Confidential Limit (UCL) at 10.243. The 95% Confidential limit of LC_{90} as Lower Confidential Limit (LCL) at 10.801 and Upper Confidential Limit (UCL) at 22.213. The regression equation is X = 3.858 and Y = 4.136. The Chi-square at value at 0.014 (Table 1).

Erode: The LC_{50} values was observed at 6.205 nematodes and LC_{90} at 10.629 nematodes in Erode species the larval stage of these insects were highly susceptible to *Steinernema* sp. causing 64.4% to 87% mortality at 48 hours exposure for treatments between T_7 - T_{11}, whereas from low susceptibility 1% to 54% mortality percentage was recorded for *S. litura* in treatments T_1 - T_6. The 95% confidential limit of LC_{50} as Lower Confidential Limit (LCL) at 4.808 and Upper Confidential Limit (UCL) at 7.765. The 95% Confidential limit of LC_{90} as Lower Confidential Limit (LCL) at 8.810 and Upper Confidential Limit (UCL) at 14.407. The regression equation is X = 4.772 and Y = 4.451. The Chi-square value at 0.231 (Table 2).

Karur: The LC_{50} values was observed at 5.567 nematodes and LC_{90} at 12.408 nematodes in Karur species the larval stage of these insects were highly susceptible to *Steinernema* sp. causing 55.4% to 86% mortality at 48 hours exposure for treatments between T_7 - T_{11}, whereas from low susceptibility 4% to 45% mortality percentage was recorded for *S. litura* in treatments T_1 - T_6. The 95% confidential limit of LC_{50} as Lower Confidential Limit (LCL) at 3.491 and Upper Confidential Limit (UCL) at 7.832. The 95% Confidential limit of LC_{90} as Lower.

Madathukulam: The LC_{50} values was observed at 4.219 nematodes and LC_{90} at 7.740 nematodes in Madathukulam species the larval stage of these insects were highly susceptible to *Steinernema sp*. causing 58.4% to 83% mortality at 48 hours exposure for treatments between T_7 - T_{11}, whereas from low susceptibility 3% to 54% mortality percentage was recorded for *Spodoptera litura* in treatments T_1 - T_6. The 95% confidential limit of LC_{50} as Lower Confidential Limit (LCL) at 2.996 and Upper

Confidential Limit (UCL) at 5.539. The 95% Confidential limit of LC_{90} as Lower Confidential Limit (LCL) at 6.241 and Upper Confidential Limit (UCL) at 10.993 The regression equation is X = 4.491 and Y = 3.853. The Chi-square value at 0.845 (Table 4).

Table: 1. Larvicidal activity of *Steinernema* sp. (Palladam) against 4th Instar larvae of *Spodoptera litura.*

S. No.	Treatments	Nematodes (IJ)	No. of larva	Mortality (%) Mean±SD	LC_{50}	95% Confidential limit		LC_{90}	95% Confidential Limit		Regression Equation	Chi Square value
						LCL	UCL		LCL	UCL		
1.	T 1	1	20	2 ± 2.739	7.464	5.680	10.243	13.804	10.80122.213	X=3.858	Y=4.136	0.014
2.	T 2	5	20	6 ± 4.183								
3.	T 3	10	20	14 ± 1.183								
4.	T 4	15	20	26 ±4.183								
5.	T 5	20	20	34 ± 6.159								
6.	T 6	25	20	42 ± 4.472								
7.	T 7	30	20	58.4 ±5.941								
8.	T 8	35	20	65 ± 6.124								
9.	T 9	40	20	73 ± 5.708								
10.	T 10	45	20	82 ± 4.472								
11.	T 11	50	20	88 ± 5.708								

Values are mean ± SD for five replications. *Mortality of the larvae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table: 2. Larvicidal activity of *Steinernema* sp., (Erode) against 4th instar larvae of *Spodoptera litura.*

S. No.	Treatments	Nematodes (IJ)	No. of larva	Mortality (%) Mean±SD	LC_{50}	95% Confidential Limit		LC_{90}	95% Confidential Limit		Regression Equation	Chi Square value
						LCL	UCL		LCL	UCL		
1.	T 1	1	20	1 ± 2.236	6.205	4.808	7.765	10.629	8.810	14.407	X=4.772 Y=4.451	0.231
2.	T 2	5	20	8 ± 4.472								
3.	T 3	10	20	17 ± 5.708								
4.	T 4	15	20	28 ± 5.708								
5.	T 5	20	20	39 ± 6.519								
6.	T 6	25	20	54 ± 6.519								
7.	T 7	30	20	64.4 ± 6.269								
8.	T 8	35	20	71 ± 6.519								
9.	T 9	40	20	78 ± 5.708								
10.	T 10	45	20	83 ± 5.708								
11.	T 11	50	20	87 ± 5.708								

Values are mean ± SD for five replications. *Mortality of the larvae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table: 3. Larvicidal activity of *Steinernema* sp. (Karur) against 4th instar larvae of *Spodoptera litura.*

S.No.	Treatments	Nematodes (IJ)	No. of larva	Mortality (%) Mean±SD	LC_{50}	95% Confidential Limit		LC_{90}	95% Confidential Limit		Regression Equation	Chi Square value
						LCL	UCL		LCL	UCL		
1.	T 1	1	20	4 ± 2.236	5.567	3.491	7.832	12.408	9.532	20.650	X=3.741 Y=3.266	0.008
2.	T 2	5	20	9 ± 4.183								
3.	T 3	10	20	16 ± 6.519								
4.	T 4	15	20	26 ± 6.519								
5.	T 5	20	20	36 ± 6.519								
6.	T 6	25	20	45 ± 7.905								
7.	T 7	30	20	55 ± 7.469								
8.	T 8	35	20	66 ± 6.519								
9.	T 9	40	20	76 ± 4.183								
10.	T 10	45	20	80 ± 7.071								
11.	T 11	50	20	86 ± 6.519								

Values are mean ± SD for five replications. *Mortality of the larvae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table: 4. Larvicidal activity of *Steinernema* sp., (Madathukulam) against 4th instar larvae of *Spodoptera litura.*

S.No.	Treatments	Nematodes (IJ)	No. of larva	Mortality (%) Mean±SD	LC_{50}	95% Confidential Limit		LC_{90}	95% Confidential Limit		Regression equation	Chi Square value
						LCL	UCL		LCL	UCL		
1.	T 1	1	20	3 ± 2.739	4.219	2.996	5.539	7.740	6.241	10.993	X=4.491 Y=3.853	0.845
2.	T 2	5	20	11 ± 4.83								
3.	T 3	10	20	20 ± 5								
4.	T 4	15	20	30 ± 5								
5.	T 5	20	20	41 ± 4.183								
6.	T 6	25	20	54 ± 4.183								
7.	T 7	30	20	58.4 ± 7.765								
8.	T 8	35	20	63 ± 8.367								
9.	T 9	40	20	71 ± 4.183								
10.	T 10	45	20	78 ± 5.708								
11.	T 11	50	20	83 ± 5.708								

Values are mean ± SD for five replications. *Mortality of the larvae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Confidential Limit (LCL) at 9.532 and Upper Confidential Limit (UCL) at 20.650. The regression equation is X = 3.741 and Y = 3.266. The Chi-square value at 0.008 (Table – 3).

DISCUSSION

Entomopathogenic nematodes (EPN's) represent an important part of the spectrum of biopesticides. They have been used to control insect pests of high value crops (the viability higher when used in large scale) in sustainable agricultural systems. Several species of EPNs in the families Steinernematidae (*Steinernema)* and Heterorhabditidae (*Heterorhabditis*) are being produced commercially and used as biological control agents against many soil insect pests and insects in cryptic habitats in many parts of the world. In a total of 20 soil samples, of four areas namely Palladam, Erode, Karur and Madathukulam harboured entomopathogenic nematodes (EPNs), when they were baited with *Galleria mellanella* larvae the honey comb pest which is the host for EPN.

The cadavers of the *G. mellanella* larvae were black in colour (Woodering and kaya 1988). So the EPNs belonged to *Steinernema* sp. Further to confirm the genus, haemolymph of *G.mellanella* larvae infected with all the four area EPNs were streaked in NBTA media after 24 hours of infection. All the colonies of four areas were red in colour and exhibited no bioluminescence so it was further

confirmed that all the four EPNs isolated belonged to *Steinernema sp*. (Akhrust, 1996). Further biochemical tests were done and the bacteria were Gram negative, Motile, Catalase –ve (Akhrust and Boemare, 1988). The nematode after entering through the natural openings left the bacteria into the haemolymph of the pest, which spread in the haemolymph within 48 hours and caused death of the pest due to septicemia. The EPNs were treated for its efficacy to the polyphagus pest *S. litura*.

The pathogenicity of EPNs is considered to be a complex process, which depends upon many, biotic and abiotic factors like host invasion, penetration, reproduction etc. thus different nematode species have been found to differ in their pathogenicity against a specific insect host owing to one or other biotic or abiotic factor. In many studies, the virulence of EPNs has also been found to be dependent on host invasion and penetration ability of the nematodes (Yadav and Lalramliana, 2012). In our study inoculum level of 100 IJs / 20 larvae of *S. litura* has caused death in 24 hours. So this pest is very susceptible to *Steinernema* sp. Isola.ted in this study.

Yadav and Lalramliana (2012) reported that the lowest dose of IJs, which could cause larval mortality at 24 hour post-exposure in *S. thermophilum* was registered to be 50 IJs per larva. When the dose of infective juveniles was increased to 100 IJs per larva, it caused at 100 % mortality in 24 hour exposure time. Park *et al.,* 2001 reported that the *Steinernema* and *Heterorhabditis* sp were examined against tobacco cutworm, *S. litura* fabricius, *H. bacteriphora* showed 100% mortality after 20 hours against 2nd instar of tobacco cutworm. In the case of 3-4th instar, *S. carpocapsae, H. bacteriphora* and *S. monitcola* showed 100% mortality after 47 hours. *S. carpocapsae* showed 100% mortality after 73 hours against 5-6th instar tobacco cutworm.

In the present study, the virulence of *Steinernema* sp. of all the four species isolated from Palladam, Erode, Karur and Madathukulam showed LC50 value to 4th instar S. *litura* larvae to be 4.46, 6.20, 5.56 and 4.21 respectively and LC90 13.80, 10.62, 12.40 and7.74. When compared with earlier studies this report seems to have isolated a very efficient bio control agent i.e, the *Steinernema* sp. All the four *Steinernema* sp isolates of the present study has caused the death of *S. litura* in low inoculums. This shows that the pest *S.litura* to be most susceptible pest to *Steinernema* sp when compared with other lepidopteron pests of earlier studies.

LC_{50} showed that for *Leucinodes orbonalis* was 7.132 nematode the mortality range was 37.5%, 62.5%, 87.5%, 100% and 100%. Treatment T3 to T5 had low susceptibility 37.5% to 62.5% mortality was recorded for *Leucinodes orbonalis*. In the study conducted by Pachareewan *et al.,* 2005 reported *Steinernema siamkayai, S. carpocapsae* and *Steinernema riobrave* the the LC50 value for *Helecoverpa armigera* larvae was 22.5,1.2 and 1.2 IJ was enough to cause mortality at 72 hours of infection and complete mortality was achieved with 200 IJs per larva of 3rdinstar *H. armigera*.

In another study by Yadav and Lalramilana, (2012) who reported at 24 hours *S. glaseri* revealed above 90% mortality of mustard sawfly, *Athalialugens, Proximaklug* has become a serious pest of mustard and radish in several regions of India. Yadav and Lalramilana (2012) reported that the LC_{50} value 30.6 IJs per larva for *H. indica*, followed by *S. thermophilum* and *S. glaseri*, with LC_{50} values of 37.3 and 50.7 IJs per larva. Demonstrated that EPN's can act as potential biocontrol agents against mustard sawfly, *A. lugens, Proxima.* The positive relationship between the dose of infective juveniles and host mortality found in this study has also been recorded in several other studies. These EPN isolates have good potential as biocontrol agents against *mustard* sawfly, *A. lugens, Proxima.* This may be same in the present study also.

Pathogenicity studied by Divya *et al.* (2010) reported in *Hiporabidus indica* against 3 lepidoteran pest *Helicoverpa armigera*, *Spodoptera litura* and *Galleria mellonalla* about greenhouse conditions showed that 2, 3 final instar involve IJs, *H. indica* larva when exposed 24 hours period 2,3 instar larvae of all the 3 lepidoteran was more sustainable 300 IJs of *H. indica* per larvae than 4,5 larvae exposed for 24 hours. EPNs were enough to cause death of five larvae in each replicate.

CONCLUSION

The present study concluded that all the four isolates of EPN's were highly virulent for the pest *S. litura.* These EPN's can be cultured and sprayed as bio pesticide in near future. These are environmental friendly so the farming community can exploit these strains of *Steinernema.* Their virulence can also be tested at field level, which will help us to incorporate this *Steinernema* sp. in IPM programs as biopesticide.

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