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Efficacy of *Steinernema siamkayai* against certain crop pests

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ABSTRACT

The efficacy of dominant *Steinernema siamkayai* Stock, Somsook and Reid isolated from Karaikal region was tested against some of the important crop pests such as *Spodoptera litura, Plutella xylostella, Leucinodes orbonalis, Earais vitella and Cnaphalocrocis medinalis.* The dosage and time mortality relationship of S. *siamkayai* against the third, fifth larval instars and prepupal stage of *S. litura* indicated that as the dosage increased the susceptibility also increased. The relative susceptibility against the above mentioned stages of the same pest indicated that the exposure time increased the susceptibility of the insect. The same trend was registered in respect of *P. xylostella*, *L.orbonalis*, *E. vitella*, and C. *medinalis* both for dosage and time mortality relationship and relative susceptibility for different exposure periods.

Key words: EPN, *Steinernema siamkayai, Spodoptera litura, Plutella xylostella, Leucinodes orbonalis, Earias vitella, Cnaphalocrocis medinalis*

INTRODUCTION

Increased awareness over the hazards caused by the continuous and indiscriminate use of pesticides forced us to find safe and eco friendly means of insect pest management. One such way is to use entomopathogenic nematodes (EPN). EPN are potential biocontrol agents and pathogenic to wide range of insect pests (Hussaini, 2002). They have the ability to search for the hosts and relatively safe to non target organisms (Gaugler, 1981) and verte brates (Bathon,1996). *Steinernema* spp could provide high levels of control.(Mc.Graw and Koppenhofer, 2008).
Entomopathogenic nematode was used against many insect pests *viz.,* rice leaf folder, *C. medinalis* (Srinivas and Prasad, 1991); tobacco cutworm, *S. litura* (Rajkumar *et al.,* 2003); brinjal fruit borer, *L. orbonalis* (Hussaini *et al.,* 2002) diamond back moth, *P.xylostella* (Singh and Shinde, 2002); *Diaprepes abbreviatus* (Jenkins *et al*. 2007); *Bemesia tabaci* (Qiu Baoli *et al*. (2008) and sugar beet beetle (Saleh *et al*. 2009). Husaini *et al*. (2002) recorded cent per cent mortality of third instar *L. orbonalis* larvae @ 25 IJs / larva within 72 h exposure with *S. bicornutum*. The final instar larvae of *P.xylostella* were most susceptible to *H. bacteriphora* amongst different stages of insect with a LC_{50} value of 9.16 IJs/larva (Singh and Shinde, 2002).Among eight entomopathogenic nematode strains tested against final instar larvae of *P.xylostella, H.bacteriphora* was adjudged the most pathogenic on the basis of LT_{50} and LD_{50} (Shinde and Singh, 2000). Karunakar *et al.* (1999) reported that *S. litura* was highly susceptible to all the three species of entomopathogenic nematode species *viz., S. feltiae S. glaseri and H. bacterphora*. Josephrajkumar and Sivakumar (1997) reported that the native sp could be used as a soil drench against *S.litura* pupae. Morris (1985) recorded 66.6 per cent mortality of diamond back moth pupa by *S. feltiae* at a dose of 125 IJs / pupa.
Hence, to suit various agro-climatic regions of India, native isolates of EPN have to be explored, tested against crop pests and mass multiplied. One such attempt has been made in which the native isolates *viz., S. siamkayai* from Karaikal coastal ecosystem of Union Territory of Puducherry, tested for its virulence against various crop pests *viz., S.litura, P.xylostella, E. vitella, C. medinalis, and L. orbonalis.*

MATERIALS AND METHODS

Mass-multiplication of nematodes

S. siamkayai was mass multiplied *in*-vivo on *Corcyra cephalonica* by exposure method. Whatman No.1 filter paper moistened with 1 ml of *S. siamkayai* suspension in a petri plate. To this 10 C*orcyra* larvae were added. The infected larvae were placed on White trap. The emerging infective juveniles (IJs) were harvested daily until the production stopts (Woodring and Kaya, 1988).

Mass culturing of test insects

Following the standard protocol, insects like *P. xylostella* (Sairabanu, 2000) L. *orbonalis* (Patil, 1990), *E. vitella* (Gautam,1994), *C. medinalis* (Heinrichs *et al.,* 1985) and *S. litura* (Manoharan and Adiroubane, 2005) were mass multiplied and tested for the bioefficacy of EPN, *S. siamkayai*.

Efficacy of entomopathogenic nematode against crop pests

Dose-mortality relationship and time mortality tests were conducted in 9 cm diameter petri dishes lined at the bottom with a sheet of filter paper, moistened with 1 ml sterile distilled water. The dosages used were 0, 5, 10, 20, 30, 40 and 50 IJs / larva and controls were treated with distilled water only. The required concentrations are prepared by serial dilution of the stock solution containing 10,000 IJs/ml. Infective juveniles are evenly applied over the filter paper at the rate of 5 ml per filter paper. After 30 minutes, five final instar larvae of each insect species were placed in each Petri dish. Each treatment was replicated eight times and totally 40 insects were used per treatment. In case of *S. litura*, third, final instar and prepupa were tested for their mortality. Similar procedure was followed for other insects namely, *P. xylostella*, *L. orbonalis*, *E. vittella* and *C. medinalis*. Only final instar larvae were used to determine mortality.

The Petri dishes were kept in a polythene bag to avoid water loss and were placed at room temperature. The insect mortality data were recorded from 12 hours after inoculation, at an interval of 12 hours up to 48 hours (Woodring and Kaya, 1988). A control was maintained with distilled water.

STATISTICAL ANALYSIS

Estimation of LD_{50} is a relative measure of susceptibility of a host population and is convenient and commonly used index of relative efficacy (Epsky and Capinera, 1994). The LD_{50} and LT_{50} values were calculated for *S. siamkayai* using SPSS statistical package.

RESULTS AND DISCUSSION

Efficacy of *S. siamkayai* against *S. litura*

In the experiment with *S. siamkayai* against third and fifth instar larvae of *S. litura*, at lower dose (10 IJs / larva), the LT_{50} value was very much higher (Table 1) and the LT_{50} decreased with increase in dosage levels. The LT_{50} values for third instar *S. litura* larva at different doses *viz.*, 10, 20, 30, 40, and 50 IJs/larva were 71.64, 53.64, 43.78, 33.55 and 26.11 hours, respectively. No mortality of *S. litura* was observed at the dosage 5 IJs / larva. At 10 IJs/larva, the LT_{50} value was higher and the susceptibility of the larva was increased as the dosage increased.

Similar trend was also observed in respect of fifth instar larva where, the LT_{50} values at the different doses (10,20,30, 40 and 50 IJs/larva) were 74.95, 57.24, 46.03, 32.48 and 25.48 hours. It was found that the LT_{50} values decreased with increase in dosage levels of *S. siamkayai* (Table1). The LD_{50} for third instar *S. litura* larva was calculated at 12, 24, 36 and 48 hours of exposure based on the Probit

Table 1. Dosage and time mortality relationship (LT_{50}) of *Steinernema siamkayai* against different stages of *Spodoptera litura*

Life Stages	Doses (IJs/larva)	Regression equation	Chi square	LT_{50} (hrs)	Fiducial limit	
					LL	UL
Third instar	5 IJ	-	-	-	-	-
	10 IJ	Y=-6.4154+3.4776x	0.451	71.64	53.64	203.89
	20 IJ	Y=-5.8380+3.3755x	1.220	53.64	44.02	82.70
	30 IJ	Y=-5.4109+3.2976x	3.427	43.78	37.14	57.69
	40 IJ	Y=-6.7518+4.4255x	3.151	33.55	29.73	38.21
	50 IJ	Y=-7.7093+5.4412x	5.429	26.11	0.09	48.76
Fifth instar	5 IJ	-	-	-	-	-
	10 IJ	Y=-6.0867+3.2467x	1.301	74.95	54.67	246.85
	20 IJ	Y=-5.3472+3.0422x	1.878	57.24	45.50	98.88
	30 IJ	Y=-5.4168+3.2572x	2.974	46.03	38.71	62.80
	40 IJ	Y=-6.4950+4.2970x	3.242	32.48	28.66	36.97
	50 IJ	Y=-9.9811+7.0982x	4.504	25.48	3.15	37.60
Prepupa	5 IJ	Y=-6.4911+3.6309x	0.479	61.34	48.93	113.91
	10 IJ	Y=-4.6315+2.7305x	0.219	49.68	40.11	76.02
	20 IJ	Y=-3.4730+2.1935x	0.135	38.30	30.79	55.07
	30 IJ	Y=-3.3307+2.3318x	0.424	26.81	21.46	33.33
	40 IJ	Y=-3.7554+2.9024x	5.230	19.67	14.26	24.33
	50 IJ	Y=-3.3048+2.9492x	4.153	13.20	9.35	19.56

Table 2. Relative susceptibility of different stages of *Spodoptera litura* to *Steinernema siamkayai* at different exposure period

Life Stages	Exposure time (hrs.)	Regression equation	Chi square	LD_{50}	Fiducial limit	
					LL	UL
Third instar	12	-	-	-	-	-
	24	Y=-4.4313+2.6115x	1.310	49.76	40.76	69.23
	36	Y=-5.8380+3.3755x	4.302	34.59	28.76	43.87
	48	Y=-5.4109+3.2976x	9.992	22.93	15.33	33.21
Fifth instar	12	-	-	-	-	-
	24	Y=-4.1981+2.4447x	0.744	52.15	41.93	75.85
	36	Y=-3.6921+2.4604x	7.847	31.67	22.41	53.09
	48	Y=-4.3560+3.1888x	16.910	23.23	12.99	38.48
Prepupa	12	Y=-4.3885+2.5133x	1.393	55.73	44.44	83.89
	24	Y=-2.7545+1.8070x	1.807	33.45	26.74	45.11
	36	Y=-2.0095+1.6443x	3.924	16.68	12.71	21.14
	48	Y=-2.1390+2.0957x	12.226	10.49	3.38	17.28

analysis (Table 2). No mortality was observed at 12 hours after treatment even at the highest dose (50 IJ/larva). The LD_{50} values after 24, 36 and 48 hours of exposure were 49.76, 34.59 and 22.93 (IJs/larva) respectively. The LD_{50} values decreased when exposure period was increased (Table 2).

The LD_{50} values for fifth instar *S. litura* larvae at different time intervals (24, 36, 48 hours) were 52.15, 31.67 and 23.23

Table 3. Dosage and mortality relationship (LT_{50}) of *S. siamkayai* against different larvae

Pests	Doses (IJs/larva)	Regression equation	Chi χ^2	LT_{50} (hrs.)	Fiducial limit	
					LL	UL
Plutella xylostella	5	Y=-5.5436+3.2017x	1.693	53.87	43.83	84.87
	10	Y=-3.2636+1.9514x	0.061	47.04	35.96	86.40
	20	Y=-3.0858+2.1376x	1.241	27.78	21.91	35.57
	30	Y=-3.6433+2.9415x	3.777	17.32	13.34	20.71
	40	Y=-3.7896+3.3005x	6.233	14.07	10.62	18.99
	50	Y=-3.7476+3.6025x	5.316	10.97	6.58	14.06
Leucinodes orbonalis	5	Y=-5.3577+2.9263x	1.247	67.74	50.80	159.16
	10	Y=-4.2957+2.4504x	0.032	56.62	43.56	105.09
	20	Y=-3.1543+1.8772x	0.053	47.90	36.19	93.50
	30	Y=-3.1512+2.0480x	0.052	34.57	27.50	48.69
	40	Y=-3.2382+2.3174x	0.527	24.97	19.69	30.82
	50	Y=-4.2437+3.3311x	3.754	18.79	15.26	21.97
Earias vitella	5	Y=-5.5870+2.8823x	0.660	86.77	58.54	506.68
	10	Y=-5.5326+3.1787x	1.702	55.02	44.48	88.99
	20	Y=-2.5250+1.5099x	0.283	47.02	33.79	124.37
	30	Y=-3.1204+2.4005x	0.015	19.95	14.92	24.38
	40	Y=-3.6009+2.9909x	3.243	15.99	12.06	19.23
	50	Y=-3.8406+3.3999x	3.090	13.48	9.99	16.25
Cnaphalocrocis medinalis	5	-	-	-	-	-
	10	Y=-7.1806+3.9602x	0.124	65.04	51.29	139.51
	20	Y=-5.9967+3.3918x	0.881	58.62	47.01	101.21
	30	Y=-6.1174+3.6367x	1.180	48.10	40.77	65.55
	40	Y=-6.1467+3.9084x	2.63	37.38	32.75	44.29
	50	Y=-4.2976+2.9915x	1.903	27.33	22.99	32.41

(IJs/larva), respectively. No mortality was observed 12 hrs after treatment as in the third instar larval treatment. It was found that, LD_{50} was observed to be decreased as the exposure time increased (Table 2). At 24-hour exposure period, the LD_{50} value was high and the LD_{50} was decreased with increasing period of exposure time. These findings were in accordance with those of Kondo and Ishibashi (1986) and, Ricci *et al*. (1995) in *S. litura*.

Gupta *et al*. (1987) reported 66 per cent cutworm, *S. litura* mortality in tobacco. Vyas and Yadav (1992) found that, in a laboratory bioassay of *S. glaseri* against *Agrotis ipsilon* and *S. litura,* cent per cent larval mortality at 16 and 32 IJs / g of soil after 72 h of exposure period respectively. Baweja and Sehgal (1997) also observed 80 per cent mortality of *S. litura* due to *Heterorhabditis bacteriophora.* Similar results were also reported by Rajkumar *et al*. (2003). The LD_{50} values for prepupa of *S. litura* at different exposure periods (12, 24, 36 and 48 hours) were 55.73, 33.44, 16.68 and 10.49 (IJs/larva). As the exposure period increased the LD_{50} was decreased and thus susceptibility of the prepupa increased at the exposure period of 48 hours (Table 2).

It was found that the LT_{50} values for prepupa of *S. litura* at the different doses (5, 10, 20, 30, 40, 50 IJs / larva) were 61.34, 49.68, 38.30, 26.81, 19.67 and 13.20 hours, respectively. At lower dose (5 IJs) / larva), the LT_{50} value was high and as the dosage increased (50 IJs / prepupa) the LT_{50} value decreased, as the susceptibility of the prepupa was increased (Table 1). Kaya and Hara (1981) observed 63 per cent mortality of *S. exigua* pupae caused by *Neoplectana carpocapsae*. Fuxa *et al.* (1988) have observed 7-20 per cent mortality of *S. frugiperda* pupae with 30 to 60 IJs/0.7ml/pupa.

Efficacy of *S. siamkayai* against *P. xylostella*

It was found that at lower dose of 5 IJs/ larva, the LT_{50} value was high (53.87 hrs) and it decreased with increase in dosage (Table 3). At 12 hours exposure period, the LD_{50} value was high (40.11 IJs) and LD_{50} decreased with increase in exposure period (Table 4). Morris (1985) reported that LD_{50} as low as 1.30 IJs / larva of *P. xylostella;* 9.16 IJs (Shinde and Singh, 2000); 9.5 IJs / larva (Singh and Shinde, 2002) of *P. xylostella*. Susceptibility of *P. xylostella* to EPN was also reported by Ratnasinghe and Hague (1997).

The LT_{50} values for *P. xylostella* at the different doses *viz*., 5, 10, 20, 30, 40 and 50 IJ/larva were 53.87, 47.04, 27.78, 17.32, 14.07 and 10.97 hours respectively. At lower dose (5 IJ/larva) the LT_{50} value was higher. The susceptibility of the larva increased as the dosage (50 IJs/larva) increased (Table 3). At 12 hours after treatment, the LD_{50} value was high and the susceptibility of the larva was increased when exposure period was increased. While the LD_{50} values at 12, 24, 36 and 48 h were 40.11, 22.66, 10.85 and 8.08 (IJs/ larva) respectively. It was found that at lower dose (5 IJs/ larva), the LT_{50} value (86.77 hours) was high and it decreased with increase in dosage (Table 3). At 12 hours exposure period, the LD_{50} value was high (40.11 IJs) and LD_{50} decreased with increase in exposure period (Table 4). This result was in corroboration with the reports of earlier workers and it was reported that LD_{50} values was 1.3 nematodes (Morris, 1985), 9.16 IJs (Shinde and Singh, 2000) and 9.5 IJs / larva (Singh and Shinde, 2002) of *P. xylostella*.

Efficacy of *S. siamkayai* against *L. orbonalis*

At lower dose (5 IJs/ larva), the LT_{50} value (67.74 hours) was higher. The LT_{50} values decreased with increase in dosage (Table 3). The LD_{50} value at 12 hrs exposure period was 93.85 IJs and it decreased to 13.3 IJs at 48 hrs after exposure (Table 4). The median lethal dosages (LD_{50}) were assessed at 12, 24, 36 and 48 hours of exposure periods and the LD_{50} values were 93.85, 45.90, 21.73 and 13.31 (IJs/ larva) respectively. As the exposure period increases, the LD_{50} value gets decreased and the susceptibility of larva increased (Table 4). Hussaini *et al*. (2002) obtained cent per cent mortality of *L. orbonalis* with *S. carpocapsae* with 25 IJs / larva but only after 96 hrs and 50 per cent mortality with 200IJs / larva by Pramila Gupta (2003). *L. orbonalis* larva was susceptible to *S. siamkayai* in the experiment conducted to find the efficacy of *S. siamkayai* and it was concluded that at lower dose (5 IJs/ larva), the LT_{50} value (67.74 hours) was higher. The LT_{50} values decreased with increase in dosage (Table 3). At 12 hours exposure period, the LD_{50} value was high (93.85 IJ) and the LD_{50} was decreased with increase in exposure period (Table 4). This is in accordance with the results obtained by the earlier workers and cent per cent mortality obtained against 50 IJs / larva (Hussaini *et al.*, 2002) and 50 per cent mortality against 200 IJs / larva (Pramila Gupta, 2003) was recorded in *L. orbonalis*.

Efficacy of *S. siamkayai* against *E. vitella*

The LT_{50} values for *E. vitella* were 86.77, 55.02, 47.02, 19.95, 15.99 and 13.48 hours at different doses. The susceptibility of the larva was higher as the dosage increased and it was indicated with a decrease in the LT_{50} values (Table 3). The LD_{50} values for *E. vittella* at 12, 24, 36 and 48 hours of exposure periods were 48.36, 27.32, 17.54 and 12.62 (IJs/ larva). However, lower LD_{50} value was obtained at higher exposure period and the higher susceptibility was obtained at higher exposure period and hence the susceptibility was

Table 4. Relative susceptibility of different larvae to *S. siamkayai* at different exposure period

Pests	Exposure time (hrs.)	Regression equation	Chi^2	LD_{50}	Fiducial limit	
					LL	UL
Plutella xylostella	12	Y=-3.6388+2.2696x	1.771	40.11	32.99	52.98
	24	Y=-2.2609+1.6682x	3.302	22.66	17.86	29.17
	36	Y=-2.3650+2.2840x	8.157	10.85	5.72	15.88
	48	Y=-2.3043+2.5390x	10.311	8.08	3.33	12.38
Leucinodes orbonalis	12	Y=-3.5990+1.8246x	0.085	93.85	61.10	275.84
	24	Y=-2.1916+1.3188x	1.039	45.90	32.94	83.86
	36	Y=-1.1620+1.2116x	4.606	21.73	15.65	30.94
	48	Y=-1.8809+1.6732x	10.933	13.31	3.87	23.70
Earias vitella	12	Y=-4.7709+2.8323x	2.817	48.36	40.30	65.38
	24	Y=-3.0594+2.1297x	2.222	27.32	22.60	33.82
	36	Y=-3.1017+2.4932x	4.584	17.54	14.60	20.73
	48	Y=-2.7718+2.5174x	13.951	12.62	5.64	20.12
Cnaphalocrocis medinalis	12	Y=-6.3245+2.9264x	7.086	144.94	120.23	160.89
	24	Y=-3.9450+2.1208x	0.901	72.46	52.64	144.11
	36	Y=-3.5219+2.1702x	3.525	41.96	34.08	57.10
	48	Y=-3.4933+2.4583x	7.254	26.37	18.64	39.22

increased along with the exposure period (Table 4). Susceptibility of *E. vitella* to *S. siamkayai* was observed in the petridish assay method and it was concluded that at lower dose the LT_{50} value was higher and it was decreased with increase in dosage. At 12 hours exposure period, the LD_{50} value was high (48.36 IJ) and LD_{50} was decreased with increase in exposure period. This result was in conformity with the results of Pramila Gupta (2003), when S.*carpocapsae* was applied as food dip at the rate of 5-100 IJs / larva.

Efficacy of *S. siamkayai* against *C. medinalis*

It was found that the LT_{50} values for *C. medinalis* at different doses were 65.04, 58.62, 48.10, 37.38 and 27.33 hours, while at the lowest dose no mortality of larva was observed. But as the dose increased, the LT_{50} value was decreased, indicating the susceptibility of larva to the increased dosage (Table 3). The LD_{50} value at various exposure periods were 144.94, 72.46, 41.96 and 26.37 (IJs/larva). It was observed that as the exposure period increased, the nematode dose required to kill 50 per cent population was decreased, as the susceptibility of larva was high at higher exposure period (Table 4).Susceptibility of *C. medinalis* was observed only at and above 10 IJs/larva. The LT_{50} values started decreasing with increase in dosage. At 12 hours exposure period, the LD_{50} value was high and LD_{50} started decreasing with increase in exposure period.

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