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Evaluation of efficacy of *Steinernema feltiae* and isolated native strain *S. kushidai* against *Cnaphalocrosis medinalis* Guenee

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ABSTRACT : The present study was carried out to compare the efficacy of *Steinernema feltiae* and native entomopathogenic nematode, *S. kushidai* against the rice leaf folder, *C. medinalis* larva at laboratory conditions. Indigenous entomopathogenic nematode was isolated from soil sample collected from Agricultural College and Research Institute, Killikulam and it was identified as *S. kushidai* KKM strain based on its morphometric measurements. Final instar larvae of *C. medinalis* were exposed to infective juveniles (IJs) of *S. feltiae* and *S. kushidai* under laboratory conditions. LC_{50} values of *S. feltiae* on *C. medinalis* larva were 59.45, 39.42 and 16.04 IJs per larva for the exposure periods of 24, 48, and 72h, respectively. LC_{50} values of *S. kushidai* KKM strain were 58.82, 38.11 and 16.67 IJs per ml at 24, 48 and 72 h exposure periods respectively. The LT_{50} values for *C. medinalis* larva at different doses *viz.*, 20, 40, 60, 80, and 100 IJs/larva were 66.04, 49.11, 34.68, 32.14 and 24.21 h, respectively. Whereas in case of *S. kushidai* the LT_{50} values were 45.42, 41.89, 33.79, 28.33, and 22.30 hours at 20, 40, 60, 80, and 100 IJs per larva, respectively.

Key Words: Efficacy, *Steinernema feltiae, S. kushidai*, rice leaf folder, *Cnaphalocrosis medinalis*, rice.

Paddy leaffolder is one of the most important insect pests in Indian subcontinent (Gunathilagaraj and Gopalan, 1986). Out of the eight species of leaffolder, the most widespread and important one is *C. medinalis* (Guenee) (Bhatti, 1995). *C. medinalis* has been reported to attain the major pest status in some important paddy growing areas of India (Murugesan and Chelliah, 1987). Loss incurred to the growing paddy crop by leaffolder larva is insurmountable (Ahmed *et al*., 2010). Bhanuand Reddy (2008) reported that in favourable conditions, leaf folder caused severe loss in rice; loss may extend upto 63 to 80 per cent depending on agro-ecological situations as reported by Rajendran *et al*. (1986). Conventional chemical pesticides are used to protect rice against this pest; however, their use has caused concerns for food safety and environmental pollution (Kumar *et al.,* 2011; Suganthi *et al.,* 2011). Moreover, due to their frequent immigration and long incubation period, it is difficult to forecast the occurrence of rice leaf folder and effectively prevent its damage. Hence, eco-friendly biocontrol agent like entomopathogenic nematodes (EPNs) shall be employed as potential biopesticides against this important foliage pest in rice.

Materials and Methods

Mass culturing of *C. medinalis*

In the screen house at the Department of Plant Protection, Agricultural College and Research Institute, Killikulam, the rice leaffolder *C. medinalis* was mass cultured on TN-1 rice seedlings as per the standard protocol described by Heinrichs *et al.* (1985).

EPN cultures

Host insect *Corcyra cephalonica* Staint.

In the bio-control laboratory at Agricultural College and Research Institute, Killikulam, the rice moth *Corcyra cephalonica* Staint. was mass multiplied on half broken pearl millet grains by following the standard procedure given by Manjunath (1988). *C. cephalonica* larva was used as host insect for mass multiplication of EPNs.

Mass culturing of EPNs

The mother culture of *S. feltiae* in distilled water was obtained from Nematology Department, TNAU, Coimbatore. The IJs were mass multiplied on grown up larvae of *C. cephalonica* in Petri dishes in laboratory condition. Ten grown up larvae were taken in 10 cm diameter Petri dish, which was lined inside with Whatman No. 41 filter paper already inoculated with *S. feltiae* from the mother culture @ 10 IJs/larva. The dead larvae two days after inoculation were collected, washed with sterile water and kept at room temperature for further multiplication of the nematode. On fifth day the multiplied IJs were recovered by White's technique (White, 1927). Harvested IJs were stored in 0.1 per cent formalin in tissue culture flasks. The cultures were aerated once in a week and re-culture were done once in 15 days in grown up *C. cephalonica* larva.

Dosage and mortality relationship (LC_{50}) of KKM strain and *S. feltiae* against Rice leaf folder C. *medinalis*

Bioassays were conducted in 10 cm diameter Petri dishes lined inside at the bottom with a sheet of filter paper. Infective juveniles of respective EPNs were evenly applied over the filter paper at the rate of one ml per filter paper. The number of IJs was counted with the nematode counting chamber (8x8x1.5 cm) after placing the nematode suspension under Stereo Binocular Microscope. The dosages used were 20, 40, 60, 80, and 100 IJs per larva and controls were treated with distilled water only. The required concentrations were prepared by diluting or concentrating the nematode suspension with distilled water. After 5 minutes, 10 final instar larvae of leaf folder were placed in each Petri dish. Each treatment was replicated four times and totally 40 leaf folder larvae were used per treatment. The Petri dishes were kept in a polythene bag to

Table-1 : Dosage and mortality relationship (LC_{50}) of *S. feltiae* against *C. medinalis* larva.

S. No.	Exposure time period	Regression equation	Chi-square value (X^2)	LC_{50}	Fiducial Limits	
					LL	UL
1.	24 h	Y=2.52+1.14x	1.22	59.45	38.99	90.63
2.	48 h	Y=2.55+1.54x	1.85	39.42	25.86	60.09
3.	72 h	Y=3.13+1.55x	1.61	16.04	6.72	38.28

Table-2 : Dosage and mortality relationship (LC_{50}) of *S. kushidai* KKM strain against *C. medinalis* larva.

S. No.	Exposure time period	Regression equation	Chi-square value (X^2)	LC_{50}	Fiducial Limits	
					LL	UL
1.	24 h	Y=2.28+1.53x	1.90	58.82	40.06	86.36
2.	48 h	Y=2.52+1.77x	0.54	38.11	24.92	58.29
3.	72 h	Y=2.93+1.70x	1.77	16.67	7.67	36.20

Table-3 : Dosage and time mortality relationship (LT_{50}) of *S. feltiae* against *C. medinalis* larva.

S. No.	Doses	Regression equation	Chi-square value (X^2)	LT_{50}	Fiducial Limits	
					LL	UL
1.	20IJs / larva	Y=1.454+1.948x	1.685	66.04	28.811	151.392
2.	40IJs / larva	Y=1.315+2.178x	0.697	49.11	28.732	83.952
3.	60IJs / larva	Y=0.540+2.895x	4.649	34.68	26.40	45.547
4.	80IJs / larva	Y=0.679+2.866x	3.195	32.14	24.94	41.41
6.	100IJs / larva	Y+0.147+3.506x	1.435	24.21	20.187	29.039

Table-4 : Dosage and time mortality relationship (LT_{50}) of *S. kushidai* KKM strain against *C. medinalis* larva.

S. No	Doses	Regression equation	Chi-square value (X^2)	LT_{50} (hrs)	Fiducial Limits	
					LL	UL
1.	20IJs / larva	Y=1.19+2.3422x	0. 610	45.42	28.802	71.623
2.	40IJs / larva	Y=1.363+2.24x	0.512	41.89	27.272	64.330
3.	60IJs / larva	Y=0.992+2.62x	1.294	33.79	25.287	45.159
4.	80IJs / larva	Y=0.951+2.787x	2.044	28.33	22.351	35.897
6.	100IJs / larva	Y=0.294+3.494x	2.819	22.20	18.483	26.66

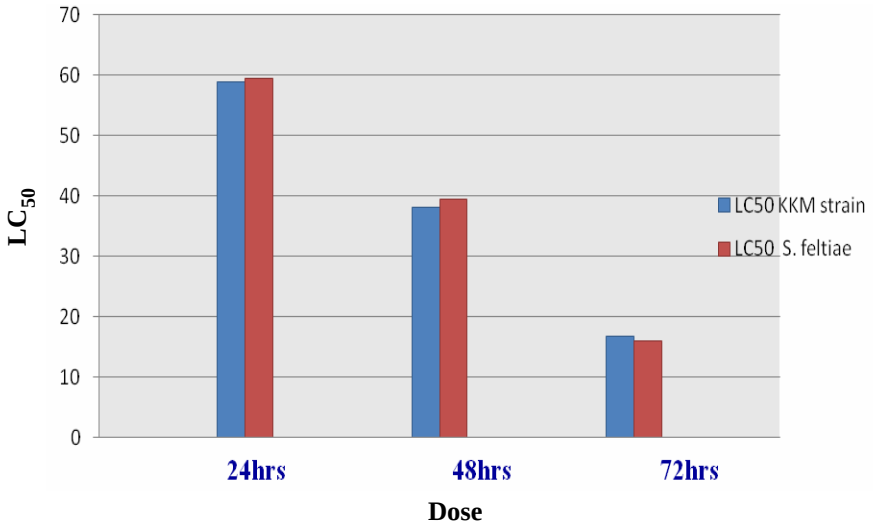

Fig-1 : Dosage and mortality relationship (LC50) of *S. feltiae* and *S. kushidai* KKM strain against *C. medinalis* larva.

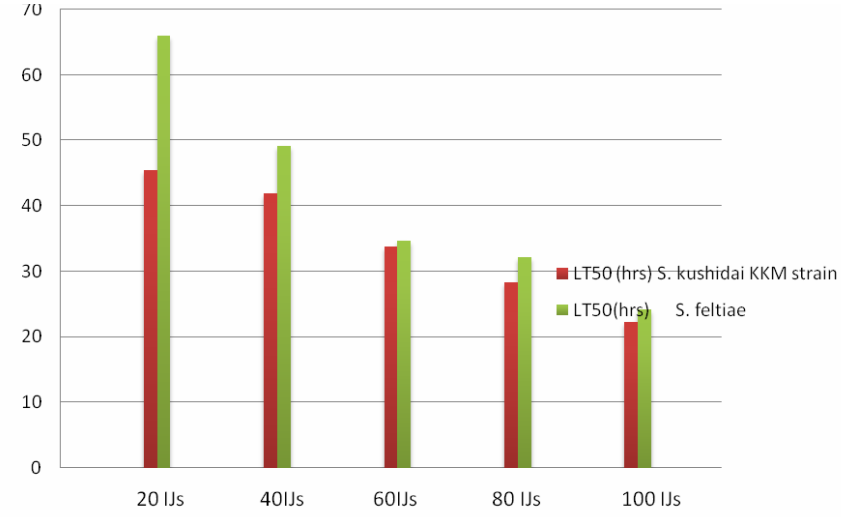

Fig.-2 : Dosage and time mortality relationship (LT50) of S. feltiae and S. kushidai KKM strain against C. medinalis larva.

avoid water loss and were placed at room temperature.

Larval mortality data were recorded in all the treatments at 24, 48 and 72 h after nematode inoculation. Finney's method of Probit analysis was followed to derive the LC50.

Median Lethal Time (LT_{50}) of KKM strain and *S. feltiae* against *C. medinalis* larva

The same methodology as described in the chapter 3.3.1 was applied for this experiment, but the larval mortality in each dosage tested was observed at eight hour interval till it reaches 75 per cent level.

Statistical Analysis

In the laboratory experiments those obtained mortality in untreated control Abbott's corrected per cent mortality worked out (Abbott, 1925).

Abbott's corrected mortality (%) =

$$\frac{\text{Mortality } (\%) \text{ in T } - \text{ mortality } (\%) \text{ in C}}{100 \ - \text{ mortality } (\%) \text{ in C}} \times 100$$

Where, T = Treatment, C= Untreated control.

LC_{50} and LT_{50} was computed by Finney's method of probit analysis (Finney, 1971).

Results and Discussion

Dosage and mortality relationship (LC_{50}) of *S. feltiae* and *S. kushidai* KKM strain against *C. medinalis*

In these experiments, there was decrease in LC_{50} values as the exposure period increased from 24 to 72 h. The LC_{50} value of *S. feltiae* on *C. medinalis* larva at 24 h exposure period was 59.45 IJs per larva and it decreased by 33.69 per cent at 48 h and by 73.02 per cent at 72 h (Fig.-1 and Table-1).

LC_{50} values were computed for *S. kushidai*at three different exposure periods *viz*., 24, 48 and 72 h after inoculation. As the exposure period increased from 24 to 72 h, there was decrease in LC_{50} values. The LC_{50} value of *S. kushidai* KKM strain for *C. medinalis* at 24 h exposure period was 58.82 IJs per larva and it decreased by 35.21 and 71.66 per cent levels at 48 and 72 h respectively (Fig.-1 & Table-2).

LC_{50} values of the isolated *S. kushidai* KKM strain at 24 and 48 h after treatment were slightly lower than those values obtained for *S. feltiae* during the same exposure periods. This implies that indigenous S. *kushidai* KKM strain is slightly more virulent than *S. feltiae* against *C. medinalis* larva. In both EPNs, LC_{50} value at 24 h of exposure time period was much higher than that of 48 h after exposure and 72h after treatment recorded the lowest median lethal dosage. Remarkable reduction in LC_{50} values were obtained for every 12h increase in exposure time. This indicated the enhancement in the susceptibility of *C. medinalis*to the two EPNs tested with increase in time period.

This general trend was also reported by Adiroubane *et al.* (2010) for *S. siamkai* against the larvae of *C. medinalis, Plutella xylostella, Leucinodes orbanalis* and *Earias vitella*

Dosage and time mortality relationship (LT_{50}) of *S. kushidai* KKM strain and *S. feltiae* against *C. medinalis* larva

In the experiment with *S. feltiae* against larvae of *C. medinalis*, at lower dose (10 IJs per larva), the LT_{50} value was very much higher and the LT_{50} decreased with increase in dosage levels. The LT_{50} values for *C. medinalis* larva at different doses *viz*., 20, 40, 60, 80, and 100 IJs per larva were 66.04, 49.11, 34.68, 32.14 and 24.21 h, respectively. At 20 IJs per larva, the LT_{50} value was higher and the susceptibility of the larva was increased as the dosage increased (Fig.-2 and Table-3). The LT_{50} values of *S. kushidai*KKM strain for *C. medinalis* larva were 45.42, 41.89, 33.79, 28.33, and 22.30 h at 20, 40, 60, 80, and 100 (IJs per larva), respectively. The susceptibility of the larva was higher as the dosage increased and it was indicated with a decrease in the LT_{50} values (Fig.-2 and Table-4).

In the present investigation, LT_{50} values of *S. kushidai* KKM strain were relatively lower with those of *S. feltiae* when tested against *C. medinalis* larva. At the dosage level of 20 IJs per larva, the median lethal time of *S. kushidai* KKM strain decreased by 31.22 per cent over *S. feltiae*. For both EPN species, LT_{50}values decreased with increase in dosage and this corroborates with the results published by Adiroubane *et al.* (2010) for *S. siamkayai* against *C. medinalis*. Median lethal time also varies with nematode species and this was also in agreement with Padmakumari *et al.* (2008) who reported that lethal time of 19.8 h for *H. indica* on *C. medinalis* larva and 37.8 h for *S.*

asiaticum in a separate bioassay study. *S. carpocapsae, S. glaseri, S. thermophilum,* and *H. indica* recorded LT_{50} of 24, 36, 42 and 36 h, respectively against *C. medinalis* larva. (Divya and Sankar, 2009).

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