



Original Research Article

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Efficacy of *Steinernema feltiae* Filipjev, Native Entomopathogenic Nematode *Kushidai* Mamiya and their Bacterial Symbionts against *Cnaphalocrocis medinalis* Guenée

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The current study highlights the importance of entomopathogenic nematode (EPN), their bacterial symbionts and their combined efficacy along with *Bacillus thuringiensis* (*Bt*) in *C. medinalis* management. In the field experiment conducted at Thenthiruperai, *Xenorhabdus bovienii+Bt* and *X. japonica* combined with *Bt* found to be more effective with 80.54 per cent and 79.37 per cent reduction in larval population over untreated control respectively. *X. japonica* combined with *Bt* and *X. bovienii* combined with *Bt*, were more effective with 69.38 per cent and 67.73 per cent reduction in leaf damage over control respectively. The native EPN *S. kushidai* showed 47.51 per cent reduction in leaf damage and it was 44.32 per cent in *S. feltiae*. In another experiment conducted at Anandanambikurichi revealed that *X. bovienii+Bt* and the *X. japonica+Bt* proved superior efficacy with 72.59 and 69.33 per cent reduction in larval population over control respectively. *S. kushidai* KKM strain was better than *S. feltiae* as it could bring 55.77 per cent reduction in the larval population of *C. medinalis*. *X. japonica+Bt* and *X. bovienii+Bt* were found to be more effective with 71.23 per cent and 68.53 per cent reduction in leaf damage over untreated control respectively.

Introduction

Insect pests are important constrain in rice cultivation over the centuries; occurrence of pest outbreaks have increased with the change of pest status (Han *et al.*, 2016; Ahmed *et al.*, 2010). For instance, formerly considered as minor pest, leaffolder *C. medinalis* appears to be more destructive and widespread insect pest throughout the rice growing regions in South and South-East Asia (Dale, 1986; Rao *et al.*, 2010; Park *et al.*, 2014). In particular, heavy use of the insecticides and fertilizer seems to favour the *C. medinalis* population outbreaks. *Cnaphalocrocis medinalis* also

induces rice sheath rot, difficulty in heading, and massive yield loss at the reproductive stage (Xiao, 1990). Although conventional chemical pesticides are used to protect rice against this pest; 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 leaffolder and effectively prevent its damage. Hence eco-friendly biocontrol agents like entomopathogenic nematodes (EPNs) and

their bacterial symbionts shall be employed as potential biopesticides against this important foliage pest in rice.

Naturally occurring entomopathogenic nematodes and their symbiotic bacteria are important biotic factor in suppression of insect pest populations in soil and cryptic habitats. The virulent species of these nematodes are commercially produced as biological control agents all over the world (Divya and Sankar, 2009).

The infective juveniles (IJs) of EPNs are microscopic organisms having 0.5 to 1.5 mm long depending on species. EPNs enter through the insect's natural body openings, the mouth, anus or respiratory inlets (spiracles) and then penetrate into the blood cavity from the gut (Divya and Sankar, 2009); *Heterorhabditis* species can also penetrate through chinks in the insect's armour (the inter skeletal membranes) by scratching away with a special tooth (Bedding and Molyneux, 1982). Once in the insect's blood, infective juvenile releases highly specialized symbiotic bacteria such as *Xenorhabdus spp.* in *Steinernema*, and *Photorhabdus spp.* in *Heterorhabditis* (Muthulakshmi *et al.*, 2011). These symbiotic bacteria multiply and rapidly kill the insect within a day or two. The bacteria then convert the insect into suitable food for the nematodes and produce a range of antibiotics (Uma *et al.*, 2010) and anti-feedants that preserve the dead insect from putrefaction while the nematodes feed and reproduce in it.

Earlier EPNs were mostly known as antagonists of soil pests, but in recent years many investigations have demonstrated that they can also be effectively used against foliar pests in various ecosystems including rice (Srinivas and Prasad, 1991; Arthurs *et al.*, 2004). With this background, present investigation is carried out to explore the

potential of EPNs and its bacterial symbionts against leaf folder.

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, and 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 culture was aerated once in a week and re-culture were done once in 15 days in grown up *C. cephalonica* larva.

Bacterial isolation

Symbiotic bacteria were obtained from the haemolymph of *C. cephalonica* larvae infected with IJs of *S. feltiae* by following standard protocol given by Kaya and Stock (1997). The dead larvae of *C. cephalonica* after 24-48 hrs of inoculation were surface-sterilized in 70 per cent alcohol for 10 min and they were opened with sterile needles and scissors without damaging the gut and a drop of the oozing haemolymph was streaked with a needle onto nutrient agar plates (37 g nutrient agar; 25 mg Bromothymol blue powder (Raymond); 4 ml of filtrates of 1% 2,3,5-Triphenyltetrazolium Chloride (BDH); 1000 ml distilled water). The agar plates after sealing with Parafilm were incubated at room temperature in the dark for 24 hrs. A single colony of bacterium was selected and it was streaked onto new plates of nutrient agar. Sub-culturing was continued until colonies of uniform size and morphology was obtained. The pathogenicity of the isolates was confirmed by inoculating (injecting) the bacterial cells into the body of *C. cephalonica* larvae and streaking the haemolymph of the infected larvae on NBTA plates. A single

colony of the bacterium was selected and inoculated into 500 ml of nutrient broth solution, containing 15 g nutrient broth and 500 ml of distilled water in a flask stoppered with sterile cotton wool. The bacterial concentration of the broth suspensions were determined by a plate count method. The concentration of the bacterial cells in the present experiment was adjusted to 1×10^9 cells per ml and wetting agent *Sandovit* 0.5 ml per litre was added as surfactant.

Experimental design

In the present study, rice varieties ADT 43, Madurai Ponni were used for field experiment. The present study was aimed to find out the influence of EPNs and their bacterial symbionts against *C. medinalis* larva and the damage inflicted to the leaves. The above rice variety was raised adopting the recommended package of agronomic practices. The experiment was conducted in a randomized block design with seven treatments replicated thrice with plot size of 5.0m x 4.0 m. Totally two field trials were conducted at two nearby villages namely Thenthiruperai and Anandanambikuruchi. Spraying was done at evening hours at 4.00 pm to 6.00 pm with hand operated high volume knapsack sprayer.

Field Trial	Place	Variety
I	Thenthiruperai	ADT - 43
II	Anandanambikuruchi	Madurai Ponni

S.No	Treatment	Dosage
1	<i>S. feltiae</i>	1×10^5 IJs/ml
2	Native EPN strain	1×10^5 IJs/ml
3	Bacterial symbionts of <i>S. feltiae</i>	1×10^9 cells/ml
4	Bacterial symbionts of Native EPN strain	1×10^9 cells/ml
5	Bacterial symbionts of <i>S. feltiae</i> + <i>Bt</i>	1×10^9 cells/ml + <i>Bt</i> 1g/lit.
6	Bacterial symbionts of Native EPN strain + <i>Bt</i>	1×10^9 cells/ml + <i>Bt</i> 1g/lit.
7	Untreated control	-

Fresh damage of leaves after treatment was taken into count for calculating the per cent leaf damage; observations were recorded from randomly selected 5 hills in each plot. The total number of leaf folder larvae in each plot was counted by visual observation.

Statistical analysis

All the field and laboratory experiment data were analysed using two factor analysis AGRES statistical package was used for data analysis.

Results and Discussion

Field efficacy of entomopathogenic nematodes and their bacterial symbionts for the management of *C. medinalis* at Thenthiruperai (Field trial I)

First field trial was conducted at Thenthiruperai village with the variety ADT 43. The leaf folder damage occurred during flower initiation stage of rice and it was ranged from 9.60 to 13.15 per cent. The pre-treatment data were well above ETL in most of the plots and they were statistically non-significant. Hence the treatments were imposed. Larval count and per cent leaf damage were considered as parameters to assess the efficacy of different treatments and the results are furnished below.

Larval count

Among the treatments, *X. bovienii* + *Bt* and *X. japonica* combined with *Bt* found to be most effective with 80.54 per cent and 79.37 per cent reduction in larval population over untreated control respectively. It was followed by 65.03 per cent reduction in *S. kushidai* KKM strain and 59.87 per cent reduction in *S. feltiae* sprayed plots. In the absence of *Bt* combination, the bacterial symbionts of *S. feltiae* and *S. kushidai* KKM

strain were found to be inferior treatments with 17.50 per cent and 13.45 per cent reduction in the *C. medinalis* larval population over untreated control respectively (Table 1).

Over all mean values of different days after treatment showed that three, five and seven days after treatments were on par with each other and the larval population in those days was 13.66, 13.47 and 13.95 per plot respectively. The larval count at a day after treatment showed the highest level of 22.75 larvae per plot.

Interaction effect between treatment and days after treatments were also statistically highly significant. *X. japonica* combined with *Bt* on third day after spray was found to show more efficacy as this treatment recorded 93.18 per cent reduction in larval population over untreated control plot in the same period of observation. Next, *X. bovienii* combined with *Bt* on fifth day after treatment showed better response as the reduction over control in this treatment was 91.97 per cent. *X. bovienii* combined with *Bt* on third day and the *X. japonica* combined with *Bt* on fifth day after treatment were statistically on par and the larval population was three per plot against 29.33 and 29 larvae in control plots.

However in the absence of *Bt*, among the two bacterial symbionts, *X. bovienii* exhibited better result as it could cause 24.14 per cent reduction in larval population over untreated control, while the *X. japonica* caused only 16.10 per cent reduction on fifth day after treatment. Among two nematode species, *S. kushidai* KKM strain caused 79.54 per cent reduction in larval population over untreated control, whereas it was 76.13 per cent reduction in *S. feltiae* sprayed plots and the difference between both the nematodes were statistically not significant.

Leaf damage

The overall mean data on above experiment revealed that the *X. japonica* combined with *Bt* and *X. bovienii* combined with *Bt* were more effective with 69.38 per cent and 67.73 per cent reduction in leaf damage over untreated control respectively. It was followed by 47.51 per cent reduction in *S. kushidai* KKM strain and 44.32 per cent reduction in *S. feltiae*, both were statistically on par with each other. *Xenorhabdus bovienii* alone and *X. japonica* alone were found to be inferior with 29.77 per cent and 26.65 per cent reduction in leaf damage over untreated control respectively (Table 2).

Over all mean values of different days after treatments showed that seven days after treatment was the most effective with 61.63 per cent reduction in leaf damage. Three and five days after treatment were statistically on par with 46.88 per cent and 50.32 per cent reduction in leaf damage respectively. The leaf damage at a day after treatment showed poor result with 31.91 per cent reduction in leaf damage over untreated control.

The interaction effect between treatments and days after treatment were statistically highly significant. *X. bovienii* combined with *Bt* on seventh day after treatment was found to be highly efficient as this recorded 83.34 per cent reduction in leaf damage over untreated control plot in the same period of observation. Next, the *X. japonica* combined with *Bt* showed better response by showing 67.74 and 80.30 per cent reduction over control on fifth and seventh day after treatment respectively. However both the bacterial symbionts without *Bt* showed poor result and among them *X. bovienii* was better with 31.24 – 33.92 per cent reduction over control during fifth to seventh day after spray. Among the two entomopathogenic nematodes, the *S. kushidai* was the most effective one with 70.01 per

cent reduction in leaf damage over control, while *S. feltiae* recorded 65.22 per cent reduction over control on seven days after treatment.

Field efficacy of entomopathogenic nematodes and their bacterial symbionts for the management of *C. medinalis* at Anandanambikurichi (Field trial II)

Second field trial was conducted at Anandanambikurichi village with the variety Madurai Ponni. The leaffolder damage occurred during vegetative growth stage and it ranged from 10.59 to 12.92 per cent. As the pre treatment data were well above ETL and they were statistically non-significant, spraying of different treatment suspensions was carried out. Larval count and per cent leaf damage were considered as parameters to assess the efficacy of different treatments and the results are furnished below (Table 3, 4).

Larval count

The mean data showed that *X. bovienii + Bt* and the *X. japonica + Bt* proved superior efficacy with 72.59 and 69.33 per cent reduction in larval population over control respectively. These bacteria in the absence of *Bt* was found to be less effective against *C. medinalis* larva and among them *X. bovienii* was the least one with 13.42 per cent reduction while the *X. japonica* showed 23.45 per cent reduction over control. Although there is no significant difference among the two EPNs, the *S. kushidai* KKM strain was numerically better as it could bring 55.77 per cent reduction in the larval population.

Over all mean values of different days after treatments showed that three, five and seven days after treatment was effective and they were on par with each other and the population on those days was 21.19, 20.86 and 18.76 larvae per plot respectively. The

larval population on the first day after spray was found higher (31.24 larvae per plot).

The interaction effect between treatments and days after treatment revealed that *X. bovienii* along with *Bt* drastically reduced the larval population of *C. medinalis* by 79.83, 79.00 and 76.52 per cent over control plot respectively and they were on par statistically on third, fifth and seventh day after spray. Next, *X. japonica* along with *Bt* at three, five, and seven days after treatment were found be effective with larval population of 9.00, 10.33 and 11.0 larvae per plot respectively. Among the two nematode species, *S. kushidai* on seventh day after treatment was the most effective nematode with 67.83 per cent reduction over untreated control and it was 64.36 per cent in *S. feltiae* on the same period of observation.

Leaf damage

Effect of entomopathogenic nematodes (EPN) and their bacterial symbionts in managing the leaf damage by *C. medinalis* was realized in all the treatments and significant differences between the days after spray was also evident. Interaction effects between treatment and days after spray were found to be non-significant.

Overall mean values of different treatments in managing the leaf damage clearly showed that the *X. japonica* along with *Bt* and *X. bovienii* along with *Bt* were the most effective with 71.23 per cent and 68.53 per cent reduction in leaf damage over untreated control respectively and they were found to be equally effective statistically. It was followed by *S. kushidai* KKM strain with 37.72 per cent reduction and *S. feltiae* with 40.02 per cent reduction over untreated control and they were found to be non significant. The efficacy of sole application of either the *X. japonica* or *X. bovienii* was inferior to all other treatments

with 19.39 - 22.28 per cent reduction in the leaf damage over control.

Similarly over all mean values of all the treatments at different days after spray showed that, seven days after treatment was effective with 60.15 per cent reduction of leaf damage. Three and five days after spray were statistically on par with 35.90 - 46.23 per cent reduction in leaf damage. The leaf damage on a day after spray was poor with 31.67 per cent reduction in leaf damage over untreated control.

Among various treatments, the two bacterial symbionts in combination with *Bt* were the most effective in reducing both the larval population and per cent leaf damage in both field trials. However, they were found to be inferior in the absence of *Bt*. The reason for enhancement in virulence of the bacterial symbionts in the presence of *Bt* has already been discussed in the laboratory studies showing similar results.

A study of Jung and Kim (2006) says that the bacterial mixture of *Bt* and symbiotic bacteria was highly synergistic against fifth instar larvae *Spodoptera exigua* and they reported that mixture of *Xenorhabdus sp.* and *Bt* induced higher mortality when compared with sole application. This study supports our results that both the symbiotic bacteria applied individually in fields exhibited poor result. Thus mixing of *Bt* with symbiotic bacteria synergistically enhanced the efficacy against rice leaf folder. Next to the bacterial symbiont, the two EPNs tested were found to be better as they could suppress the leaf folder larvae and leaf damage per cent in the field conditions. The microclimate of rice culture with high humidity and moderate temperature is conducive for the survival, movement, tracking and invasion of the host by EPN and their establishment as a bio-control agent (Sankar *et al.*, 2009).

Table.1 Efficacy of entomopathogenic nematodes and their bacterial symbionts against *C. medinalis* larva in Field trial I (village: Thenthiruperai)

TREATMENT	Dose	Larval population				Mean (T)	Percent reduction over control (%)
		I DAT	III DAT	V DAT	VII DAT		
<i>S.feltiae</i>	1×10^5 IJs/ml	24.00 (4.89) ^{GH}	7.00 (2.58) ^{DE}	7.33 (2.82) ^{DE}	8.33 (2.89) ^E	11.67 (3.30)c	59.87
<i>S. kushidai</i> KKM strain	1×10^5 IJs/ml	21.33 (4.62) ^G	6.00 (2.44) ^D	6.33 (2.51) ^{DE}	7.00 (2.64) ^{DE}	10.17 (3.05)b	65.03
<i>X. bovienii</i>	1×10^9 cells/ml	27.33 (5.23) ^{HJK}	23.33 (4.83) ^G	22.00 (4.68) ^G	23.33 (4.81) ^G	23.99 (4.89)d	17.50
<i>X. japonica</i>	1×10^9 cells/ml	28.00 (5.29) ^{IJK}	25.00 (4.99) ^{GHII}	24.33 (4.93) ^{GHI}	23.33 (4.82) ^G	25.17 (5.01)d	13.45
<i>X. bovienii +Bt</i>	1×10^9 cells/ml+Bt 1g/lit.	14.00 (3.74) ^F	3.00 (1.72) ^{ABC}	2.33 (1.49) ^{AB}	3.33 (1.80) ^{BC}	5.66 (2.19)a	80.54
<i>X. japonica+Bt</i>	1×10^9 cells/ml+Bt 1g/lit.	15.00 (3.87) ^F	2.00 (1.38) ^A	3.00 (1.72) ^{ABC}	4.00 (1.99) ^C	6.00 (2.23)a	79.37
Untreated control	-	29.66 (5.44) ^K	29.33 (5.42) ^K	29.00 (5.38) ^{JK}	28.33 (5.32) ^{IJK}	29.08 (5.39)e	-
Mean(DAT)		22.75 (4.73)b	13.66 (3.34)a	13.47 (3.36)a	13.95 (3.47)a	15.96 (3.72)	-

Figures in parentheses are square root transformed values.

In a column / row, means followed by a common letter are not significantly different at 1% level (LSD).

	T	DAT	T × DAT
Significance	0.01	0.01	0.01
CD (p=0.05)	0.20	0.15	0.40

T- Treatment

DAT- Days after treatment

Table.2 Effect of entomopathogenic nematodes and their bacterial symbionts against leaffolder damage in Field trial – I (Village: Thenthiruperai)

TREATMENT	Dose	Initial damage (%)	Leaf damage (% reduction over control)*				Mean(T)
			I DAT	III DAT	V DAT	VII DAT	
<i>S. feltiae</i>	1×10^5 IJs/ml	12.12	15.29 (21.29)IJ	46.60 (43.05) ^{EFG}	50.17 (45.09) ^{CDEF}	65.22 (54.04) ^{BCDE}	44.32 (40.87)b
<i>S. kushidai</i> KKM strain	1×10^5 IJs/ml	13.15	13.74 (19.86)J	48.92 (44.38) ^{DEF}	57.38 (49.29) ^{CDE}	70.01 (56.85) ^{ABC}	47.51 (42.59)b
<i>X. bovienii</i>	1×10^9 cells/ml	11.13	21.69 (27.25) ^{HJ}	31.24 (33.82) ^{FGH}	32.22 (34.51) ^{FGH}	33.92 (35.30) ^{FGH}	29.77 (32.72)c
<i>X. japonica</i>	1×10^9 cells/ml	12.45	17.26 (21.49) ^{IJ}	28.18 (31.69) ^{GHI}	27.16 (31.07) ^{HJ}	34.01 (35.27) ^{FGH}	26.65 (29.88)c
<i>X. bovienii +Bt</i>	1×10^9 cells/ml+Bt 1g/lit.	9.60	59.39 (50.64) ^{CDE}	60.92 (51.40) ^{CDE}	67.26 (55.64) _{ABCD}	83.34 (66.02) ^A	67.73 (55.93)a
<i>X. japonica+Bt</i>	1×10^9 cells/ml+Bt 1g/lit.	11.77	64.07 (53.66) ^{BCDE}	65.43 (54.14) ^{BCDE}	67.74 (56.31) ^{ABC}	80.30 (63.81) ^{AB}	69.38 (56.98)a
Untreated control	-	11.46	-	-	-	-	-
Mean(DAT)			31.91 (32.37) c	46.88 (43.08) b	50.32 (45.32) b	61.63 (51.88) a	47.56 (43.16)

Figures in parentheses are arcsine transformed values.

In a column / row, means followed by a common letter are not significantly different at 1% level (LSD).

* In a column / row, means followed by a common letter are not significantly different at 5% level (LSD).

	T	DAT	T × DAT
Significance	0.01	0.01	0.05
CD (p=0.05)	5.90	4.82	11.80

T- Treatment

DAT- Days after treatment

Table.3 Efficacy of entomopathogenic nematodes and their bacterial symbionts against *C. medinalis* larva in Field trial II (village: Anandanambikurichi)

TREATMENT	Dose	Larval population				Mean(T)	Per cent reduction over control (%)
		I DAT	III DAT	V DAT	VII DAT		
<i>S.feltiae</i>	1×10^5 IJs/ml	35.33 (5.92) ^{HJ}	12.00 (3.45) ^{BCD}	13.33 (3.65) ^D	13.66 (3.69) ^D	18.58 (4.18)b	53.49
<i>S.kushidai</i> KKM strain	1×10^5 IJs/ml	33.00 (5.74) ^{FGHI}	12.33 (3.50) ^{CD}	13.00 (3.56) ^D	12.33 (3.48) ^{BCD}	17.67 (4.07)b	55.77
<i>X. bovienii</i>	1×10^9 cells/ml	37.67 (6.13) ^{HJK}	35.67 (5.97) ^{HJK}	32.33 (5.68) ^{FGHI}	32.67 (5.71) ^{FGHI}	34.59 (5.87)d	13.42
<i>X. japonica</i>	1×10^9 cells/ml	34.00 (5.83) ^{GHIJ}	31.33 (5.60) ^{FGH}	29.00 (5.38) ^{FG}	28.00 (5.29) ^F	30.58 (5.53)c	23.45
<i>X. bovienii +Bt</i>	1×10^9 cells/ml+Bt 1g/lit.	18.00 (4.24) ^E	8.00 (2.87) ^A	8.33 (2.88) ^A	9.00 (2.95) ^{AB}	10.95 (3.23)a	72.59
<i>X. japonica+Bt</i>	1×10^9 cells/ml+Bt 1g/lit.	18.67 (4.31) ^E	9.00 (2.98) ^{ABC}	10.33 (3.21) ^{ABCD}	11.00 (3.31) ^{ABCD}	12.25 (3.45)a	69.33
Untreated control	-	42.00 (6.48) ^K	39.67 (6.30) ^{JK}	39.67 (6.30) ^{JK}	38.33 (6.19) ^{IJK}	39.95 (6.32)e	-
Mean(DAT)		31.24 (5.52)b	21.19 (4.38)a	20.86 (4.38)a	18.76 (4.37)a	23.51 (4.66)	-

Figures in parentheses are square root transformed values.

In a column / row, means followed by a common letter are not significantly different at 1% level (LSD).

	T	DAT	T × DAT
Significance	0.01	0.01	0.01
CD (p=0.05)	0.27	0.20	0.53

T- Treatment ; DAT- Days after treatment

Table.4 Effect of entomopathogenic nematodes and their bacterial symbionts against leaffolder damage in Field trial – II (Village: Anandanambikurichi)

TREATMENT	Dose	Initial damage (%)	Leaf damage (% reduction over control)				Mean(T)
			I DAT	III DAT	V DAT	VII DAT	
<i>S.feltiae</i>	1×10 ⁵ IJs/ml	12.16	17.13 (23.90)	20.55 (23.49)	56.09 (48.69)	66.29 (54.62)	40.02 (37.68)b
<i>S. kushidai</i> KKM strain	1×10 ⁵ IJs/ml	10.59	23.45 (28.47)	24.48 (29.61)	38.43 (37.94)	64.54 (53.75)	37.72 (37.44)b
<i>X. bovienii</i>	1×10 ⁹ cells/ml	12.41	8.84 (15.87)	19.21 (25.24)	22.37 (27.36)	27.11 (26.63)	19.39 (23.77)c
<i>X. japonica</i>	1×10 ⁹ cells/ml	12.88	15.41 (22.48)	17.74 (23.97)	22.28 (22.77)	40.85 (36.79)	22.28 (26.51)c
<i>X. bovienii +Bt</i> 1g/lit.	1×10 ⁹ cells/ml+Bt 1g/lit.	12.92	60.76 (51.28)	65.77 (54.28)	66.59 (55.13)	80.99 (64.36)	68.53 (56.26)a
<i>X. japonica+Bt</i> 1g/lit.	1×10 ⁹ cells/ml+Bt 1g/lit.	12.77	64.44 (53.43)	67.62 (55.52)	71.60 (55.08)	81.19 (64.45)	71.23 (57.87)a
Untreated control	-	11.29	-	-	-	-	-
Mean(DAT)			31.67 (32.57)	35.90 (35.35)	46.23 (41.16)	60.15 (50.1)	43.48 (39.80)
			c	b	b	a	

Figures in parentheses are arcsine transformed values.

In a column / row, means followed by a common letter are not significantly different at 1% level (LSD).

	T	DAT	T × DAT
Significance	0.01	0.01	NS
CD (p=0.05)	8.95	7.31	17.90

T- Treatment; DAT- Days after treatment

The bacterial symbionts in combination with *Bt* could produce good results in short period of time. EPNs would have taken some time to release the symbiont from its bacterial pouch. However, the symbionts along with *Bt* would have readily reached insect haemocoel in short period of time to produce good results. Between the two EPNs and their bacterial symbionts tested the native *S. kushidai* and its bacterial symbiont *X. japonica* yielded good results against rice leaffolder larva. This statement is further confirmed by getting lower values of LC₅₀ and LT₅₀ for *S. kushidai* in our earlier laboratory studies in the present research work.

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