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Virulence of *Xenorhabdus bovienii* and *Xenorhabdus japonica* against *Cnaphalocrocis medinalis* Guenee

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The symbiotic bacteria associated with entomopathogenic nematodes can be exploited as potential bio control agents against insect pests. *X. bovienii,* the symbiotic bacterium of *S. feltiae* in combination with *Bt* in the inoculation method, resulted in cent per cent larval mortality of *C. medinalis* on 2.5 days after treatment. However in the sole application of *X. bovienii* even at the highest dosage level of 10^8 cells per ml it showed the lowest larval mortality of eight per cent. *X. japonica* the symbiotic bacterium of *S. kushidai* KKM strain in the inoculation method, at 10^8 cells per ml with *Bt* combination recorded complete larval mortality at 72 h after treatment. Whereas in the absence of *Bt*, it recorded only 3.13 per cent mortality at the same dosage level of 10^8 cells per ml. Hence, we speculate that the addition of *Bt* synergise the virulence of symbiotic bacteria on *C. medinalis.*

Key words: Efficacy, *Xenorhabdus bovienii, Xenorhabdus japonica, Cnaphalocrocis medinalis*

Rice (*Oryza sativa* L.) is staple food of most of the people living in the developing countries, primarily in Asia. There are 2.4 billion consumers of rice in the world, of which over 95 per cent are in Asia. According to United Nations Food and Agricultural Organization (FAO), global food production must increase by 70 per cent to feed the world posing a great challenge to the rice scientists (Seck, 2011). Insect pests are considered as major constraints for increasing rice productivity in tropical world since, the warm and humid climate of tropics is quite congenial for outbreak of the pests. Adoption of high yielding varieties coupled with high fertilizer consumption has brought significant changes in rice pest status. Formerly considered as minor pest, leaffolder appears to be more destructive and widespread insect pest throughout the rice growing regions in South and South-East Asia (Rao *et al.,* 2010). India has a great potential to exploit beneficial nematodes for the suppression of insect pests attacking various crops particularly in rice. 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. 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.

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Material 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 leaf folder *C. medinalis* was mass cultured on TN-1 rice seedlings as per the standard protocol described by Heinrichs *et al.,* (1985)

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 h 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 sterile needle onto nutrient agar plates (37 g nutrient agar; 25 mg Bromothymol blue powder (Raymond); 4 ml of filtrate of 1% 2,3,5-Triphenyltetrazolium Chloride (BDH); 1000 ml distilled water). The agar plates after sealing with parafilm were incubated at 28°C 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 plugged with sterile cotton wool. The bacterial concentration of the broth suspensions were determined by 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.

Efficacy of symbiotic bacteria against C. medinalis larva

A broth suspension containing 1×10^9 cells/ml of *X. bovienii* and native EPN strain was prepared as explained earlier and it was serially diluted to the following five concentration levels. One gram of commercial product of *Bacillus thuringiensis kurstaki* (Lipel SP, Agri Life SOM Phytopharma India Ltd., Hyderabad) containing 1×10^{11} cells per gram was added uniformly to each of the bacterial symbiont dilution. One ml each of the suspension was applied by micropipette on the screen house reared grown up *C. medinalis* larva kept placed on 10 cm diameter Petri dishes @ 10 larvae per Petri dish and sealed with parafilm. The experiment was replicated four times and the larval mortality was recorded after 24-48 h.

Treatments

The treatment shedule followed for the eperiments are T1 – 1×10^8 cells / ml + Bt one g. L^{-1}, T2 – 1×107 cells / ml + Bt one g. L^{-1}, T3 - 1×10^6 cells / ml + Bt one g. L^{-1}, T4 - 1×10^5 cells / ml + Bt one g. L^{-1}, T5 – 1×10^4 cells / ml + Bt one g. L^{-1}, T6 – 1×10^8 cells alone, T7 – Bt one g. L^{-1}, T8 – Bt 2g. L^{-1} and T9 – Untreated control. All the treatments were replicated four times and the statistical design followed is CRD. Experimental data were analysed using two factor analysis in AGRES statistical package. Mortality in untreated control was corrected using Abbott's corrected per cent mortality (Abbott, 1925).

$$\text{Abbott's corrected mortality (\%)} = \frac{\text{Mortality (\%) in T} - \text{mortality (\%) in C}}{100 - \text{mortality (\%) in C}} \times 100$$

Where, T = Treatment, C= Untreated control.

Results and Discussion

Efficacy of X. bovienii against C. medinalis larva

In this experiment, up to 36 h after treatment, there was no significant larval mortality in all the treatments.

Table 1. Efficacy of *X. bovienii* against *C. medinalis* larva

Treatment (X. bovienii)	Per cent mortality Hours after treatment(TxH)					Mean(T)
	12	24	36	48	60	
10^8 cells / ml + *Bt* one g. L^{-1}	0 (4.05)^{l}	5 (9.77)^{kl}	10 (15.49)^{kl}	65 (54.25)^{bcde}	100 (88.72)^{a}	36 (32.74)a
10^7 cells / ml + *Bt* one g. L^{-1}	0 (4.05)^{l}	0 (4.05)^{l}	10 (15.49)^{jk}	60 (51.36)^{cde}	100 (88.72)^{a}	34 (33.31)a
10^6 cells / ml + *Bt* one g. L^{-1}	0 (4.05)^{l}	0 (4.05)^{l}	5 (9.77)^{jk}	50 (45.33)^{cdef}	80 (66.84)^{b}	27 (27.15)b
10^5 cells / ml + *Bt* one g. L^{-1}	0 (4.05)^{l}	0 (4.05)^{l}	0 (4.05)^{l}	35 (36.37)^{fgh}	75 (60.61)^{bc}	22 (21.83)c
10^4 cells / ml + *Bt* one g. L^{-1}	0 (4.05)^{l}	0 (4.05)^{l}	0 (4.05)^{l}	25 (30.07)^{hi}	45 (42.41)^{efg}	14 (16.92)de
10^8 cells / ml	0 (4.05)^{l}	0 (4.05)^{l}	0 (4.05)^{l}	15 (21.21)^{ij}	25 (30.07)^{hi}	8 (12.69)e
Bt one g. L^{-1}	0 (4.05)^{l}	0 (4.05)^{l}	0 (4.05)^{l}	30 (33.22)^{gh}	50 (45.29)^{de}	16 (18.14)cd
Bt 2 g. L^{-1}	0 (4.05)^{l}	0 (4.05)^{l}	5 (9.77)^{kl}	35 (33.54)^{gh}	55 (48.48)^{def}	19 (19.98) cd
Control	0 (4.05)^{l}	0 (4.05)^{l}	0 (4.05)^{l}	0 (4.05)^{l}	0 (4.05)^{l}	0 (4.05)f
Mean (H)	0 (4.05)d	0.56 (4.69)cd	3.33 (7.86)c	35.00 (34.37)b	58.88 (52.80)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	H	T×H
Significance	0.01		0.01
CD (p=0.05)	4.52	3.37	10.10

T- Treatment; H – Hours after treatment

However during 48 h after treatment, the first two higher dosage levels (10^8 and 10^7 cells / ml + Bt one g. L^{-1}) of the bacterial symbiont combined with Bt at one g. L^{-1} inflicted 60–65 per cent mortality and both were found to be statistically on par. In these two treatments, the larval morality reached cent per cent on 2.5 days after treatment. This was followed by 75-80 per cent mortality in the next two dosage levels 10^6 and 10^5 cells / ml + Bt one g. L^{-1}) in the same period (Table 1).

Overall mean values of different treatments clearly showed that as the dosage level of the *X. bovienii* + Bt decreased from 10^8 cells per ml to 10^4 cells per ml, there was a gradual decrease in the larval mortality from 36 to 14 per cent. The higher dosage levels 10^8 and 10^7 cells per ml along with Bt were found to be more effective with 36 and 34 per cent mortality, respectively and both the treatments were statistically on par. This was followed by 106 cells per ml bacterial suspension with *Bt* combination, which was effective with 27 per cent mortality. *Xenorhabdus bovienii* at 10^5 cells per ml + *Bt* inflicted 22 per cent mortality, while Bt at one gram and two gram per lit. Sole application of *X. bovienii* recorded 16 and 19 per cent larval mortalities, respectively. However, the sole application of the symbiotic bacterium *X. bovienii* even at the highest dosage level of 10^8 cells per ml inflicted the lowest larval mortality of eight per cent.

The overall mean values of different hours after treatment showed a positive trend in mortality level in *C. medinalis* larvae. The observations revealed that 60 h after treatment was effective with 48.33 per cent mortality followed by 35.00 per cent at 48 h after treatment. However the mortality level recorded during 24 and 36 h were 0.56 and 3.33 per cent respectively. No mortality was observed in 12 h after treatment.

Efficacy of X. japonica against C. medinalis larva

The difference among treatments and different h after treatment were found significant. No mortality was observed until 12, 24 and 36 h after treatment in all the treatments. The highest dosage of *X. japonica* (10^8 cells per ml) with *Bt* combination recorded complete mortality at 72 h after treatment. Over all mean values showed that *X. japonica* at 10^8 cells per ml combined with *Bt* was superior among all the treatments as it recorded 39.58 per cent larval mortality. *Xenorhabdus japonica* at 10^7 cells per ml combined with *Bt* exhibited 31.25 per cent mortality, while at 10^6 cells per ml + *Bt* showed 29.27 per cent larval mortality and these two treatments were on par with the mortality level (23.96 %) occurred in sole treatment of *Bt* two gram per lit. *Xenorhabdus japonica* alone even at the highest dosage of 10^8 cells per ml showed poor larval mortality of 3.13 per cent and it was statistically on par with *Bt* one gram per lit. (4.17 %) (Table 2).

Table 2. Efficacy of *X. japonica* against *C. medinalis* larva

Treatment (X. japonica)	Per cent mortality Hours after treatment(TxH)						Mean(T)
	12	24	36	48	60	72	
10^8 cells / ml + *B t* one g. L^{-1}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	62.5 (56.17)^{bcd}	75 (63.67)^{b}	100 (88.72)^{a}	39.58 (36.79)a
10^7 cells / ml + *B t* one g. L^{-1}.	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	31.25 (31.24)^{fg}	62.5 (56.17)^{bcd}	93.75 (81.62)^{a}	31.25 (30.20)b
10^6 cells / ml + *B t* one g. L^{-1}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	18.75 (20.93)^{gh}	62.5 (52.81)^{bcd}	93.75 (81.62)^{a}	29.27 (27.92)bc
10^5 cells / ml + *B t* one g. L^{-1}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	6.25 (10.62)^{hi}	37.5 (35.00)^{ef}	68.75 (56.57)^{bcd}	18.75 (19.06)d
10^4 cells / ml + *B t* one g. L^{-1}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	12.5 (17.19)^{hi}	31.25 (34.07)^{efg}	50 (45.31)^{de}	15.63 (18.12) d
10^8 cells / ml	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	18.75 (23.76)^{gh}	3.13 (7.34)e
B t one g. L^{-1}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	6.25 (10.62)^{i}	18.75 (20.31)^{fgh}	4.17 (7.96) e
B t 2 g. L^{-1}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	12.5 (14.36)^{hi}	56.25 (49.05)^{cd}	75 (60.33)^{bc}	23.96 (22.65)cd
Control	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)^{i}	0 (4.05)e
Mean(H)	0 (4.05)d	0 (4.05)d	0 (4.05)d	15.97 (18.07)c	36.80 (34.38)b	57.63 (51.43) 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	H	T×H
Significance	0.01	0.01	0.01
CD (p=0.05)	5.58	4.56	13.67

T- Treatment ; H- Hours after treatment

In this bio assay, the bacterial suspension was applied over the body of *C. medinalis* larva. The entry of the symbiotic bacteria and *Bt* into insect body might be through natural openings like mouth, anus, spiracles or they might have penetrated through larval skin. *Xenorhabdus* is motile gram negative aerobic bacteria having numerous peritrichous flagella for swarming motility under moist conditions (Balcerzak, 1991; Givaudan *et al.,* 1995; Forst and Nealson, 1996). Mahar *et al.,* (2005) and Sahina *et al.,* (2011) could observe penetration of *X. nematophilus* into the body of *G. mellonella* and vine mealy bug.

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