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Research Note

In vitro pathogenicity of *Bacillus thuringiensis* against tea termites

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ABSTRACT: Tea is an economically important plantation crop of Assam, India. Termites are one of the predominant pests causing damage to tea, thereby affecting the productivity and quality. *Bacillus thuringiensis* strains are widely used as microbial control agents for different insect pests. In the present study *B. thuringiensis* and *B. thuringiensis* subsp. *israelensis* were evaluated *in vitro* for their pathogenicity against two species of tea termites, *viz*., *Microtermes obesi* Holmgren and *Microcerotermes beesoni* Snyder. *Bacillus thuringiensis* strains caused mortality of above 80% in both the termite species. *B. thuringiensis* subsp. *israelensis* was found to be more virulent compared to *B. thuringiensis* against the termites.

KEY WORDS: *Microcerotermes beesoni, Microtermes obesi*, median lethal time

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INTRODUCTION

Termites are highly organized social insects present in all natural warm, terrestrial environments and feed on cellulose. Several termite species play an important ecological role by contributing significantly to most of the world's ecosystems. They help to recycle the woody and other plant material. Their tunneling action helps to aerate soils. Termite activity causes patchy changes that improve the soil composition and fertility (Roonwal, 1978a). Known primarily for their destruction of woody plant parts, termites have been considered one of the important pests of tea, causing great damage (>30% of total crop) to both young and mature tea plantations (Choudhury *et al*., 2005).

Bacillus thuringiensis (Berliner) is a spore-forming, gram-positive bacterium of ubiquitous occurrence, with as many as 50 serotypes or 63 serovars (Thiery and Frachon, 1997). There are several reports on the efficacy of entomopathogenic bacteria and viruses against caterpillar pests of tea (Kariya, 1977; Sato *et al*. 1986; Tabashnik *et.al.* 1990; Tabashnik *et al*.1991). The efficacy of *B. thuringiensis* on tea insect pests has been reported by Muraleedharan and Radhakrishnan (1989) and Muraleedharan (1993).

Bacillus thuringiensis has been shown to cause 75 – 100% mortality in *Reticulitermes flavipes, R. virginicus* and *R. hesperus* (Isoptera: Rhinotermitidae) when tested *in-vitro* (Symthe and Coppel, 1965; Stadykov, 1970; Khan *et al*., 1985; Grace and Ewart, 1996). Castilhos-Fortes *et al*. (2002) evaluated the susceptibility of *Nasutitermes ehrhardti* (Isoptera: Termitidae) to 55 strains of *B. thuringiensis* and seven strains were found pathogenic to the termite species. However, no field data on *B. thuringiensis* efficacy for termite control is available so far (Milner and Staples, 1995).

Microcerotermes beesoni Snyder and *Microtermes obesi* Holmgren were found to be very common in the tea growing areas under study and considered for experimentation. In the present study, the potentiality of two strains of *B. thuringiensis* were evaluated against the termite pests (*M. beesoni* and *M. obesi*) of tea growing areas of southern Assam. Field visits were made periodically in the tea growing areas to collect the termite nests. Collected termites with their nests were maintained under laboratory condition. Two isolates of *B. thuringiensis*, *viz*., *B. thuringiensis* (MTCC-1953) and *B. thuringiensis* subsp. *israelensis* (MTCC-869) were obtained from the Microbial type culture collection, Institute of Microbial Technology, Chandigarh.

The *Bt* strains were grown in Usual Glicosed Medium (Castilhos-Fortes *et al*. 2002) at 28°C for 48 hr. The suspension was centrifuged at 5000 rpm and washed 3 times with sterilized water. The concentration of bacterial spores was determined in a Neubaeur chamber using a phase contrast microscope. Bioassays were done by using different concentrations (10^4, 10^5, 10^6 cells ml^{-1}). The suspensions

Table 1. Per cent mortality of worker caste of *M. beesoni* by *B. thuringiensis* isolates

Bacterialstrain	Conc.	% mortality after treatment* (± S.E.)					LT_{50} (Days)	$X^2$for LT_{50}
		1st day	2nd day	3rd day	4th day	5th day		
Control	3.33 ± 1.11	7.78 ± 0.64	12.22 ± 0.64	18.89 ± 1.28	21.11 ± 1.11			
B. thuringiensis sub sp. *israelensis*	10^4	21.11 ± 1.11	40.00 ± 0.64	53.33 ± 1.11	82.22 ± 0.64	92.22 ± 1.28	2.84	4.83(P<0.05)
	10^5	30.00 ± 1.66	52.22 ± 1.69	61.11 ± 0.64	88.89 ± 1.11	100.00 ± 0.00	1.89	
	10^6	36.67 ± 1.69	62.22 ± 1.11	78.89 ± 1.11	93.33 ± 1.28	100.00 ± 0.00	1.63	
B. thuringiensis	10^4	8.89 ± 0.64	22.22 ± 1.69	41.11 ± 0.64	58.89 ± 0.64	68.89 ± 1.28	3.85	5.85(P<0.02)
	10^5	11.11 ± 0.64	31.11 ± 1.28	48.89 ± 0.64	67.78 ± 0.64	81.11 ± 1.11	3.36	
	10^6	13.33 ± 1.11	40.00 ± 1.11	55.56 ± 1.69	74.44 ± 1.69	90.00 ± 1.28	2.88	
CV(%)		7.94	8.35	9.07	10.25	10.83		
CD (*P* = 0.05)		4.58	5.03	5.68	6.49	7.06		

*Mean of three replicates with 30 termites per replicate

Table 2. Per cent mortality of worker caste of *M. obesi* by *B. thuringiensis* isolates

Bacterialstrain	Conc.	% mortality after treatment* (± S.E.)					LT_{50} (Days)	$X^2$for LT_{50}
		1st day	2nd day	3rd day	4th day	5th day		
	Control	3.33 ± 1.11	8.89 ± 0.64	14.44 ± 0.64	20.00 ± 1.11	22.22 ± 1.69		
B. thuringiensis sub sp. *israelensis*	10^4	24.45 ± 1.28	44.45 ± 1.69	57.78 ± 1.28	86.67 ± 1.69	95.55 ± 1.11	2.46	8.87(P<0.01)
	10^5	32.22 ± 1.69	55.55 ± 1.11	65.56 ± 1.69	90.00 ± 1.11	100.00 ± 0.00	1.73	
	10^6	38.88 ± 1.28	64.44 ± 1.69	80.00 ± 1.28	95.55 ± 0.64	100.00 ± 0.00	1.51	
B. thuringiensis	10^4	10.00 ± 0.64	24.45 ± 1.28	44.44 ± 0.64	60.00 ± 1.11	70.00 ± 1.28	3.47	7.63(P<0.01)
	10^5	12.22 ± 1.11	33.33 ± 1.28	50.00 ± 1.69	69.89 ± 0.64	82.22 ± 1.11	3	
	10^6	15.55 ± 1.28	42.22 ± 0.64	58.89 ± 1.11	78.89 ± 1.28	91.11 ± 1.69	2.62	
CV(%)		8.23	8.76	9.31	10.73	11.13		
CD (*P* = 0.05)		4.64	5.11	5.83	6.54	7.28		

*Mean of three replicates with 30 termites per replicate

were applied to sterilized sawdust, which was then offered as food source to the termites. After the evaporation of excess moisture, 2g of the sawdust was placed on the Petri plates covered with moist Whatman No.1 filter paper. Thirty equal sized active termite workers were then released on Petri plates. For every treatment, three replicates were used for each isolate. Distilled water was used as control instead of suspension of *B. thuringiensis*. Insect mortality was recorded daily from the date of onset of the bioassay.

The data obtained were analysed to calculate the mean and SE. The CV% and CD (P = 0.05) values were also calculated. The median lethal time was calculated by using probit analysis (Finney, 1971).

The results showed that *B. thuringiensis* subsp. *israelensis* was more virulent compared to *B. thuringiensis* to the worker castes of both the termite species *in vitro*. At the lowest concentration (10^4 cells ml^{-1}), *B. thuringiensis* subsp. *israelensis* caused 21.11% mortality on the 1st day of observation, while *B. thuringiensis* caused only 8.89% mortality of the worker castes of *M. beesoni* (Table 1). The bacterial concentrations 10^5 and 10^6 cells/ml caused 100% mortality of the workers of *M. beesoni* with the strain of *B. thuringiensis* subsp. *israelensis* while *B. thuringiensis* caused only 81.11% and 90.00% of mortality on the 5th day of observation. Both the *B. thuringiensis* strains were also found to cause significant mortality to the worker castes of *M. obesi* (Table 2). The LT_{50} values calculated were 2.84 and 3.85 days for *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* against *M. beesoni* at the lowest concentration (10^4cells/ml) used. The LT_{50} values were found to decrease with the increase in concentration. In case of *M. obesi*, the LT_{50} values for *B. thuringiensis* subsp. *israelensis* and *B. thuringiensis* at the lowest concentration used (10^4 cells ml^{-1}) were 2.46 and 3.47 days, respectively. Considering the LT_{50} values, *B. thuringiensis* strains were found to be more virulent to *M. obesi* than *M. beesoni*.

References on the efficacy of *B. thuringiensis* against termites are scarce and there is little data available. The toxic effects of *B. thuringiensis* against several termites have been verified, but the authors (Cowie *et al.*, 1989) do not report the subspecies used. There were wide differences in the specificity among the isolates, also individual isolates are found to be active against different insect orders, including Isoptera (Hernandez, 1988; Caetano *et al.*1998). Castilhos-Fortes *et al.* (2002) reported the susceptibility of *Nasutitermes ehrhardti* to different strains of *B. thuringiensis.* Khan *et al*. (1977) isolated *B. thuringiensis* from the naturally infected nymphs of the termite *Bifiditermes beesoni*, causing high mortality of *Heterotermes indicola* when the bacterium was used against it. The present work has added and confirmed the susceptibility of two isopteran species to *B. thuringiensis* subsp. *israelensis*.

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