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EVALUATION ON THE TOXICITY EFFECT OF FOUR *BACILLUS THURINGIENSIS* STRAINS AGAINST THE TEAK SKELETONISER, *PALIGA DAMASTESALIS* WALKER (LEPIDOPTERA: PYRALOIDEA: CRAMBIDAE)

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INTACHAT, J., MASTURA, M. & STAINES, H. 2000. Evaluation on the toxicity effect of four *Bacillus thuringiensis* strains against the teak skeletoniser, *Paliga damastesalis* Walker (Lepidoptera: Pyraloidea: Crambidae). Four *Bacillus thuringiensis* (Bt) strains, namely HD-1, Florbac, SN-2 (Bt subspecies *aizawai* strain) and SN-5, were screened against the 3rd instar of the teak skeletoniser, *Paliga damastesalis*. A total of 750 larvae were tested in the laboratory at 25 ± 1 °C. Mortality after 24, 48 and 72 h exposure was analysed using logistic regression. Bt subspecies *aizawai* (strain SN-2) was found to be the most effective in controlling the teak skeletoniser larvae.

Key words: Teak skeletoniser - *Paliga damastesalis* - *Tectona grandis* - Bt strains - toxicity - Malaysia

INTACHAT, J., MASTURA, M. & STAINES, H. 2000. Penilaian kesan ketoksikan empat strain *Bacillus thuringiensis* terhadap perangka daun jati, *Paliga damastesalis* Walker (Lepidoptera: Pyraloidea: Crambidae). Empat strain *Bacillus thuringiensis* (Bt), iaitu HD-1, Florbac, SN-2 (strain subspesies Bt *aizawai*) dan SN-5, diuji terhadap instar ketiga perangka daun jati, *Paliga damastesalis*. Sebanyak 750 larva diuji di dalam makmal pada suhu 25 ± 1 °C. Kematian selepas pendedahan selama 24, 48 dan 72 jam dianalisis menggunakan regresi logistik. Subspesies Bt *aizawai* (strain SN-2) didapati paling berkesan dalam mengawal larva perangka daun jati.

Introduction

Ever since teak was planted widely in Malaysia in the early 1990s, leaves of young teak saplings have been observed to be constantly skeletonised by the larvae of a moth. The moth *Paliga damastesalis* Walker (Lepidoptera: Pyraloidea: Crambidae), commonly known as the teak skeletoniser, was later found to be different but closely related to the teak skeletoniser species in India that causes similar damage on teak leaves (Intachat 1998). They seem to be present wherever teak is planted in Malaysia, especially in Sabah causing, usually, low levels of defoliation. Attacks by this moth are seasonal (Chey 1996) but can be severe particularly in Sabah during dry spells.

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With the current increased interest in teak planting in Malaysia, which extends to small-scale plantings, the populations of this moth are expected to increase. Constant defoliation, especially on young teak saplings, may have adverse effects on their growth. It is therefore important that a suitable, environmentally friendly control be applied to protect young teak plantings from heavy defoliation caused by the moth. One possible approach is biological control using a microbial pesticide.

Preparations of the bacterium *Bacillus thuringiensis* Berliner (Bt) have been widely used as a microbial pesticide since the 1960s in the United States. Besides exotoxins, Bt also produces a glycoprotein entomotoxins called δ-endotoxins (Singleton & Sainsbury 1987). The nature of the endotoxins differs among Bt subspecies and isolates, with the characteristics of these specific endotoxins determining their target organism. Among the best known and most widely used Bt insecticides are those formulated from Bt var. *kurstaki* isolates that are pathogenic and toxic only to larvae of butterflies and moths (Lepidoptera). Bt var. *aizawai* is another Bt that kills caterpillars (Weinzerl & Solter 1995).

As a biological control approach is suitable for forest environments, the usage of Bt to control major forest pests from the order Lepidoptera may be an effective solution. However, because Bt strains are specific, we evaluated the toxicity of four different Bt strains in search of one, which might effectively control *P. damastesalis*. No previous analysis has investigated the effects of various Bt strains on this species of teak skeletoniser.

Materials and methods

The micro-organisms used in this study consisted of four strains of Bt: HD-1, Florbac, SN-2 and SN-5. All the strains were obtained from the Department of Microbiology, Faculty of Life Science, Universiti Kebangsaan Malaysia. Strain HD-1 was previously obtained from the *Bacillus* Genetic Stock Center, The Ohio State University Columbus, U.S.A. Florbac strain was re-isolated from the commercial preparation by Florbac FC, Novo Nordisk, Denmark. Strain SN-2 is a radiation-resistant mutant strain of a re-isolated strain (subspecies *aizawai*) from a commercial preparation (Bacillex, Shionogi, Japan) (Jangi & Hashim 1983) and SN-5 strain is a local isolate of Bt subspecies *kurstaki*.

Media were sterilised by autoclaving at 120 °C for 15 min and all subsequent manipulations were carried out in a glass laminar flow cabinet. Liquid transfers were made with sterilised pipette tips. The bacterial strains were grown and maintained on nutrient agar slants and were stored at 4 °C under aerobic conditions. Bt strains were subcultured by inoculating a loopful into 10 ml of nutrient broth and incubated for 48 h at 30 ± 2 °C. The resultant inoculums were then adjusted further to obtain the turbidity comparable to that of McFarland Standard No. 0.5 (Vandepitte *et al.* 1991) before they were ready to be tested. McFarland No. 0.5 was chosen since this is the lowest bacterial suspension standard that is equivalent to the density of bacteria at 150×10^5 cells per ml (Anonymous 1995).

Teak leaves were cut into round discs of 9.0 cm diameter. The teak discs were then washed in a 10% solution of sodium hypochlorite (NaOCl), rinsed in a series of distilled water, and air-dried. Distilled water or nutrient broth (both being the control treatments) or one of the four strains of bacteria suspensions was then sprayed on

both sides of the teak discs using an ordinary handspray (Taiwan SC-202). The total amount of two sprays on each teak disc sprayed by the same person was found to be equivalent to 1 ml. Five 3rd instar larvae (as described in Intachat 1999) obtained from laboratory rearing and starved for three hours were then introduced to each of the treated teak discs that were kept individually in Petri dishes at 25 ± 2 °C in an incubator. For each of the six treatments, a total of 25 Petri dishes (replicates) were used and hence a total of 125 larvae were tested for each treatment.

The mortality rate of the larvae in each replicate of each treatment was recorded after 24, 48 and 72 h. Mortality rates for some treatment/time combinations were less than 10%, so the normal approximation to the binomial distribution and hence the use of the arc sine transformation was invalid. Stepwise logistical regression was used to determine the relative importance of the crossed factors exposure time and treatment and the nested factor dish (replicate). Only factors that significantly reduced (at the 5% significance level) the residual deviance were included in the model. Attempts at model simplification by combining treatment levels were made to determine significant differences among treatments. Standard diagnostic checks showed no departures from the usual model assumptions.

Results and discussion

Mortalities were observed in groups of larvae treated among the four strains within the first 24 h. Infected larvae showed a loss in appetite, ceased moving and became flaccid and brownish in colour. Fluid of disintegrating internal tissues liberated from the broken skin was also observed in dead larvae. Since the δ-endotoxins produced by Bt are not a contact poison, the larvae must first ingest the leaf disc coated with Bt to be effective and death may occur within several hours thereafter. Ingestion by susceptible larva will degenerate the mid-gut epithelium and paralyse the gut. This may kill the larvae directly or they may stop feeding and die within 48 to 72 h from the effects of lethal septicaemia (blood poisoning) (Singleton & Sainsbury 1987, Weinzerl & Solter 1995).

Table 1. Mean mortality (%) for each treatment after 24, 48 and 72 h exposure. The fitted value for each treatment/time combination is shown in parentheses.

Treatment code	Treatment	Time		
		24h	48h	72h
T1	Control 1 (distilled water)	2.4 (1.76)	9.6 (7.78)	23.2 (25.65)
T2	Control 2 (nutrient broth)	0.8 (1.57)	7.2 (6.97)	24.0 (23.45)
T3	HD-1	1.6 (3.85)	10.4 (15.84)	51.2 (43.51)
T4	Florbac	4.0 (3.85)	17.6 (15.84)	41.6 (43.51)
T5	SN-2	5.6 (5.79)	20.8 (22.43)	56.0 (54.19)
T6	SN-5	4.8 (2.39)	13.6 (10.34)	26.4 (32.06)

Observation at 24 h showed a higher percentage of mortality for SN-2 and SN-5 (5.6 and 4.8% respectively) as compared to Florbac and HD-1 (1.6 and 4.0% respectively) (Table 1). A slight variation, however, occurred after 48 h exposure (Table 1) whereby Bt strain Florbac (17.6% mean mortality) outperformed SN-5 (13.6%). At this exposure time, SN-2 still recorded the highest mortality amongst the four with 20.8%. Variation in activity was again detected at 72 h exposure (Table 1). Whilst SN-2 maintained its good performance, HD-1 outperformed Florbac by 9.6 %. The toxic cumulative effect is consistent amongst the four strains tested with increment in mortality on prolonged exposure.

Stepwise logistic regression showed that exposure time was the most important single factor [$(\chi^2\ (2) = 322.4;\ p \ll 0.01$]. Adding treatment produced a significantly better model [$(\chi^2\ (5) = 62.9;\ p \ll 0.01$]. However, adding the nested factor dish did not produce a significantly better model [$\chi^2\ (144) = 50;\ p > 0.05$] nor did adding the interaction term between time and treatment [$(\chi^2\ (10) = 16.6;\ p > 0.05$] (see Table 2 for the analysis of deviance).

The fitted model including the factors time and treatment was

$$\log_e\left(\frac{p}{1-p}\right) = -4.022 + 1.549\text{tim2} + 2.958\text{tim3} - 0.119\text{T2} + 0.803\text{T3} + 0.803\text{T4} + 1.232\text{T5} + 0.313\text{T6}$$

where p = probability of mortality for an individual

timx = 1 for time x and 0 otherwise.

Tx = 1 for treatment x and 0 otherwise (see legend to Table 1 for treatment codes)

So, for example, the predicted mortality for treatment 5 (T5 =SN-2) after 72 h (time 3) is given by

$$\log_e\left(\frac{p}{1-p}\right) = -4.022 + 1.549(0) + 2.958(1) - 0.119(0) + 0.803(0) + 0.803(0) + 1.232\ (1) + 0.313\ (0)$$

$$= -4.022 + 2.958(1) + 1.232\ (1)$$

$$= 0.168$$

Transforming this gives $p = \frac{e^{0.168}}{1 + e^{0.168}} = 0.542 = 54.2\%$

Fitted values for all treatment/time combinations are shown in Table 1.

Table 2. Analysis of deviance for the experiments

Model	Deviance explained	df	Deviance difference	Df difference	p value
Null	0	0			
Time	322.4	2	322.4	2	< 0.01
Time + treatment	385.3	7	62.9	5	< 0.01
Time + treatment + dish (nested in treatment)	435.3	151	50	144	> 0.05
Time* treatment	401.9	17	16.6	10	> 0.05

Table 3. Analysis of deviance to investigate the subgroups within the treatments

Model	Deviance explained	df	Deviance difference	df difference	p value
Time + treatment	385.3	7	62.9		
Time + treatment with (T1, T2, T6) combined	381.6	5	3.7	2	> 0.05
Time + treatment with (T1, T2, T6) combined and (T3, T4) combined	381.6	4	0	1	> 0.05
Time + treatment with (T1, T2, T6, T3, T4) combined	353.3	3	28.3	2	< 0.01
Time + treatment with (T1, T2, T6) and (T3, T4, T5) combined	374.7	3	6.9	1	< 0.01

Note: See Table 1 for treatment codes.

The effect of longer exposure is likely to be due to a higher amount of leaf consumed by the larvae in order to acquire more toxic substance. The fitted values shown in Table 1 suggest that some treatments may not be significantly different. For example, SN-5 does not appear to show substantially greater mortality than either of the two control treatments. This can be tested formally by combining these treatment levels in the model and determining if this produces a significantly larger residual deviance. This analysis showed that there was no significant difference among the two controls and SN-5 [χ^2 (2) = 3.70; $p > 0.05$]. Further simplification showed that there was no significant difference between HD-1 and Florbac. Any further attempts at model simplification produced a significantly worse model (see Table 3 for full details). Our statistical results, together with the results from Table 1, show that among the four strains tested, strain SN-2 had a significantly greater mortality than strains HD-1 and Florbac which in turn had a significantly greater mortality than the remaining treatments. The insignificant dish effect shows that the results are reproducible whilst the insignificant exposure time/treatment interaction shows that all treatments increase mortality over time in a consistent manner.

Conclusion

The differences in Bt strains tested in the laboratory showed that SN-2, a radiation-resistant mutant of a commercial strain (subspecies *aizawai*), was superior. Although the physiological mechanism for the strain is not known, SN-2 has demonstrated that it is the better biological control agent for the control of the teak skeletoniser, *P. damastesalis*. In addition, due to the high number of different classes of *cryI* genes (codes for δ-endotoxins) for SN-2, it has also been predicted that the pest will take a longer time to develop resistance to prolonged use in the field (Mahadi *et al.* 1998).

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