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Effect of *Bacillus thuringiensis* Berliner on Size, Weight and Fat Content of Citrus Leaf Caterpillar, *(Papilio demoleus* L.)

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ABSTRACT

Studies on the effect of *Bacillus thuringiensis* on citrus leaf caterpillar, *Papilio demoleus* revealed the changes in size, weight and lipid content. A decrease in body length and weight to about 47.8 and 46.1 per cent was observed. Gradual decrease in total lipid content was noticed.

INTRODUCTION

Considerable work has been made with *Bacillus thuringiensis*, pathogenic for most of the lepidopterous insects. A number of symptoms caused by this crystal-forming bacteria in affected larvae has been described, including, sluggishness regurgitation, diarrhoea, cessation of feeding, shrinking and shrivelling, retardation in growth, and finally death. Recently investigations have been undertaken to study the effects of crystal-forming *B. thuringiensis* on the larvae of citrus leaf eating caterpillar, *Papilio demoleus* with regard to histo-pathology and physio-pathology (Narayanan and Jayaraj, 1974 a, b). In the present paper further observation on the effect of *B. thuringiensis* on the size, weight and lipid content of *P. demoleus* has been presented.

MATERIALS AND METHODS

Size: Studies were made on 12 days old larvae, treated with 1 in 40 dilution of Thuricide 90 TS Flowable containing 15 billion viable spores per gram, by way of coating on the citrus leaf. Ten larvae each in control and treated group were kept individually and the length of each determined by using a plastic metric scale. The larvae to be measured was slightly pressed to the scale and reading taken.

Weight: Three sets, consisting five larvae per set were selected from both treated and untreated groups and the wet weight was determined in a weighing bottle. The mean wet weight per larvae was calculated and expressed as mg per larvae.

Total fat: Groups of five larvae were homogenised in chloroform-methanol (2:1 v/v) after the method of Folch *et al.* (1957) and extracted at 60°C for 5 minutes (Orr, 1964). The insoluble material was separated by centrifuging at 5000 rpm for 10 minutes. The supernatant liquid was collected in a separating funnel. The precipitate was resuspended in 5 ml of chloroform-methanol and the extraction procedure repeated. The

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supernatant was added to the original one in the separating funnel and partitioned against 3 per cent (W/V) acqueous sodium chloride (Schaefter, 1968). The chloroform phase was drawn off and the acqueous phase washed twice with chloroform. The combined chloroform phase was dried under reduced pressure and the lipid residue was dissolved in a minimum quantity of diethyl ether and transferred to a weighing bottle. The ether was allowed to evaporate completely and the dry lipid residue was weighed. The total fat content was expressed as mg/g wet weight of larvae as outlined by Jacob (1972).

RESULTS AND DISCUSSION

The data on the mean length of healthy and treated larvae are presented in Table 1. It will be observed that the length of healthy larvae increased steadily with advancing age. Thus from 36.3 mm observed at 8 hours, the length of healthy larvae increased to 42.0 mm at 48 hours. The infected larvae showed a gradual reduction in length. Comparing the length of healthy and treated larvae it is seen that there was no significant difference at 8 hours and 12 hours, after treatment, whereas infected larvae showed a significant reduction in length from 24 hr onwards. It may be seen that the healthy larvae increased in wet weight with advancing age, the treated larvae recorded a significant decrease in weight (Table 2). The observation recorded (Table 3) revealed that the average fat content was higher in healthy larvae, 39.45 mg/g than the treated one 7.27 mg/g. A comparative examination of the fat content in both the group revealed that there is a gradual increase in fat content in the case of healthy and gradual decrease in the treated during the period under observation.

TABLE 1. Mean length of healthy and *B. thuringiensis* treated larvae of *P. demoleus*

Test group	Period after treatment (in hours)	Mean length (in mm)	Standard error	Level of significant difference	Per cent decrease over control
Healthy	8	36.3	0.30	Non-significant	0.27
Treated		36.2			
Healthy	12	37.3	1.47	Non-significant	1.3
Treated		36.8			
Healthy	24	39.3	1.50	P<0.01	35.3
Treated		25.4			
Healthy	36	40.1	1.10	P<0.01	45.1
Treated		22.0			
Healthy	40-43 (moribund)	42.0	0.407	P<0.01	47.8
Treated		21.9			

TABLE 2. Changes in wet weight of healthy and *B. thuringiensis* treated larvae of *P. demoleus*

Period after treatment (in hours)	Mean wet weight (in mg)		Per cent reduction in weight
	Healthy	Treated	
8	623.3	594.2	4.6
12	666.6	462.3	30.6
24	890.7	427.5	52.0
36	984.9	392.2	60.1
40-48 (moribund)	1036.0	385.2	62.8
Mean	840.3	452.3	46.1

C. D. (P = 0.01)	Healthy Vs. Treated	3.96
C. D. (P = 0.01)	Periods	6.27
C. D. (P = 0.01)	Interactions	8.80

TABLE 3. Changes in fat content of healthy and *B. thuringiensis* treated larvae of *P. demoleus*

Period after treatment (in hours)	Fat content (in mg/g)		Per cent reduction over healthy
	Healthy	Treated	
12	19.19	12.12	36.8
24	39.65	7.04	82.2
36	46.29	5.05	89.0
40-48 (moribund)	52.69	4.90	90.7
Mean	39.45	7.27	81.5

Though cessation of feeding, shrinking and shrivelling has been reported in *Bacillus* treated larvae in most of the cases (Heimpel and Angus, 1963), no precise information is available on the effect of *B. thuringiensis* on the larval size, weight and fat content. The present study showed that there did not exist much difference in the length of healthy and treated larvae until 24 hr after treatment. But at 24—36 hr and at moribund stage, the mean length was drastically lower in treated larvae compared to the healthy ones. The results thus indicate that in the infected larvae there was a retardation of growth. This should naturally be expected since the larvae stop feeding after initial meal. The observation that the mean wet weight of treated larvae was markedly lower than that of healthy during the period under observation show further retarding effect of

B. thuringiensis on the growth of the larvae. The pattern of fat accumulation observed in the healthy larvae of *P. demoleus* follow the report in *Bombyx mori* (Niemierko *et al.* 1956), *Hyalophora cercopia* (Gilbert and Schneiderman 1961), *Spodoptera littoralis* (Jacob, 1972), wherein fat content increased sharply with advancing in age. There was practically no study on the effect of *B. thuringiensis* on the fat content. But, however, studies on other infectious disease caused by *Serratia marscens* in *Spodoptera littoralis* (Govindarajan, 1973) and nuclear polyhedrosis infection in *S. littoralis* (Jacob, 1972), showed comparable quantitative and qualitative changes in the total lipid content. This observation plus the observations on the length and weight and feeding activity (Narayanan and Jayaraj, 1974b), clearly show that *B. thuringiensis* infection had a tremendous retarding effect on the growth rate of the host larvae.

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