POTENTIATION OF B. THURINGIENSIS AGAINST S. LITTORALIS AS AFFECTED BY ITS HOST PLANTS

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SUMMARY: Second instar larvae of the cotton leaf worm Spodoptera littoralis (Boisd.) showed obvious differences in their susceptibility to Bacillus thuringiensis when fed on treated foliage of different host plants. Rapid growth on lettuce plant allowed the larvae to tolerate more doses of the pathogen by developing into more resistant stages. On the other hand, plants which do not support full growth as onions and cabbage, showed a great stress on the insect. Slower rate of insect development caused its exposure for long periods to the impact of the disease. The feeding behavior of young larvae on different host plants was also considered as factors influencing the efficacy of B. thuringiensis.

Key Words: Potency, bacillus thuringiensis, spodoptera littoralis.

INTRODUCTION

Spodoptera littoralis is a polyphagous insect that attacks various plant species belonging to different plant families but in varying degrees (15).

In the last decade, the application of microbial control agents particularly *Bacillus thuringiensis* against agricultural insects in Egypt received a great attention. Field trials with *B. thuringiensis* have been attempted in Egypt against *S. littoralis* and other lepidopterous insect pests (18). However, little has been devoted to investigate the host plant-insect pathogen interaction. Kushner and Harvey (8), Smirnoff (20) and Smirnoff and Hatchison (21) mentioned that some antibiotic substances in the leaf extract of some plants inhibit the parasporal inclusion synthesis in crystalliferous bacteria. McGaughey (11) and Matter (9) found that those diets increasing or decreasing the rate of development of the pest accounted for the low and high efficacy of *B. thuringiensis*, respectively. Similar findings were reported by Hellipas and Zebitz (6) and Salama and Sharaby (19). They found that some plant extracts, acting as deterrents, potentiated the effect of *B. thuringiensis* on the target insects.

The present study aims to explore the effect shown by some host plants on the potential activity of *B. thuringiensis* against the cotton leaf worm *S. littoralis*.

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MATERIALS AND METHODS

A mother culture of *S. littoralis* was raised on a semi-artificial diet (14) under controlled laboratory conditions of 25±2°C for several generations. The culture was oriented to provide the required experimental individuals.

The bacterial formulation SAN 415 I (kindly provided by Sandoz LTD, Agro development, Basle, Switzerland) based on the strain HD-Σ of *B. thuringiensis* var. Kurstaki with 32000 IU/mg, was used in the experiments.

Two experiments were made, the first one was conducted on March 1990 using five winter host crops namely, lettuce (Lactuca sativa), onion (Alium cepa), clover (Trifolium alexandrinum), sweet potato (lpoma batatas), and cabbage (Brassica oleracea var. Brunswick). The second experinent was carried out on June 1990 using two summer host crops namely cotton (Gossypium barbadense) and soybean (Glycine hispida). The tested host plants represent a broad spectrum of acceptability to the target insect (15).

Bioassay methodology

Second instar larvae with uniform size (average weight 3.5 mg/larva) were used in the experiments for the different assays. Fifty larvae distributed in five plastic vials (10/each) were used for each concentration level per host plant. An orientation test was made for approximating the toxicity range of *B. thuringiensis* for each treated plant. Six concentration levels were made at half dosage rate and used for each plant.

In each case, the host plant was sprayed to the point of run off with either water or a suspension of the desired tested dose of the pathogen in water, air dried, then offered to the larvae and kept under constant conditions (27±1°C) for two days. Survivors of each treatment were then transferred with fresh untreated foliage of the corresponding plant to clean sterilized glass jars and kept under the same conditions. Mortality counts / replicate /concentration/host plant were recorded six days after treatment. Percent mortality was calculated for each concentration level and corrected for the corresponding natural mortality according to Abbott (1). The mortality data were subjected to statistical analysis (5) and the LC50 and slope values were established.

The effect of the pathogen on the growth rate during the control period was tested in a separate experiment. According to Salama *et al.* (15), Dimetry (4) and preliminary experiments, three plants (lettuce, cotton and onion) were selected to represent different rates of larval growth (high, moderate and ceasing effect). The same methodology was undertaken except that two suitable concentrations of the pathogen were only tested. The survivors of larvae/replicate/treatment were identified for the different instars. The percentage of occurrence of each instar among survivors of each treatment and the average weight/larva/treatment were calculated.

Table 1: Susceptibility of second instar larvae of *S. littoralis* to *B. thuringiensis* when fed on the foliage of winter and summer host plants.

Host plant	LC50±S.E.	Slope±S.E.
Winter Crops		
Lettuce (Lactuca sativa)	331.3±61.3	2.80±0.20
Onion (Allium cepa)	100.1±26.7	1.65±0.06
Clover (Trifolium alexandrinum)	161.9±32.6	1.93±0.28
Sweet potato (Ipomea batatas)	142.7±16.8	2.11±0.11
Cabbage (Brassica oleracea var. Brunswick)	98.3±27.2	2.34±0.54
Summer Crops		
Cotton (Gossypium barbadense)	208.4±28.2	2.27±0.13
Soybean (Glycine hiopida)	145.7±13.8	2.57±0.05

RESULTS AND DISCUSSION

Assay of B. thuringiensis on different host plants

The data given in Table 1 illustrate the assay of *B. thuringiensis* when applied on the foliage of five winter and two summer host plants. The LC50 of *B. thuringiensis* scorded the highest value (331.3 ug/ml) when lettuce was used as a diet. This was followed by cotton, clover, soybean, sweet potato, onion and cabbage in a descending order.

At the 1% level of significance, differences between lettuce and either of the following plants are ranked as follows: cabbage=onion>sweet potato=soybean>clover. The difference between lettuce and cotton or cotton and either of onion or cabbage was only significant at 5% level.

Effect of untreated and Bacillus-treated host plants on the larval growth rate

The data given in Table 2 revealed marked differences in the growth rate of *S. littoralis* larvae when fed on different untreated host plants during the control period (six days). When onion was used as a diet, more than 40% of the tested larvae ceased growth, about 55% developed into the third instar, while a negligible percentage (ca. 2%) reached the fourth instar. On the other hand, 60 and 68.5% of the larvae developed to the fourth instar when they were fed, for the same period, on untreated lettuce and cotton plants, respectively.

Table 2: Growth of second instar larvae of *S. littoralis* (six days period) on different untreated or *B. thuringiensis* treated plants.

B. thuringiensis concentration (ug/ml)	Host plant	No. survivors	% survivors	Percentage of occurrence of different larval instars six days post treatment				Average larval % weight±S.E. (mg/l)	Reduction in weight compared to control
				Second	Third	Fourth	Fifth		
125	L	133	88.7	4.5	22.6	50.4	22.6	199.2±14.6	13.9
	O	43	28.7	65.1	34.9	-	-	14.3±0.5	42.8
	C	112	74.7	10.7	62.5	26.8	-	87.4±3.9	44.8
500	L	94	62.7	-	47.9	52.1	-	162.8±5.8	29.6
	O	13	8.7	76.9	23.1	-	-	9.3±0.4	62.8
	C	48	32.0	18.8	66.7	14.6	-	61.1±7.3	61.4

Regarding the effect of *B. thuringiensis* on the growth rate of insects, the data (Table 2) showed that the pathogen caused various levels of retardation in larval development when they fed, for the same period (six days), on different host plants. In case of lettuce, more than 50% and about 25% of the tested larvae reached the fourth and the fifth instars, respectively whether they fed on untreated foliage or foliage treated with *B. thuringiensis* at the 125 ug/ml concentration level. Slight retardation of growth was only detected when the pathogen concentration increased to 500 ug/ml. Retarded effect of the pathogen on insect growth was more pronounced when onion was used as a diet. More than 50% of the tested larvae reached the third instar in the check, while only about 35 and 23% reached the same instar when fed on foliage treated at the rate of 125 and 500 ug/ml, respectively.

The retarding effect of *B. thuringiensis* on insect growth was reported by many authors working on different lepidopterous pests (2, 9,17, 22, 23).

The data given in Table 2 also illustrated the retarding effect of the pathogen on larval growth as exemplified by the percentage of reduction in the average weight or larvae due to bacterial treatment. It was evaluated, for each host plant, according to the following equation:

$$\text{Percent reduction in larval weight due to bacterial treatmant} = \frac{\text{Average wt. in the check - average wt. in the treated group} \times 100}{\text{Average wt. in the check}}$$

Regarding the effect of the host plant on larval weight, it was found that the average weight of larval raised for six days on untreated lettuce was about 1.46 and 9.25 times that raised for the same period on cotton and onion, respectively, while the average weight of larvae fed on cotton was about 6.3 times that fed on onion. These figures indicated that the growth rate of *S. littoralis* larvae was high, moderate and low after being fed on lettuce, cotton or onion, respectively. Dimetry (4) claimed that lettuce plant ranked the first with respect to the host plants supporting growth of *S. littoralis*.

Bacterial treatment of the foliage of lettuce, cotton and onion at the rate of 125 ug/ml caused 13.9, 42.8 and 44.8% reduction in the average weight of larvae as compared with the corresponding check, respectively. At higher rate of 500 ug/ml, the corresponding reductions were 29.6, 62.8 and 61.4%, respectively. These figures indicate that *B. thuringiensis* has similar retarding effects on the larvae fed on either cotton or onion plants, while it possesses the least effect on larvae fed on lettuce plant.

In conclusion, the young larvae of *S. littoralis* showed various susceptibilities to the pathogen when fed on the foliage of lettuce or onion and cabbage. These findings are in agreement with results of Salama *et al.* (16) who found that the ether extract of cabbage caused about three fold increase in the efficacy of *B. thuringiensis* against *S. littoralis* larvae, while lettuce extract produced negligible effects.

It seems that some diets may change the defense mechanism in insects and hence, alter its susceptibility to different biological agents (13). The chemical constituents in the host plants may affect the microflora in the insect gut and hence, acted on the process of microbial intoxication (8, 20,21). Hellpap and Zebitz (6) and Salama and Sharaby (19), attributed the increase in potency of *B. thuringiensis* vs. the larvae of *S. frugiperda* or *S. littoralis* to the presence of some deterrent substances in the diet which caused retardation of development. Accordingly, plants which do not support growth as onion, might have deterrent substances which prolonged the duration of the susceptible stage and thus, exposing it for longer periods to the impact of the disease, while those plants supporting growth as lettuce might increase the defense on insect larvae by developing, shortly after treatment, into more resistant instars capable of tolerating the dose of the pathogen. The increase in tolerance to *B. thuringiensis* with larval development has been recorded by many authors (2, 3, 7, 10-12).

The physical nature of some plants might play a great role in potentiating the efficacy of the pathogen. In case of onions, the young larvae were observed feeding on epidermal layer of the foliage. This encouraged the larvae to consume large area of the treated surface of the foliage and hence, increasing the uptake of the pathogen irrespective of the reduced amounts of food ingested from that crop.

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