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Susceptibility of the Cotton Leafworm, *Spodoptera littoralis* 3rd Instar Larvae to some Bio-insecticides (Lepidoptera: Noctuidae)

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ABSTRACT

Four bio-insecticides were tested against the cotton leafworm, *Spodoptera littoralis* (Boisd.) 3rd larval instar under simulation field conditions. The tested products were: Bioranza (*Metarhizium anisopliae*), Biovar (*Beauveria bassiana*), Delfin WG, (*Bacillus thuringiensis* var. *kurstaki*) and Neem-Azal-T/S. Biovar product induced the highest percent mortality, followed by Bioranza, Delfin then Neem product. When *Bacillus* and Neem were applied in different sequential manner, results indicated that when *Bacillus* preceded Neem application, the percentage of mortality increased; but when Neem product preceded *Bacillus* application, the percentage of mortality reduced clearly.

Key Words: Bio-insecticides, *Spodoptera littoralis, Metarhizium anisopliae*, *Bacillus thuringiensis*, *Beauveria bassiana*, Neem-Azal-TS.

INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a major pest on cotton, corn, peanuts, vegetables, lucerne, clover, ornamentals, wild plants, weeds and shade trees, soybean and other plants cultivated in Egypt, as well as in Mediterranean and Middle East countries (Champion *et al.*, 1977).

Chemical control of *S. littoralis* has been extensively used in Egypt especially in cotton fields. Until 1968, *S. littoralis* was held in check by methyl-parathion, but then resistance of the insect to this compound was developed. Since then, numerous other organo-phosphorus, synthetic pyrethroid and other insecticides were used, with the appearance of resistance and cross resistance in many cases (Issa *et al.*, 1984a; 1984b; Abo-El-Ghar *et al.*, 1986).

Numerous studies have been carried out using safe alternatives to chemicals. Treatment with *Bacillus thuringiensis* has been used (Navon *et al.*, 1983), but only some strains are effective since *S. littoralis* is resistant to many strains (Salama *et al.*, 1989). Trials to use entomopathogenic fungi were applied too (Shelton *et al.*, 1998; Vandenberg *et al.*, 1998 & 1999, Abdel-Razek *et al.*, 2006 and Farag, 2008).

Researches had almost focused on the relatively easily produced asexual spores (conidia) of the hyphomycete genera *Metarhizium*, *Beauveria*, *Verticillium* and *Paecilomyces*. These fungi often have a wide host range although there is a considerable genetic diversity within species and some show a high degree of specificity (Driver *et al.*, 2000).

Efficacy of biological control agents, particularly *Bacillus thuringiensis*, with chemical insecticides or natural entomostatic plant extracts was investigated by many authors (Facknath, 1999; Jayanthi and Padmavathamma, 2001; and El-Mandarawy *et al.*, 2007).

The objective of the present work is to examine the efficacy of some bio-pesticides, when applied separately or in a sequential manner against the 3rd instar *S. littoralis* larvae.

MATERIALS AND METHODES

Bio-pesticides

The commercial formulations of the tested bio-pesticides that were used under laboratory conditions (27±2°C and 75±5% RH) are:

1- Bioranza, based on the entomopathogenic fungus, *Metarhizium anisopliae*.
2- Biovar, based on the entomopathogenic fungus, *Beauveria bassiana*. These commercial formulations were manufactured and produced by "The Kingdom of Bahrain, Ministry of Municipalities Affairs and Agriculture Wealth Directorate". The active ingredient concentration is $32x10^6$ viable spore/mg for both fungi, which was formulated as WP; and the recommended concentration is 200g/100L against several insect pests and mites.
3- Delfin WG, based on the entomopathogenic bacterium *Bacillus thuringiensis* var. *kurstaki*, produced by [Sandoz, Agro. Div., 4002 Basel, Switzerland], with $52x10^3$ SU/mg of toxicity based on *Spodoptera exigua*. The formulation was kept under 5°C till used.

4- Neem-Azal-T/S, based on azadirachtin [1%], the active ingredient, extracted from *Azadirachta indica* (Meliaceae) trees, is produced by the TRIFOLIO-M GmbH, company, Germany.

Test insect

Newly moulted 3rd instar *S. littoralis* larvae were obtained from a standard laboratory culture and placed in 2 litres glass jars (20 larvae/jar) and kept starving for about four hours. Groups of 5 jars each were prepared. Each group was used/concentration /treatment. The jars of each group were supplied with sprayed cotton leaves grown in planted pots, to the point of run off with the respective concentration of each treatment or sprayed with water for the check group, using small hand atomizer and kept under the laboratory conditions (27±2°C and 75±5% RH). The leaves were replaced every other day with the corresponding treated leaves till the end of the experimental period to simulate field exposure (Matter *et al.*, 1999).

The used concentrations of the tested commercial formulations were based on the recommended rates in field applications. Delfin & Neem-Azal-TS did not cause reasonable effects, the experimental work was extended to test how far the results will be when both of those agents are applied together in different sequential applications at 2 days intervals as follows: the larvae were exposed to castor oil leaves treated with the bacterial formulation at the LC_{25} and/or LC_{50} levels either one day after or before being exposed to Neem-Azal-TS treated leaves. Results of mortality percentages for separate treatment of each bio-agent or of both treatments in a sequential manner were calculated, 7 days after the last treatment (Matter and Zohdey, 1981).

Statistical analysis

For the first experiment, all data were subjected to analysis of variance, using F-test, means were compared by applying Duncan's Multiple Range test. Where in the second treatment, interaction between Delfin and Neem product was evaluated using Benz formula (Benz, 1971).

$$P_{A+B} = P_A + P_B (1- P_A /100)$$

Where: P_{A+B} = expected mortality of the mixture.
$P_A + P_B$ = separate corrected mortality of the two bio-insecticide.

Expected mortality = (A + B)- ((A* B))/100

Where: A= % mortality of the first bio-insecticide
B= % mortality of the second bio-insecticide

. RESULTS AND DISCUSSION

Data in table (1), show that *S. littoralis* 3rd instar larvae were susceptible to the tested bio-pesticides at the recommended concentrations. *B. bassiana* was more efficient than other tested products, followed by *M. anisopliae*, with statistically significant difference between each other and also with other treatments 7 days post treatments. Both of *B. thuringiensis* and Neem products induced less mortality, which did not exceed 42 and 38%, respectively; being insignificantly different between each other (P>0.05). It is well known that Neem has antifeedant effects on some insects (Weathersbee and Mckenzie, 2005).

Table (1): Susceptibility of *Spodoptera littoralis* 3rd instar larvae to some bio-insecticides treatments at the recommended rates of field application

Treatments (recommended concentration)	Mean percent mortality ± SE
Metarhizium anisopliae (200g/100L)	67.40±2.18[b]
Beauveria bassiana (200g/100L)	81.80±2.27[a]
Bacillus thuringiensis var. *kurstaki* (0.50-1.25 Lbs./Acre)	42.00±1.41[c]
Neem Azal-TS (5ml/L)	38.00±0.71[c]
Check	4.00±4.00[d]
F-value	157.639**

Means in a column followed with the same letter are not significantly different at 5% level of probability.
** = Highly significant

When both formulations, the bacterial and Neem-Azal-TS were applied at two concentration levels [LC_{25} and LC_{50}] in a sequential manner at two days interval, the observed effects almost surpassed the corresponding expected ones when the application of the pathogen preceded Neem treatment particularly at LC_{50} level (Table 2). The appeared antagonistic effect, when Neem treatment preceded bacterial one was most probably due to the deterrent effect of Neem which reduced the acquired bacterial dose.

While the synergistic effect obtained when bacterial application preceded Neem treatment was almost due to the fact that the infected larvae faced another stress factor from Neem which delayed development into more resistant instars that can tolerate the bacterial dose. It is well known that some percentages of *Bt*-treated larvae can tolerate certain bacterial doses and develop into more resistant strains, but when those survivors of larvae faced stress factors like starvation or different effects of Neem treatment, they will be kept within their susceptible instars till death.

Table (2): Susceptibility of *Spodoptera littoralis* 3rd instar larvae to Delfin and Neem treatments applied in different sequential manner

A- When Delfin preceded Neem Treatment						
Delfin		Neem Azal-TS		Delfin/Neem Azal-TS		(%) Increase (+) or Decrease (-) than expected
Concentration	% Mortality	Concentration	% Mortality	% Mortality		
				Expected	Observed	
LC_{50}	42	LC_{50}	38	64.40	88.00	+ 36.65
LC_{50}	42	LC_{25}	28	58.24	68.00	+ 16.76
LC_{25}	20	LC_{50}	38	50.40	54.00	+ 7.14
LC_{25}	20	LC_{25}	28	42.40	36.00	-15.09
B- When Neem preceded Delfin Treatment						
Delfin		Neem Azal-TS		Neem Azal-TS/Delfin		(%) Increase (+) or Decrease (-) than expected
Concentration	% Mortality	Concentration	% Mortality	% Mortality		
				Expected	Observed	
LC_{50}	38	LC_{50}	42	64.40	48.00	- 25.55
LC_{50}	38	LC_{25}	20	50.40	34.00	- 32.54
LC_{25}	28	LC_{50}	42	58.24	46.00	- 21.02
LC_{25}	28	LC_{25}	20	42.40	30.00	- 29.25

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