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Impact of Spinosad on Some Enzymatic Activities of the Cotton Leafworm

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Abstract: Spinosad is a fermentation product of *Saccharopolyspora spinosa* Martz and Yao with biological activity against a range of insects including Lepidoptera. The cotton leafworm, *Spodoptera littoralis* (Boisd.) is the most destructive phytophagous lepidopterous pests in Egypt for it causes various ravages not only for cotton plants, but also other field crops and vegetables. This study was conducted in order to evaluate the effect of spinosad which is a new mode of action of insecticides on the activity of esterases (acetylcholine and aliphatic esterase), non-specific esterases (α-and β-esterase), phosphatases (acid and alkaline phosphatase), transaminases (aspartate and alanine transaminase) and carbohydrate hydrolyzing enzymes (amylase and invertase) on 4th instar larvae of the laboratory and the field strains of the cotton leafworm *S. littoralis* (Boisd.). It can concluded that, the change of response to spinosad could be associated with the decrease in AchE activity, likewise the tested compound caused a disturbance in the activities of the other tested enzymes either with increase (Ali-E, β-E and amylase activities) or with decrease (ALT).

Key words : Spinosad, *Spodoptera littoralis* (Boisd.), enzymatic activities

INTRODUCTION

The environmental hazards of conventional insecticides are necessitates entrance other new mode of action of insecticides that are effective, safer for human and negligible effects on ecosystem. Spinosad is an extract of the fermentation broth of soil actinomycete bacterium, *Saccharopolyspora spinosa* Martz and Yao, containing a naturally occurring mixture of two macrocyclic lactones, spinosyn A and spinosyn D. Spinosad has rapid contact and ingestion activity in insects, causing excitation of the nervous system, leading to cessation of feeding and paralysis (Thompson and Hutchins, 1999). Spinosad acts primarily on the insect's nervous system at the nicotinic acetylcholine receptor (nAChRs) and also exhibits activity on the gamma-aminobutyric acid receptor GABA (Salgado, 1997; Sparks *et al*., 2001). It shows exceptional activity against Lepidoptera, Thysanoptera, Diptera and Coleoptera, and is selective to many beneficials and nontarget insects (Dutton *et al*., 2003). The cotton leafworm, *Spodoptera littoralis* (Boisd.) is the most destructive phytophagous lepidopterous pests in Egypt for it causes various ravages not only for cotton plants, but also other field crops and vegetables. The objective of this research was to evaluate the impact of spinosad on some enzymatic activities, such as esterases (acetylcholine and aliphatic esterase), non-specific esterases (α-and β-esterase), phosphatases (acid and alkaline phosphatase), transaminases (aspartate and alanine transaminase) and carbohydrate hydrolyzing enzymes (amylase and invertase) on 4th instar larvae of the laboratory and the field strains of the cotton leafworm *S. littoralis* (Boisd.) to use it in ICM programmes but, to conserve this product, it is essential that it is used carefully within well planned resistance management strategies.

MATERIALS AND METHODS

Spinosad is produced by the fermentation process from a soil actinomycete, *Saccharopolyspora spinosa* Martz and Yao. Spinosad is obtained from a whole broth extraction, following fermentation of the organism on a feedstock of water, vegetable flours, sugar and animal fat. The commercial product is a mixture of spinosyn A and spinosyn D (BCPC, 2004).

Trade name: Tracer 24% SC (Dow AgroSciences).

Formula: C41H65NO10 (spinosyn A) + C42H67NO10 (spinosyn D)

Activity: Microbial insecticides (macrocyclic lactone insecticides)

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Cotton leafworm strains: A laboratory strain of the cotton leafworm *S. littoralis* (Boisd.) was maintained under constant conditions of 25°C±1 and 70±5% RH and kept of any contamination with chemicals till the time of study in order to obtain a susceptible and homogenous strain. A field strain was collected as egg-masses from Dakahlia Governorate in June, 2004. The obtained egg-mass of cotton leafworm strain were reared in the laboratory as described by El-Defrawi *et al.* (1964).

Preparing samples for enzyme assays: Caster-bean leaves were dipped for 30 sec in an aqueous solution of spinosad at the LC_{50} level (21.061 and 26.969 ppm for the laboratory and the field strains of *S. littoralis*, respectively), then left to dry for 1 h in room temperature before being offered to the 4th instar larvae. Larvae were fed for 24 h on the treated leaves, then transferred to fresh untreated leaves for three days. Haemolymph was obtained by removing one of the prolegs by forceps and applying gentle. Pressure was on the larvae with the fingers and take the haemolymph by syringe. The haemolymph was collected in cold tubes and stored in a refrigerator until the enzyme activities were determined (Sookar *et al.*, 1999; Abd El-Mageed, 2002).

Determination of enzyme activities: Acetylcholine esterase (AchE) and aliphatic esterase (Ali-E) were measured according to the method described by Simpson *et al.* (1964). Alpha esterases (α-E) and beta esterases (α-E) were determined according to the method of Van Asperen (1962). Acid phosphatase (AC-P) and alkaline phosphatase (Alk-P) were determined according to the method described by Powell and Smith (1954). Aspartate transferase (AST)[also known as glutamic oxaloacetic transaminase (GOT)] and Alanine transaminase (ALT) [also known as Glutamine pyruvic transaminase (GPT)] were determined colourimetrically according to the method of Reitman and Frankel (1957). Invertase and amylase based on the digestion of sucrose and starch, which were determined spectrophotometrically according to the method described by Ishaaya and Swiriski (1970).

RESULTS AND DISCUSSION

Determination of esterases activities

Acetylcholine Esterase (AchE): The results obtained in Table 1 noticed that spinosad gave decrease in the acetylcholine esterase (AchE) activity - 39.29% lower than control in the laboratory strain of *S. littoralis* (Boisd.), also the field strain gave the same trend of response but with high level of decrease in the enzyme activity - 52.73% lower than control.

Table 1: Esterases activity in haemolymph of the 4th instar larvae of laboratory and field strains of *Spodoptera littoralis* (Boisd.) After treatment with LC_{50} of spinosad

	Acetylcholine Esterase		Aliphatic Esterase	
	Laboratory strain	Field strain	Laboratory strain	Field strain
Activity	0.68	0.52	3.9	4.3
Control	1.12	1.1	3.2	3.22
Control (%)	-39.29	-52.73	21.88	33.54

Control (%) = (Test - Control)/Control×100

Table 2: Non specific esterases activity in haemolymph of the 4th instar larvae of laboratory and field strains of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of spinosad

	Alpha Esterase		Beta Esterase	
	Laboratory strain	Field strain	Laboratory strain	Field strain
Activity	3.80	4.14	0.66	1.10
Control	4.00	4.00	0.28	0.99
Control (%)	-5.00	3.50	135.71	11.11

Control (%) = (Test - Control)/Control×100

Aliphatic esterase (Ali-E): The data obtained in Table 1 revealed that spinosad gave increase in the aliphatic esterase (Ali-E) activity higher than control in the laboratory strain of *S. littoralis* (Boisd.), it was 21.88%, while recorded the highest increase in the field strain reached 33.54% higher than control.

Determination of non-specific esterases activities

Alpha esterase (α-E): Spinosad recorded the limited decrease or increase in alpha esterase (α-E) activity Table 2. Concerning the laboratory strain of *S. littoralis* (Boisd.) the compound gave a little decrease in α-E activity, it was -5.00% lower than control. In contrary spinosad gave an increase in α-E activity on the field strain, with value 3.50% higher than control.

Beta Esterase (α-E): From the results obtained in Table 2 it could be noticed that spinosad gave increase in beta esterase (α-E) activity reached 135.71% higher than control in the laboratory strain of *S. littoralis* (Boisd.), also the field strain gave the same trend of response but with less level of increase in the β-E activity, it was 11.11% higher than control.

Determination of phosphatase activities

Acid phosphatase (AC-P): Data showed in Table 3 revealed that spinosad gave a negligible effects in acid phosphatase (AC-P) activity with values -0.45 and 0.48 % in both the laboratory and the field strains of *S. littoralis* (Boisd.), respectively.

Alkaline phosphatase (Alk-P): Studying the effect of spinosad on alkaline phosphatase (Alk-P) activity in the

Table 3: Phosphatase activity in haemolymph of the 4th instar larvae of laboratory and field strains of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of spinosad

	Acid phosphatase		Alkaline phosphatase	
	Laboratory strain	Field strain	Laboratory strain	Field strain
Activity	4.38	4.16	6.14	7.12
Control	4.40	4.14	8.80	6.80
Control (%)	-0.45	0.48	-30.23	4.71

Control (%) = (Test - Control)/Control×100

Table 4: Transaminase activity in haemolymph of the 4th instar larvae of laboratory and field strains of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of spinosad

	Aspartate transaminase		Alanine transaminase	
	Laboratory strain	Field strain	Laboratory strain	Field strain
Activity	8.00	8.60	3.90	4.12
Control	8.60	8.22	4.55	4.88
Control (%)	-6.98	4.62	-14.29	-15.57

Control (%) = (Test - Control)/Control×100

laboratory strain of *S. littoralis* (Boisd.) revealed that the tested compound gave high reduction in the enzyme activity, it reached -30.23% lower than control, while in the field strain spinosad caused a little increase in Alk-P activity, it was 4.71% higher than control (Table 3).

Determination of transaminases enzymes activities

Aspartate transaminase (AST): Data in Table 4 indicated that spinosad gave a little of decrease in aspartate transaminase (AST) activity lower than control in the laboratory strain of *S. littoralis* (Boisd.), it was -6.98%, while the tested compound gave a little increase in AST activity in the field, it reached 4.62% higher than control.

Alanine transaminase (ALT): Spinosad gave the same pattern of changes in alanine transaminase (ALT) activity on the laboratory and the field strains of *S. littoralis* (Boisd.) with values -14.29 and -15.57% lower than control, respectively (Table 4).

Determination of carbohydrate hydrolyzing enzymes activities

Amylase: The results obtained in Table 5 noticed that spinosad gave an increase in the amylase activity 46.15% higher than control in the laboratory strain of *S. littoralis* (Boisd.), also the field strain gave the same trend of response but with less level of increase in the enzyme activity 22.39% higher than control.

Invertase: Data in Table 5 revealed that spinosad gave a reduction in invertase activity on the laboratory strain of *S. littoralis* (Boisd.), reached -28.89% lower than control. On the other hand the tested compound gave an increase in the enzyme activity in the field strain, it was 5.98% higher than control.

Table 5: Carbohydrate hydrolyzing enzymes activity in haemolymph of the 4th instar larvae of laboratory and field strains of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of spinosad

	Amylase		Invertase	
	Laboratory strain	Field strain	Laboratory strain	Field strain
Activity	1.90	1.64	3.20	3.90
Control	1.30	1.34	4.50	3.68
Control (%)	46.15	22.39	-28.89	5.98

Control (%) = (Test - Control)/Control×100

The previous researchers mentioned that, spinosad has unique mode of action may reduce the probability of being cross-resistant to other cholinesterase inhibitor insecticides (Liu *et al*., 1999). Moreover, its rapid contact and ingestion activity in insects, causing excitation of the nervous system, leading to cessation of feeding and paralysis (Thompson and Hutchins 1999). Spinosad insecticides act not only on the nAChRs in the CNS of insects, inducing long-term release of the ACh, but also on the GABA receptors, affecting the function of GABA-gated chloride ion channels ((Ishaaya *et al*., 2001;Wu and Fu, 2003). Reviewing the present results as added data, it could concluded that, the change of response to spinosad could be associated with the decrease in AchE activity, likewise the tested compound caused a disturbance in the activities of the other tested enzymes either with increase (Ali-E, β-E and amylase activities) or with decrease (ALT). Finally, to conserve this product, it is essential that to be used carefully within well planned resistance management strategies (Dutton *et al*., 2003).

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