J. Agric. Sci. Mansoura Univ., 34 (5): 5209 - 5219, 2009

INVESTIGATION OF RESISTANCE MECHANISMS IN SPINOSAD AND ABAMECTIN RESISTANT STRAINS OF COTTON LEAFWORM, *Spodoptera littoralis* (BOISDUVAL)

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ABSTRACT

The role of mixed function oxidases (MFO), hydrolytic cleavage enzymes and glutathione-mediated reactions in resistance of cotton leaf worm (CLW) *Spodoptera littoralis* (Boisd.) toward spinosad and abamectin were investigated using spinosad dipping resistant strain (SDRS), spinosad feeding resistant strain (SFRS) and abamectin dipping resistant strain (ADRS) and compared with the parent field strain (PS). Piperonyl butoxide (PB), MDPOC, triphenyl phosphate (TPP) and diethyl maleate (DEM) as mixed function oxidase, esterase and glutathione transferase inhibitors,respectively, were used in this investigation. The effect of cuticle permeability on the two mentioned insecticides was also studied by injecting the fifth instar larvae of *S. littoralis* with the two insecticides and treating them topically. Results emphasized that no role of cuticle penetration was found as resistance mechanism in the two spinosad resistant strains (SDRS and SFRS). While, cuticle permeability of ADRS was considered a responsible factor for the resistance of cotton leaf worm to abamectin. Values of synergistic ratio (SR) of spinosad in SDRS were 0.89, 1.11, 0.80 and 1.78 with PB, MDPOC, TPP and DEM, respectively. While SR values of spinosad in SFRS with the same corresponding synergists were 1.70, 1.96, 1.22 and 2.05, respectively. No significant differences were found between spinosad toxicity alone or with any of tested synergist in both strains SDRS and SFRS. These results suggest that metabolic detoxification enzymes play very limited role in the resistance mechanism(s) to spinosad in cotton leafworm (CLW). Values of SR in ADRS with the same corresponding synergists were 3.33, 2.29, 3.08 and 3.33, respectively. While SR values in the parent field strain were 1.08, 1.00, 0.97 and 1.03, respectively, with the same corresponding synergists. These results indicated high activity of detoxifying enzymes in ADRS compared with the parent field strain.

INTRODUCTION

Four mechanisms of insecticide resistance are the most important and famous; uptake and penetration, excretion, detoxication, and insensitive target site. The biochemical mechanisms (i.e. enhanced activity of detoxification enzymes and target site insensitivity) are frequently reported to be the most important ones (Brattsten *et al.*, 1986; Mullin and Scott, 1992). Insecticide synergists are very helpful in proving preliminary evidence of their involvement in resistance mechanisms (Scott, 1990; Bernard and Philogene, 1993; Ishaaya, 1993). Price (1991) reported that studies on reduced penetration as a resistance mechanism usually carried out by direct method using radiotracers. The radiolabelled insecticide is applied to the insect and then at various time intervals, insects are surface rinsed with an appropriate solvent to remove unpenterated radiolabel. This technique has always been the subject of debate since choice of solvent may extract penetrated label or

may force surface label deeper into the tissues. However, in many cases, especially when small insects are studied, this may be the only practical method of investigation. Our laboratory doesn't have the radiolabeled insecticides. So, we used indirect another alternative method to determine the role of cuticle permeability as a factor in insecticide resistance mechanisms. The same dose of tested insecticide was used topically and injectionally to the fifth instar larvae of resistant and parent strains of cotton leafworm. This study aimed to investigate the resistance mechanism(s) in spinosad and abamectin resistant strains of CLW *S. littoralis*.

MATERIALS AND METHODS

1-1 Chemicals used as insecticides

1-1-a Spinosyns

Spinosad (SC 24 %, Dow AgroSciences Co.)
mixture of 50–95 % of (*2R, 3aS, 5aR, 5bS,9S,13S,14R,16aS,16bR)-2-(6*-deoxy-*2,3,4*-tri-*O*-methyl-*α-L*-mannopyranosyloxy)-*13-(4* dimethylamino-*2,3,4,6*-tetradeoxy-*β-D*-erythropyranosyloxy)-*9*-ethyl *2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b*-hexadecahydro-*14*-methyl-*1H-8*-oxacyclododeca[b]as-indacene-*7,15*-dione (spinosyn A) and 50-5% of *(2S,3aR,5aS,5bS,9S,13S,14R,16aS,16bR)-2-(6*-deoxy *2,3,4*-tri-*O*-methyl-*α-L*-mannopyranosyloxy)-*13-(4*-dimethylamino*2,3,4,6*-tetradeoxy-*β-D*-erythropyranosyloxy)-*9*-ethyl *2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b*-hexadecahydro-*4,14* dimethyl-*1H-8*-oxacyclododeca[b]as-indacene-*7,15*-dione (spinosyn D)

1-1-b Avermectins

Abamectin (EC 1.8 %, Roan Agrochemicals Co.)
mixture of *(10E,14E,16E,22Z) (1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S)-6'-[(S)*-sec-butyl]-*21,24*-dihydroxy-*5',11,13,22*-tetramethyl-2-oxo-(*3,7,19* trioxatetracyclo[*15.6.1.1*^{4,8}.*0*^{20,24}]pentacosa-*10,14,16,22*-tetraene)-*6*-spiro-*2'-(5',6'*-dihydro-*2'*H-pyran)-*12*-yl *2,6*-dideoxy-*4-O-(2,6*-dideoxy-*3-O*-methyl-*α-L*-arabino-hexopyranosyl)-*3-O*-methyl-*α-L*-arabino-hexopyranoside (avermectin B1a) and *(10E,14E,16E,22Z)-(1R,4S,5'S,6S,6'R,8R,12S,13S,20R,21R,24S)-21,22*-dihydroxy-*6'*-isopropyl-*5',11,13,22*-tetramethyl-*2*-oxo-(*3,7,19* trioxatetracyclo[*15.6.1.1*^{4,8}.*0*^{20,2}
^{4}]pentacosa-*10,14,16,22*-tetraene)-*6*-spiro-*2'-(5',6'*-dihydro-*2'H*-pyran)-*12*-yl*2,6*-dideoxy-*4-O-(2,6*-dideoxy-*3-O*-methyl-*α-L*-arabino-hexopyranosyl)*3-O*-methyl-*α-L*-arabino-hexopyranoside(avermectinB1b).

1-2- Chemicals used as synergists

1-2-a Organophosphorus compound

Triphenyl phosphate (TPP) (TG, 99.9 % purity, Sigma Chemical Co.).
O,O,O- tripheny phosphate

1-2-b- Methylenedioxy phenyl compounds

Piperonyl butoxide (PB) (90 % purity, Sigma Chemical Co).
α-[*2*-(*2*-butoxyethoxy)ethoxy]- *4, 5*-(methylenedioxy)-*2*-propyltoluene.
MDPOC (TG , > 98 % purity)

methylenedioxyphenyl oxime-carbamate *3,4* methylendioxybezaldehyde *O*- (metylcarbamoyl)oxime

This compound synthesized and tested as oxidases inhibitor by El-Ghareeb (1993)

1-2-c- Maleate compound

Diethyl maleate (DEM) (TG, > 98% purity, Sigma Chemical Co.)

1.3.Chemicals used as Surfactant

Triton X_{100} (100 % purity, BDH Chem, Ltd. Poole England) Iso-octylphenoxy polyethoxy ethanol polyethoxy.

2- Insects

Different strains of cotton leafworm S. littoralis were used:-

2-1- Parent field strain (PS)

The parent field strain of cotton leafworm, *S. littoralis* was brought as eggs and new hatches larvae from the laboratory of Alexandria university and kept away from insecticidal contamination. The source of this strain collected from cotton fields as big catches from different Lower Egypt Governorates. The supplied insects were reared without exposure to insecticide in the laboratory of Plant Protection at Assiut University for two years to be stable then divided into sub-strains to start the present study.

2-2- Spinosad dipping resistant strain (SDRS)

This strain was obtained by selecting a part of the parent field strain (≈4,000 larvae) with spinosad (LC50 of the previous generation) solution. Selection pressure was applied using dipping technique of 4th instar larvae for 25 generations.

2-3- Spinosad feeding resistant strain (SFRS)

This strain was obtained by selecting a part of the parent field strain (≈4,000 larvae) with spinosad (LC50 of the previous generation) solution. Selection pressure was applied using feeding method to 4th instar larvae for 23 generations.

2-4-Abamectin dipping resistant strain (ADRS)

This strain was obtained by selecting a part of the parent field strain (≈4,000 larvae) with abamectin (LC50 of the previous generation) solution. Selection pressure was applied using dipping technique of 4th instar larvae for 25 generations.

3- Determination of the role of cuticle permeability in resistance mechanism

According to El-Ghareeb, 1994 and Young *et al.*, 2000, one dose was done topically and injectionally by Micro-applicator using the 5th instar larvae with average weight of 180 mg of parent field strain, spinosad dipping resistant strain (SDRS), spinosad feeding resistant strain (SFRS) and abamectin dipping resistant strain (ADRS) were used in this application.

In the injection application, the larvae were injected with 1 µl of spinosad representing 70.59 Ug/larva in each of SDRS and SFRS, while it was 1 µl of abamectin representing 18 and 1.8 Ug/larva in ADRS and PS, respectively. Injection was carried out at pre-true legs using hydrometric needle (26-gauge) syringe that inserted in hemocel. Any larva that bled was removed.

In the topical application technique, the larvae were topically treated

with the same dosage of the tested insecticides on the thorax dorsum. A duplication consisting of 50 larvae per dose was used.

The control was carried out in the same replicates and under the same conditions except the larvae was treated only with the solvent. Every replicate of the treated and control larvae were put in Petri-dishes containing filter paper and fresh castor bean leaves. The Petri-dishes were incubated under optimum conditions for 48 hrs till the results were recorded. The mortalities were counted and the data were corrected using Abbott formula (Abbott, 1925).

4- Role of synergism in resistance mechanism

Two mixed function oxidase inhibitors, piperonyl butoxide (PB) (Casida, 1970), and MDPOC (El-Ghareeb, 1993); the esterase inhibitor, triphenyl phosphate (TPP) (Wood *et al.,* 1984), and the glutathione transferase inhibitor, diethyl maleate (DEM) (Raffa and Priester, 1985) were used. The effect of these enzyme inhibitor synergists on the toxicity of spinosad and abamectin toward the resistant strains of cotton leafworm as indirect line were studied to identify the resistance mechanisms. Synergism techniques had done with identical to the larval-dip and the leaf- dip bioassays with the exception that the larvae were treated with tested synergist prior exposure to the tested insecticides (spinosad or abamectin) (Moulton *et al.*, 1999).

Synergists were prepared as fixed solutions of 20 µg/½µl for TPP, PBO and MDPOC and 5 µg/½µl (sub-lethal dose) for DEM solved in acetone.

Half µl of synergist solution was applied to the thoracic dorsum of the 4th instars larvae (38 mg) by Micro-applicator equipment, using hydrometric needle (20-gauge). Groups of synergist-treated larvae were put in Petri-dishes and hold on temperature of about 26±2 C° for 2 hrs prior to treatment with the tested insecticides. After that the larvae were treated by 6-7 serial concentrations of tested insecticide by both techniques mentioned before (larval-dip bioassay and leaf dip bioassay).

The treated larvae were put in Petri-dishes which was supplied with fresh castor bean leaves and held in the incubator for 48 hrs at 26± 2 C°, 12:12 L:D and 65± 5 RH. Control larvae were treated with ½ µl of acetone only and were supplied with fresh castor bean leaves and held under the optimum conditions. Synergistic ratios (SR) were calculated as LC_{50} of the insecticide alone/ LC_{50} of the same insecticide + synergist

RESULTS AND DISCUSSION

1- Role of cuticle permeability in insecticide Resistance

Fifth instar larvae of resistant and parent strains were treated by the same dose of spinosad and abamectin using surface topical and injection methods as an indirect method to investigate the role of cuticle permeability as resistance mechanism in the resistant tested strains. The single dose which used topically or injectionally of spinosad against the 5th instar larvae of the same strain were the following: 1 ug /larva in parent field strain(PS), 70 ug / larva in spinosad dipping resistant strain (SDRS) and 70 ug / larva in spinosad feeding resistant strain (SFRS). Table (1) shows that mean

percentages of mortality in parent field strain were 31.10 and 33.30 using topical and injection method, respectively. The statistical analysis cleared that no significant difference between the mortality percentages resulted by topical and injection methods of treatment with spinosad insecticide in parent field strain. Also, the mean of mortality percentages (Table 1) in treated larvae of SDRS with spinosad were 43.11 and 46.67 using topical and injection methods, respectively. Whereas % mortality with the same dose using the prementioned corresponding methods were 55.20 and 57.14, respectively in case of SFRS.

Table 1. Percent mortalities resulted from treatment of fifth instar larvae of parent field strain(PS), spinosad dipping resistant strain(SDRS), spinosad feeding resistant strain(SFRS) and abamectin dipping resistant strain (ADRS) of cotton leafworm, *S. littoralis* with spinosad and abamectin.

Insecticide	Dose (ug/larva)	Strain(av. weight = 180 mg/larva)	Method	
			Topically	Injection
Spinosad	1.00	PS	% Mortality ±SE[a] 31.10±4.61	% Mortality ± SE[a] 33.30±3.33
	70.59	SDRS	43.11±0.60	46.67±2.23
	70.59	SFRS	55.20±5.10	57.14±2.80
Abamectin	1.8	PS	33.33±1.40	35.31±0.33
	18	ADRS	13.33±3.33*	63.33±1.33*

a, SE : standard error.
* significant difference (P<0.05, based on T-test).

Statistically, no significant difference was found in means of percentage mortality between injection and topical application methods in the two spinosad resistant strains. These results indicated that there is no role of reduced penetration of spinosad through the cuticle as resistance mechanism in the two spinosad resistant strains of cotton leafworm. Other mechanism(s) may be involved in the two spinosad resistant strains of cotton leafworm.

The present results are in agreement with those obtained by Young *et al.*(2000) who reported that no differences in the rate of penetration of 2'-O-(C14)-methyl spinosyn A across the cuticle of susceptible and selected larvae of tobacco budworm.

Also, the mean percentages of mortality of treated parent field strain with abamectin were 33.33 and 35.31 with topical and injection techniques, respectively, in which no significant difference in the mortality percentage was obtained between topical and injection methods in parent field strain. Meanwhile, the mean percentages of mortality of treated larvae in abamectin dipping resistant strain were 13.33 and 63.33 by topical and injection methods, respectively, in which there was significant difference in mortality percentages of (ADRS) between topical and injection application methods. This suggests that reduced penetration of abamectin is considerd as resistance mechanism(s) in abamectin resistant strain of cotton leafworm.

The present results are in coincident with that obtained by Konno and Scott (1991) who found that the abamectin resistance mechanism in housefly, *M. domestica* was associated with a 2.4-fold decreased rate of

cuticle penetration and altered abamectin binding. On the other hand, the data disagree with the results of Argentine *et al.*(1992) who reported that penetration and excretion factors play no significant role in resistance mechanisms in abamectin in Colorado potato beetle resistant strain.

2-Role of synergism in spinosad and abamectin resistance mechanisms of CLW, *S. littoralis*:

Three major enzyme systems are responsible for detoxification of insecticides. These enzymes are mixed function oxidases(MFO), hydrolytic cleavage enzymes and glutathione-mediated reactions, (Kuhr and Dorough, 1976; Pimprikar and Georghiou, 1979; Fest and Schmidt, 1982 and Casida *et al.*, 1983). The fundamental investigation of synergism has led to much better appreciation of detoxication mechanism in insects and of the basic biochemical processes involved in insecticides resistance. This indirect line of evidence for resistance mechanisms of cotton leafworm strains was used.

2-a- Spinosad resistant strains

Results in tables 2&3 showed that values of synergistic ratio (SR) of spinosad with all synergists in parent field strain were ranged from 0.97 to 1.39. These results indicate that spinosad was insensitive to be metabolized by oxidases, esterases or glutathione transferases in parent field strain of cotton leafworm. In the spinosad dipping resistant strain (SDRS), SR Values of spinosad were 0.89, 1.11, 0.80, 1.78 with PBO, MDPOC, TPP and DEM, respectively (table 2). While these values were 1.70, 1.96, 1.22 and 2.05, respectively, in spinosad feeding resistant strain (SFRS) with the same corresponding synergists (Table 3). This exhibit that there was little difference in SR values between parent strain and each of the two spinosad resistant strains for the same synergists. On the other hand, comparing the LC_{50} values of spinosad without synergist and spinosad with the same synergist in spinosad resistant strains (SDRS, SFRS) by F-test analysis, it is clear that no significant differences were found between spinosad toxicity alone or with any synergist in SDRS or SFRS. This suggest that metabolic detoxification enzyme systems (oxidases, hydrolyses and transferases) play very limited role in the resistance mechanism(s) in spinosad resistant strains SDRS and SFRS. Few studies on resistance mechanisms to spinosad in insects are available. In beet armyworm, *S. exigua* , no role of enzymatic degradation with MFOs, esterases or GSTs was found as resistance mechanism in spinosad resistant strain with 70 fold resistance (Moulton *et al.* 1999).In the same species, Zhang *et al.*, (2003) stated that no obvious relationship between the sensitivity of the beet armyworm to spinosad and the activities of endogenous enzymes of protective system. Treatement with sublethal dose of spinosad, caused no significant difference in the activities of superoxide dismutase, catalase and peroxidase in vivo in the third-instar larvae of the beet armyworm between the control and treated larvae within 0-24 hr. On the other hand, results obtained by Wang *et al.*, (2003) indicated that spinosad inhibited polyphenol oxidase (PPO) activities of the third-instar larvae of the beet armyworm in vitro. Meanwhile in vivo, 0.1 ~ 0.8 mg spinosad L^{-1} induced increasing PPO activity within 4 hrs and then inhibited its activity after 12 hrs. The doses 1.0x10-3 ~1.0 mg spinosad L^{-1} had no effect on the carboxyl esterase activity in vitro. While carboxyl esterase activity in vitro was

significantly increased when third-instar larvae of the beet armyworm were fed on leaves treated with 0.05 mg spinosad L^{-1} . Wang *et al.*, (2005) found that PBO synergist had significant synergistic effect on spinosad against beet armyworm resistant strain and the activity in vitro of microsomal-o-demethylase and glutathione S-transferase was 5.2 and 1.0-fold for resistant and susceptible strain, respectively. The author thought that the detoxifying enzymes (oxidases and glutathione transferases) may have minor roles in resistance mechanism to spinosad in the beet armyworm resistant strain.

In the present study, concerning the penetration and synergism results, it is indicated that penetration and detoxification enzymes(esterases, oxidases and glutathione transferases, did not play significant role in resistance mechanism(s) to spinosad. Therefore, this finding suggest that the insensitive target site(s) of spinosad may be the major mechanism of resistance in the two spinosad resistant strains of cotton leafworm. Young et *al.*(2000) reported that altered target site, excretion and sequesteration may explain the resistance to spinosad in tobacco budworm (RR= 245 fold). Interestingly, the mode of action of spinosad in insects appears to attack the nicotinic acetylcholine receptor and gamma-aminobutyric acid receptor (GABA) (Salgado et *al.* 1997 and Watson, 2001). Also the abamectin modes of action are effective on the GABA receptor on insects (Duce and Scott, 1985). Therefore, if the GABA receptor became insensitive or altered by spinosad selection, it may be the same site insensitive to some other insecticides like abamectin.

Table2: synergistic effect of PB, MDPOC, TPP and DEM with spinosad against spinosad dipping resistant strain (SDRS) and the parent field strain (PS) of cotton leafworm, *S. littoralis*, using the larval-dip technique.

	Insecticide + synergist	Spinosad alone	+PB	+MDPOC	+TPP	+DEM
SDRS	LC_{50}[a]	17627.75	19865.40	15831.36	23492.77	9897.08
	* 95% CL	13935.27 - 22232.53	7000.26- 56332.85	6549.04- 1700249.80	14531.38- 49192.37	6682.69- 15521.65
	Slope±SE[b]	2.85±0.47	1.59±0.30	1.47±0.19	1.34±0.37	2.10±0.36
	Synergistic Ratio (SR)[c]	.....	0.89	1.11	0.80	1.78
PS	LC_{50}[a]	162.03	113.32	115.0	155.40	126.50
	*95% cl	39.99- 275.29	44.49- 203.87	95.91- 136.95	123.02- 194.21	69.50- 209.4
	Slope±SE[b]	1.42±0.49	1.30±0.34	2.0±0.61	2.77±0.44	1.96±0.26
	Synergistic Ratio (SR)[c]	.....	0.97	1.39	1.03	1.26

a, a.i.: active ingredient, µg ml-1
b, SE: standard error
c. SR: LC50 of the spinosad alone on the resistant strain/LC50 of the spinosad on the resistant strain with the synergist
***: 95% confidence limit**

Table3. synergistic effect of PB, MDPOC, TPP and DEM with spinosad against spinosad feeding resistant strain (SFRS) and the parent field strain (PS) of cotton leafworm, *S. littoralis*, using the leaf-dip technique.

	Insecticide + synergist	Spinosad alone	+PB	+MDPOC	+TPP	+DEM
SFRS	LC_{50}[a]	8847.36	5201.52	4500.07	7244.50	4322.96
	*95% CL	6215.02 - 15119.35	3010.69- 6878.58	2334.04- 5244.75	5102.12- 11140.69	1976.63- 6632.17
	Slope±SE_b	1.95±0.42	2.26±0.52	1.09±0.21	1.89±0.44	1.40±0.35
	Synergistic Ratio (SR)c	.....	1.70	1.96	1.22	2.05
PS	LC_{50}[a]	101.87	90.0	95.21	94.52	115.6
	*95% CL	30.51- 194.17	234.20- 700.59	123.79- 252.05	65.96- 694.91	71.44- 176.89
	Slope±SE_b	1.27±0.31	1.46±0.033	2.14±0.43	1.70±0.29	1.89±0.43
	Synergistic Ratio (SR)c	.....	1.31	1.06	1.07	0.88

a, a.i.: active ingredient, µg ml-1

b, SE: standard error

c. SR: LC50 of the spinosad alone on the resistant strain/LC50 of the spinosad on the resistant strain with the synergist

*: 95% confidence limit

2-b- Abamectin dipping resistant strains

Values of SR of PBO, MDPOC, TPP and DEM synergists were 3.33, 2.29, 3.08 and 3.33 against abamectin dipping resistant strain (ADRS), respectively. While they were 1.08, 1.00, .97 and 1.03, respectively, against parent field strain with the same corresponding synergists (Table 4). This means that SR values of abamectin were higher in abamectin dipping resistant strain than parent field strain by 3.08, 2.29, 3.18 and 3.23 fold for PBO, MDPOC, TPP and DEM , respectively. These results indicated high activity of detoxifying enzymes (oxidases, esterases and glutathione transferases) in abamectin resistant strain compared with the parent field strain . The LC_{50} values of abamectin insecticide in ADRS statistically by T-test analysis showed that there were high significant differences between the toxicity of abamectin alone and abamectin with synergists used in the experiment. These results emphasized that the metabolic detoxification enzymes (oxidases, esterases, and glutathione tranferases) play significant role as resistance mechanism in abamectin cotton leafworm resistant strain.

Concerning synergism and penetration results in the present study, metabolic detoxification enzymes; oxidases, esterases and glutation-s-transferases play a major role as resistance mechanism(s) of cotton leafworm to abamectin. Besides, reduced larval cuticle permeability of abamectin resistant strain is considered as a factor responsible for the resistance of cotton leafworm to abamectin. Clark *et al.* (1995) reviewed that a variety of biochemical and pharmacokinetic mechanisms may contribute to avermectin resistance in arthropods. Resistance of *P. xylostella* to abamectin was believed to be associated, in part with monooxygenases, esterases, and glutation-s-transferases (Wright, 1986 and Wu *et al.*, 2001) and also related to carboxylesterase and mixed function oxidase activity (Liang *et al.*, 2001).

Table 4. synergistic effect of PB, MDPOC, TPP and DEM with abamectin against abamectin dipping resistant strain (ADRS) and the parent field strain (PS) of cotton leafworm, *S. littoralis*, using the larval-dip technique.

	Insecticide + synergist	Abamectin alone	+PB	+MDPOC	+TPP	+DEM
ADRS	LC_{50}[a]	1600.80	480.20	700	520.01	481.10
	*95% CL	1491.33 - 1993.62	390.24- 590.4	360.08- 1358.0	315.43- 744.63	345.37- 600.13
	Slope±SE_b	4.13±1.3	2.22±0.49	4.20±0.89	2.59±0.69	2.76±0.79
	Synergistic Ratio (SR)c	.....	3.33	2.29	3.08	3.33
PS	LC_{50}[a]	84.46	78.00	84.20	86.91	81.31
	*95% CL	34.15- 203.67	43.33- 140.40	84.71- 149.39	61.75- 451.83	67.52- 248.55
	Slope±SE_b	1.25±0.38	3.21±0.39	3.05±0.72	1.83±0.69	2.26±0.34
	Synergistic Ratio (SR)c	.....	1.08	1.00	0.97	1.03

a, a.i.: active ingredient, µg ml-1
b, SE: standard error
c. SR: LC50 of the spinosad alone on the selected strain/LC50 of the selected strain with the synergist
*: 95% confidence limit

In conclusion, resistance of the two spinosad resistant cotton leaf worm strains in the present study may be due to insensitive or altered target site(s) that affected by spinosad selection. While reduced cuticle penetration and enhanced metabolic detoxification (high activity of oxidases, esterases and glutathione-s-transferases) may be play a major role as resistance mechanisms in cotton leaf worm to abamectin.

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ميكانيكية مقاومة دودة ورق القطن لمبيدى الاسبينوساد والابامكتين

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تم دراسة دور الانزيمات المؤكسدة متعددة الوظائف والانزيمات المحللة والانزيمات الناقلة فى مقاومة دودة ورق القطن لمبيدى الاسبينوساد والابامكتين باستخدام المثبطات الانزيمية التالية :اثنين من مثبطات انزيمات الاكسدة وهم البيرونيل بيتوكسيد (MDPOC , PB)وواحد من مثبطات انزيمات التحلل وهو TPPوواحد من مثبطات الانزيمات الناقلة وهو داى ايثيل مليت . (DEM)بالاضافة الى دراسة تأثير دور تغذية جليد الحشرة للمبيد وذلك بمعاملة العمر الخامس ليرقات دودة ورق القطن معاملة سطحية وبنفس الجرعة تمت معاملة بالحقن فى كلتا السلالات المقاومة والسلالة الحساسة٠كانت قيم معدل التنشيط لمبيدى الاسبينوساد فى السلالة المقاومة للاسبينوساد بالغمر هى ٨٩ر ٠، ١١ر٠٨ ر ٠، ٧٨ر ١على الترتيب مع منشطات MDPO, , DEM , TPP PB,بينما كان معدل التنشيط للاسبينوساد على السلالة المقاومة بالتغذية لنفس المنشطات وبنفس الترتيب هو ٧ر ١، ٩٦ر ١، ٢٢ر١، ٠٥ر٠ ٢ لايوجد اختلافات معنوية بين سمية الاسبينوساد بمفرده وسمية الاسبينوساد باستخدام المنشطات على سلالتين الاسبينوساد المقاومتين بالغمر والتغذية٠وهذه الدراسة تقترح ان انزيمات الهدم لها دور محدود فى مقاومة دودة ورق القطن لمبيد الاسبينوساد٠قيم معدل التنشيط فى سلالة دودة ورق القطن المقاومة لمبيد الابامكتين مع نفس المنشطات السابقة وبنفس الترتيب هى ٣٣ر ٣، ٢٩ر ٢، ٠٨ر ٣، ٣٣ر ٣وكان معدل التنشيط على سلالة الاباء الحساسة هو ٠٨ر ١، ٠ر ١، ٩٧ر ٠، ٠٣ر ١على الترتيب وهذه توضح النتائج أن نشاط الانزيمات فى السلالة المقاومة للابامكتين أعلى من السلالة الحساسة٠تشير نتائج مدى نفاذية الجليد للمبيد أن ليس هناك دور لنفاذية الجليد لجسم الحشرة فى مقاومة دودة ورق القطن المقاومة لمبيد الاسبينوساد بينما هناك دور لنفاذية الجليد فى المقاومة لمبيد الابامكتين.