EFFICACY AND PERSISTENCE OF METHOXYFENOZIDE AGAINST THE COTTON LEAFWORM *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

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INTRODUCTION

Many compounds are known to interfere with insect development, in particular with larval ecdysis. Ecdysone receptor agonists or moulting accelerating compounds (MACs) are non-steroidal ecdysone analogs and mimic the natural function of the endogenous moulting hormone 20-hydroxy-ecdysone. MACs bind to ecdysone receptor-ultraspiracle protein heterodimer complexes. This ligandheterodimer complex then transactivates a cascade of genes by binding to a DNA ecdysone response element initiating molting. Chemically MACs are described as substituted dibenzoylhydrazines directly acting on ecdysone receptors (Nauen and Bretschneider, 2002). Methoxyfenozide is an insect growth regulator that acts as an agonist of 20-hydroxyecdysone, a key hormone in the molting process (Wing, 1988). Methoxyfenozide (RH-2485) is the latest compound in this class to be developed commercially and is the most potent analog to date against larval Lepidoptera (Ishaaya *et al.* 1995; Le *et al.* 1996; Trisyono and Chippendale1997 and Smagghe *et al.*, 1999). The first sales of methoxyfenozide occurred in 1999, and the first full registrations in the United States were granted for cotton and pome fruits in 2000. Intoxicated larvae cease feeding soon after ingestion and eventually die due to a premature molt. Although methoxyfenozide is highly effective against beet armyworm larvae, this compound has little impact on beneficial arthropods and because its unique mode of action, pest cross resistance to this insecticide is not likely (Wing *et al.*, 1988; Blanco *et al.*, 2000). Therefore, this insecticide is beneficial in integrated pest management systems and preservation of the insect growth regulators is an important concern. Methoxyfenozide appear very good for inclusion in IPM in cotton (Elzen and Elzen, 1999). The purpose of these studies was to examine the efficacy of methoxyfenozide, Runner® to 2nd and 4th instars larvae of the cotton leafworm *Spodoptera littoralis* (Boisd.) and assessment the effect of methoxyfenozide on some biochemical activities on *S. littoralis* (Boisd.)

also evaluate the initial as well as the residual efficacy of methoxyfenozide against these instars larvae with two rates of application 200 and 400 ml/feddan on cotton. In addition assessment the photodegradation of methoxyfenozide.

MATERIAL AND METHODS

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

Bioassay tests:

Test solutions were prepared by making serial dilutions based on ppm of commercial formulation of methoxyfenozide (Runner 24% sc) in water. Castor leaves were dipped for 30 seconds in each concentration then left to dry for one hour. The 2nd and 4th instars larvae of tested strain were confined with treated leaves in glass jars covered with muslin for 24 hrs. Treated leaves were then removed and fresh untreated leaves provided for another days. Test also included a non treated control in which leaves were dipped in water (as a check). Three replicates (each of 20 larvae) were tested for each concentration. Daily inspection was carried out for all treatments and mortality percentages were recorded until the 4th day after treatment. The average of mortality percentage was corrected using Abbott's formula (1925). The corrected mortality percentage of each compound was statistically computed according to Finney (1971). From which the corresponding concentration probit lines (LC-p lines) were estimated in addition to determine 25, 50 and 90% mortalities, slope values of tested compound were also estimated.

Preparing samples for enzyme assays:

Caster leaves were dipped for 30 seconds in an aqueous solution of methoxyfenozide at the LC_{50} level, then left to dry for 1 hour in room temperature before being offered to the 4th instar larvae. Larvae were fed for 24 hours 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 (Sooker *et al.*, 1999 and Abd El-Mageed, 2002).

Determination of enzyme activities:

Aliphatic or carboxyl esterase (Ali-E) was measured according to the method described by Sympthon *et al.* (1964). Alpha esterases (α-E) and beta esterases (β-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 Frankle (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).

Evaluation of residual efficacy:

With the purpose of evaluate the initial as well as the residual efficacy of methoxyfenozide against the 2nd and 4th instars larvae of the cotton leafworm *S. littoralis* (Boisd.) under field condition of Aga district Dakahlia Governorate to evaluate the residual performances in cotton field (Variety Giza 86) during the period from 9th till 24th September, 2004. Four replicates (42 m^2 each) were used / plot. A knapsack sprayer provided with one nozzle delivering (200 liters water / feddan). Samples of leaves were collected at random from each of treated and untreated plants. Samples were taken immediately after one hour of spraying (zero time) and then after 1, 2, 4, 6, 8, 10 and 15 days from application. The collected leaves were instantly transferred to the laboratory and introduced to each of eight groups larvae for each starved 2nd and 4th instars larvae in glass jars covered with muslin cloth, each jar contained twenty larvae and three replicates. Mortality percentages were assessed following feeding period for 24 hrs on treated leaves and three days on untreated leaves for each time intervals tested and corrected with the same previous technique.

Assessment the photodegradation of methoxyfenozide:

This part of study was conducted in order to investigate the role of direct sunlight, ultraviolet rays (UV) and infrared rays (IR) exposure on the degradation of methoxyfenozide.

Procedure: 0.05 ml of methoxyfenozide formulation (equal 120 ppm when dissolved in 100 ml distilled water) was dissolved in adequate amount of acetone and spread as thin film as uniformly as possible on the surface of glass Petri dishes

(10 cm i.d.). The solvent was left to dry at room temperature, then the Petri dishes were divided into three groups and subjected to different treatments, where the first one was exposed to direct sunlight for 1, 3, 6, 9, 12 and 15 days, this test was conducted from the period from 21st September till 6th October, 2004 and the maximum temperature ranged between 30 and 36°C during this period. the second group of Petri dishes were exposed to the short wave of UV rays (1520 W lamp) at a distance of 30 cm for 1,3,6,12 and 24 hours , and the third group of Petri dishes were exposed to IR rays (1580 W lamp) at a distance of 30 cm for 1,3,6,12 and 24 hours.

The methoxyfenozide residues were dissolved and transferred quantitatively from Petri dishes using 100 ml distilled water and finally determined by previous bioassay technique by compared the percentage mortalities resulted from the degradation of methoxyfenozide under direct sunlight, UV and IR rays for each time intervals to the susceptibility LC-p lines of the 4th instar larvae of field strain of *S. littorals* to methoxyfenozide then equivalent toxicity was calculated in ppm. The half life period of residue for methoxyfenozide was calculated using the equation of Moye *et al.*, (1987).

$T\ ½ = Ln_2 / K` = 0.6932 / K`.$ $\quad K` = 1/ tx\ .\ Ln\ a/bx$

Where: K = Rate of decomposition. t_x = Time in days.

a = Initial residue. b_x = Residue at x time.

RESULTS AND DISCUSSION

Susceptibility of the field strain of cotton leafworm to methoxyfenozide:

The present data showed in Table (1) and Figure (1) revealed that the susceptibility of 2nd and 4th instars larvae of the cotton leafworm *S. littoralis* (Boisd.) to methoxyfenozide. It was found that the most effective of methoxyfenozide reached after 4 days from treatment (Feeding period for 24 hrs on treated leaves and three days on untreated leaves) which giving LC_{25} value 0.041 and 4.967 ppm , LC_{50} value 0.878 and 60.785 ppm and the corresponding LC_{90} reached 303.324 and 7087.305 ppm for both 2nd and 4th instars larvae, respectively.

Biochemical impacts:

Data in Table (2) revealed that enzymes activity in haemolymph of the 4th instar larvae of *S. littoralis* (Boisd.) after treatment with LC_{50} of methoxyfenozide following period for 24 hrs on treated leaves and three days on untreated leaves.

Methoxyfenozide gave a decrease in aliphatic esterase (Ali-E), alpha esterase (α-E), Aspartate transferase (AST) and Alanine transaminase (ALT) activities with values of -3.11, -3.00, -0.97 and -18.03 % lower than control, respectively . In contrary the compound gave an increase in beta esterases (β-E), acid phosphatase (AC-P), alkaline phosphatase (Alk-P), Invertase and amylase activities with values of 13.13, 23.67, 2.65, 29.85 and 4.89 % higher than control , respectively.

TABLE (I)

Susceptibility of 2nd and 4th instar larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) filed strain to methoxyfenozide after feeding period for 24 hrs on treated leaves and three days on untreated leaves.

Parameters	2nd instar larvae	4th instar larvae
LC_{25} Its confidence limits at 95%	0.041 0.016 0.106	4.967 2.394 9.947
LC_{50} Its confidence limits at 95%	0.878 0.344 2.240	60.785 27.650 187.860
LC_{90} Its confidence limits at 95%	303.324 118.893 773.846	7087.305 1443.747 99986.168
Slope	0.505 ± 0.073	0.620 ± 0.084

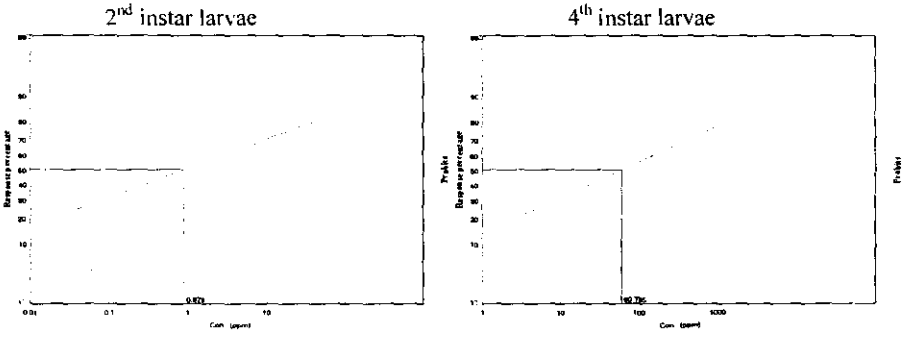

Fig. (1): Log concentration probit lines of susceptibility of 2nd and 4th instar larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) field strain to methoxyfenozide after feeding period for 24 hrs on treated leaves and three days on untreated leaves.

TABLE (II)

Enzymes activity in haemolymph of the 4th instar larvae of *Spodoptera littoralis* (Boisd.) after treatment with LC_{50} of methoxyfenozide following period for 24 hrs on treated leaves and three days on untreated leaves.

	Esterases			Phosphatase		Transaminase		Carbohydrate hydrolyzing enzymes	
	ALI-E	Non specific-E		AC-P	ALK-P	AST	ALT	Amylase	Invertase
		(α-E)	(β-E)						
Activity	3.12	3.88	1.12	5.12	6.98	8.14	4.00	1.74	3.86
Control	3.22	4.00	0.99	4.14	6.80	8.22	4.88	1.34	3.68
% compared to control	-3.11	-3.00	13.13	23.67	2.65	-0.97	-18.03	29.85	4.89

% compared to control = (Test - Control) / Control x 100

Evaluation of residual efficacy:

The residual efficacy on the 2nd and 4th instars larvae of cotton leafworm *S. littoralis* (Boisd.) intervals days with two rates of application 200 and 400 ml / feddan are tabulated in Table (3). Summarized results showed that the initial efficacy on the 2nd instar larvae as determined one hour after spray with two tested rates were 94.915and 98.305%, respectively. The latent toxicity effects of methoxyfenozide were decreased to reach 45.763 and 71.186% at 15 days from application with two tested rates, respectively. The Mean of residual efficacy were 65.38 and 88.14% and also the mean of mortality percentage were 69.07 and 89.41 % on 2nd instar larvae from two tested rates of application with methoxyfenozide, respectively.

Regarding the residual efficacy on the 4th instar larvae, it was appeared from following results that all tested rates of methoxyfenozide gave less initial or latent efficacy compared with the efficacy on 2nd instar larvae. The initial efficacies as determined one hour after spray were 38.983 and 57.627% with two tested rates of application, respectively. The latent effect of methoxyfenozide indicated that the lowest efficacy of methoxyfenozide (20.339 and 27.118 % with two tested rates of application, respectively) recorded at 15 days. The Mean of residual efficacy were 29.06 and 45.16% and also the mean of mortality percentage were 30.29 and 46.72 % on 4th instar larvae from two tested rates of application with methoxyfenozide, respectively. The correlation coefficient between the residual efficacy and time elapsed after application showed to be negative.

TABLE (III)

The bioresidual efficacy of methoxyfenozide to the 2nd and 4th instars larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) at time intervals in days with two rates of application.

Time after insecticidal application (days)		Mortality percentage			
		2nd instar larvae		4th instar larvae	
		200 ml/fed.	400 ml/fed.	200 ml/fed.	400 ml/fed.
Initial mortality (Zero time)		94.915	98.305	38.983	57.627
Residual mortality	1	93.220	96.610	35.593	54.237
	2	93.220	96.610	32.203	56.780
	4	59.322	94.915	30.508	50.847
	6	57.627	93.220	30.508	47.457
	8	57.627	89.830	28.813	44.068
	10	50.847	74.576	25.423	35.593
	15	45.763	71.186	20.339	27.118
Mean of residual mortality		65.38	88.14	29.06	45.16
General mean of % mortality		69.07	89.41	30.29	46.72
r		- 0.838	- 0.800	- 0.852	- 0.728

Zero time after one hour from application.

Effect of direct sunlight, UV and IR rays on photodegradation of methoxyfenozide:

The percent study to investigate the effect of direct sunlight, UV rays and IR rays on the stability and photodegradation of methoxyfenozide.

Data presented in Table (4) showed that the percentage of degradation for methoxyfenozide after being exposed to direct sunlight increased gradually. The results clearly showed that the concentration of remaining for methoxyfenozide was equivalent 118.614 ppm with degradation percentages 1.155 % after three days of exposure to direct sunlight. The concentration of remaining for methoxyfenozide was decreased gradually from 90.650 to 40.759 ppm with degradation percentages 24.458 and 66.034 % after 6 and 15 days from exposure to direct sunlight, respectively. The rate of decomposition (K) of methoxyfenozide was 0.044, the corresponding half life value ($T_{1/2}$) was15.828 days.

Concerning the effect of exposure to UV rays, data in Table (5) showed that the degradation percentages of methoxyfenozide are affected greatly by exposure to UV rays. The concentration of remaining for methoxyfenozide was equivalent 118.614 ppm with degradation percentage 1.155 % after one hour of

exposure. The concentration of remaining was decreased slowly to 118.614, 90.650, 90.650 and 53.206 ppm with degradation percentages 1.155, 24.458, 24.458 and 55.662 % after 3, 6, 12 and 24 hours from exposure to UV rays, respectively. The rate of decomposition (K) of methoxyfenozide was 0.024; the consequent half life value ($T_{1/2}$) was 29.004 hours.

TABLE (IV)

The mortality percentage and equivalent residues toxicity of methoxyfenozide degradation resulted from the effect of exposure to direct sunlight.

Time of exposure in days	Mortality percentage	Equivalent residues toxicity (ppm)	% Degradation of equivalent residues toxicity
0	57.265	120.000	---
1	57.143	118.614	1.155
3	57.143	118.614	1.155
6	54.286	90.650	24.458
9	51.429	69.444	42.130
12	48.571	53.206	55.662
15	45.714	40.759	66.034
K		0.044	
T1/2(days)		15.828	

K = Rate of decomposition.

$T_{1/2}$ = Half-life period (days) of the equivalent toxicity.

In case of exposure to IR rays, data in Table (5) showed that the concentration of remaining for methoxyfenozide was 118.614 ppm after six hours of exposure with degradation percentage 1.155 %. The concentration of remaining was decreased from 60.785 to 2.183 ppm with degradation percentages 118.614 to 69.444 % at 24 hours after exposure to IR rays, respectively. The rate of decomposition (K) of methoxyfenozide was 0.013; the corresponding half life value ($T_{1/2}$) was 54.505 hours.

In this respect, investigators consider that nonsteroidal ecdysone agonists are a new group of insect growth regulators. Methoxyfenozide proved to be the most potent on last-instar larvae of the cotton leafworm *S. littoralis* (LC_{50} = 44.6 (38.0-51.8 mg/litre). The results obtained so far should bring further insights in the selective activity of this new group of insect growth regulators and their potency against caterpillar (Carton *et al.*, 2000a&b). Oberlander *et al.*, (1998) found that the weights of control larvae of *Plodia interpunctella* increased by approximately 400%

by day 2, compared with only a 50% increase in weight when the larvae were treated with 25 ppm of RH-2485 (methoxyfenozide). Similarly, mortality in control larvae was less than 10%, but was as much as 90-100% in larvae reared on diet treated with methoxyfenozide . Pineda *et al.*, (2000) cited that methoxyfenozide (RH-2485) caused more than 90% recorded mortality of newly emerged larvae of *S. littoralis* (Boisd.) at 10 mg a.i. / litre. It could be discussed the enzyme results in the light of the findings of El-Kordy *et al.* (1995) found a significant reduction in the level of GPT (Official name:ALT) on the 4th and 6th instars larvae of *S. littoralis* after the treatment with IGR compounds (Pyriproxfen, Flufenoxuron and Teflubenzuron). Abd El-Mageed (2002) cited that the change of response to chlorfluazuron associated with the increase in β-E, AC-P and Alk-P activities and decrease in Ali-E, α-E, AST, ALT, invertase and amylase activities.

TABLE (V)

The mortality percentage and equivalent residues toxicity of methoxyfenozide degradation resulted from the effect of exposure to UV and IR rays.

Time of exposure in hour	UV rays			IR rays		
	Mortality percentage	Equivalent residues toxicity (ppm)	% Degradation of equivalent residues toxicity	Mortality percentage	Equivalent esidues toxicity (ppm)	% Degradation of equivalent residues toxicity
0	57.265	120.000	---	57.265	120.000	---
1	57.143	118.614	1.155	57.143	118.614	1.155
3	57.143	118.614	1.155	57.143	118.614	1.155
6	54.286	90.650	24.458	57.143	118.614	1.155
12	54.286	90.650	24.458	54.286	90.650	24.458
24	48.571	53.206	55.662	51.429	69.444	42.130
K		0.024			0.013	
$T_{1/2}$(hrs)		29.004			54.505	

K = Rate of decomposition

$T_{1/2}$ = Half-life period (hours) of the equivalent toxicity.

The primary route of intoxication for these IGRs is ingestion. Acute doses induce a prompt cessation of feeding followed by eventual death through induction of a premature larval molt Chronic doses have a chemosterilizing effect by disrupting both oogenesis and spermatogenesis (Wing *et al.*, 1988 and Smagghe and Degheele 1994a&b). Smagghe *et al.* (2000) cited that *S. littoralis* final instars were treated with RH-5849, tebufenozide, halofenozide and methoxyfenozide. These 4 ecdysone agonists induced evagination in cultured imaginal discs, acting as agonists of 20-hydroxyecdysone. The level of toxicity (methoxyfenozide most toxic and RH-5849 least toxic) correlated with the ability to induce evagination . In India

Santharam *et al.* (2004) indicated that methoxyfenozide applied as foliar spray at 200 g a.i. ha^{-1} reduced the larval population of both *H. armigera* and *S. litura* on groundnut significantly and increased pod yield. No phytotoxic symptom was observed when the chemical was applied at a higher rate of 600 g a.i. ha^{-1}. The phototransformation on soil was slow with a half life of 173 d under conditions of 12 h light: 12 h dark. Phototransformation would not be a route of transformation of methoxyfenozide on soil or in water (PMRA, 2004).

Bariselli *et al.* (2003) mentioned that the new products, methoxyfenozide one of them, generally had good larvicidal activity, particularly when compared to the organophosphorus insecticides. No phytotoxic effects to foliage or fruit were reported in provided efficacy trials when Intrepid 240 F insecticide (methoxyfenozide) was applied alone. Finally, it could be concluded that the important role that methoxyfenozide could play in developing integrated crop management (ICM) on cotton in Egypt.

SUMMARY

Methoxyfenozide is an insect growth regulator that acts as an agonist of 20-hydroxyecdysone, a key hormone in the molting process. The purpose of these studies was to examine the efficacy of methoxyfenozide, (Runner®) to 2nd and 4th instars larvae of the cotton leafworm *Spodoptera littoralis* (Boisd.) and assessment the effect of methoxyfenozide on some biochemical activities on 4th instars larvae of *S. littoralis* (Boisd.) and evaluate the initial as well as the residual efficacy of methoxyfenozide against these instars larvae with two rates of application 200 and 400 ml/feddan on cotton. In addition assessment the photodegradation of methoxyfenozide . It was found that the most effective of methoxyfenozide reached after 4 days (24 hrs on treated leaves and three days on untreated leaves), LC_{50} values were 0.878 and 60.785 ppm , the corresponding LC_{90} reached 303.324 and 7087.305 ppm for both 2nd and 4th instars larvae, respectively. Methoxyfenozide gave a decrease in aliphatic esterase (Ali-E), alpha esterase (α-E), Aspartate transferase (AST) and Alanine transaminase (ALT) activities and an increase in beta esterases (β-E), acid phosphatase (AC-P), alkaline phosphatase (Alk-P), Invertase and amylase activities than control. The mean of mortality percentage on 2nd and 4th instars larvae were (69.07 and 89.41 %) and (30.29 and 46.72 %) from two tested rates of application with methoxyfenozide, respectively. The correlation coefficient between the residual efficacy and time elapsed after application showed to be negative. This study was conducted in order to investigate the role of direct sunlight,

ultraviolet rays (UV) and infrared rays (IR) exposure on the stability of methoxyfenozide. The rates of decomposition (K) of methoxyfenozide were 0.044, 0.024 and 0.013, the corresponding half live value ($T_{1/2}$) of the equivalent residues toxicities were 379.872, 29.004 and 54.505 hours under exposure to direct sunlight, (UV) and (IR). So, it could be concluded that the important role that methoxyfenozide could play in developing integrated crop management (ICM) on cotton in Egypt.

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