

Toxicological and biochemical studies of Methylamine Avermectin, a new type of bioinsecticide against the cotton leafworm, *Spodoptera littoralis* (Biosd).

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ABSTRACT

Larvicidal efficacy of a new semi-synthetic avermectin derivative Methylamine avermectin (Radical 0.5% EC) was determined against larval instars of the Egyptian cotton leafworm, *Spodoptera littoralis* (Biosd.) in the laboratory, field and semi field experiments. 2nd and 4th instar larvae showed greatest susceptibility to the Radical in the laboratory experiment. The LC_{50s} values of the 2nd and 4th larval instar after 48 hours were 0.005 and 0.008 ppm, respectively. Radical was tested with recommended dosage (200 ml / 100 liter water) in field; it caused 84.6% reduction of pest population up to day 8 post-treatment. On the other hand, the semi field application of the same recommended dose on the 2nd instar larvae showed general mean 73.6% mortality, 7 days after post-treatment. Also, some biochemical changed in the 4th instar larvae after 48 hours of treatment with tested bioinsecticide were measured. It's clear from the results that activities of trehalase, invertase and acetylcholine esterase were increased in all treatments. Tested bioinsecticide reduced the activity of alkaline phosphatase at all doses compared to untreated larvae. No significant changes in acid-phosphatase activities were observed at all treatment doses. On studying the effect of esterases isozymes patterns, there were no differences in number and position of esterases isozymes between untreated and treated larvae in the whole larval body tissues although each band different in its concentration. The toxicity of the formulation to some beneficial predators was also evaluated in the field. There was no detectable effect of these bioinsecticide on naturally occurring beneficial species.

Key Words: Methylamine Avermectin, Bioinsecticide, *Spodoptera littoralis*, Enzyme, Predators

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is a series pest in Egypt cause a considerable damage to many vegetables and crops (Hafez and Hassan, 1969& Ibrahim and Tawfik,1975).This pest has acquired resistance to different insecticides commonly used in Egypt in chemical used control programs on various crops (El-Guindy *et al.*, 1982 and 1989; El-Baramawy *et al.*, 1991/1992 and Rashwan *et al.*, 1991 and 1992). Therefore, there is always need for finding out new materials having specific modes of actions to replace the conventional insecticides.

Methylamine avermactine (Radical) is a novel semi-synthetic derivative of natural product abamactin in avermectin family. Abamactins (Avermectin B₁) are a fermentation product from the soil microorganisms, *Streptomyces avermectitis* (Burg *et al.*, 1979). Avermactins have been shown to be effective against broad spectrum of

arthropod pests (Putter *et al.*, 1981). This materials act by interfering with the action of gamma aminobutyric acid (GABA) (Fritz *et al.*, 1979).

It blocks post- synaptic potentials of neuromuscular junctions, leading to paralysis. Avermectin B₁ has been shown to inhibit pheromone production (Wright 1984) and inhibit feeding (Pienkowski and Mehring 1983). Radical shows high potential effect against lepidopterous larvae (Mrozik 1994) and White *et al.*, (1997). Abamectin also is more environmentally acceptable because it binds to soil, does not bioaccumulate, and degrades rapidly (Lasota and Dybas 1991).

The aim of this research is to evaluate the effect of radical on larvae of *Spodoptera littoralis* (Boisd.) in laboratory, field, and semi-field strains and study the total protein and carbohydrate contents and the biochemical activity of some enzymes in fourth larval instars, which treated by LC₅₀ and sub lethal dose.

MATERIALS AND METHODS

Insecticide:-

The bioinsecticide, Radical 0.5 % EC was provided by a trade Mark or Agromen Chemicals Co. Ltd.,-China. Radical 0.5 % EC, common name Methylamine Avermectin " 4-deoxy-4 (Methylamine)-(4 R) Avermectin Benzoate (salt) ".

Test Insects:-

A laboratory and Field strain of *Spodoptera littoralis* (Boisd.) was reared on castor bean leaves at $27 \pm 2^\circ \text{C}$ and $65 \pm 5\%$ R.H.

Laboratory Bioassay-

Different concentrations of Radical 0.5 % EC were prepared in distilled water. For each concentration, leaves of castor bean were washed, dried and immersed in tested solution for 20 seconds, then allowed to dry under laboratory conditions. On drying, the treated leaves were placed in individual Petri dishes (5-cm diameter) each treatment (concentration) was replicated three times (20 larvae/replicate), including controls. 2nd and 4th instar larvae were placed on each leaf (replication) and thus total numbers of tested larvae per concentration were 60. For the control test, larvae were fed on leaves immersed in distilled water after drying. The bioassays were kept at constant conditions of temperature $27 \pm 2^\circ \text{C}$. Mortality was assessed after 48 h exposure to bioinsecticides.

Biochemical Studies:-

The biochemical studies of 4th larval instars were measured after 48 hours of treatment. Total carbohydrate and protein contents were measured according to the methods described by Singh and Singh (1977) and Bradford (1976), respectively. Determination of trehalase, and invertase enzymes according to the method described by Ishaaya and Swiriski (1976). Acetylcholine esterase was measured according to method described by Simpson *et al.*, (1964). Acid and alkaline phosphatase activities were determined by the method described by Laufer and Schin (1971).

Electrophoretic Separation of Esterases:-

Esterase's patterns of larval body tissues of treated and untreated larvae of cotton leafworm were separated by using poly-acrylamide gel electrophoresis into groups based on their relative mobility using - naphthal acetate as a substrate, according to Sell *et al.*, (1974)

Field Experiments:-

Field experiments were conducted at Kaha Research Station, Toukh district, Qalyobia Governorate Egypt, during the cotton season 2008 to evaluate the bioactivity of radical against *S. littoralis*. The field area was cultivated with Giza 86

cotton variety on March 27, 2008 and the normal agricultural practices were applied. The experimental area was divided into plates of 1/16 feddan (262.5 m²). The treatment was arranged in randomized complete blocks design (RCBD) with four replicates each. Application of insecticide was on July 11. A motor sprayer was used. The volume of spray solution was 40 liters / feddan. The number of larvae were recorded on one meter lengthwise for five times (four at corners and the last one on plot center), before the spray and on 2, 4, 6 and 8 days after the spraying. Percentage of reduction in the larval population of *S. littoralis* population was calculated according to Henderson and Tilton (1955).

Semi Field Experiment:-

From the same experiment area of the treated cotton leaves were collected after zero time, 1, 2, 3, 4, 5, 6 and 7 days and transfer directly to the laboratory for feeding the second larval instars of *S. littoralis* to estimate the mortality percent.

Statistical Analysis:-

The percentage of mortality was corrected according to the Abbott formula (Abbott, 1925) for correction wherever required. Probit analysis was determined to calculate LC₅₀ (Finney, 1971), through software computer program. Statistical significant differences between individual means were determined by one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

1-Laboratory Bioassay:-

Results presented in Tables (1 and 2) summarized the efficacy of Radical at different concentrations against the 2nd and 4th larval instars of *S. littoralis*. It is cleared from the obtained results that the different applied concentrations of the present bioinsecticide clearly affected the percentage of larval mortality, increasing gradually with an increase with the tested concentration. The total mortality of the 2nd larval instars was detected at concentrations 0.1 ppm Table (1). Where 43.3 and 46.7 % mortality were observed at concentrations 0.005 and 0.003 ppm, respectively. Data in Table (2) showed 100% mortality of 4th larval instars at 0.2 ppm. and 28.3% mortality at 0.003 ppm.

The Probit analysis of the data indicated that the LC₅₀ values of the 2nd and 4th larval instars after 48 hours were 0.005 and 0.008 ppm, respectively.

These results indicated that the Radical bioassay under laboratory was effective against *S. littoralis*. The 2nd instar larvae was more sensitive to the bioinsecticide than 4th instar. Thus, the bioinsecticide Radical showed some promise for the control of this serious pest. David *et al.*, (1985) suggested that the death of insect occur due to nonfunctional mouthparts because of the physiological action of avermectins, rather than antifeedant activity.

The relative toxicity of topically applied avermectin B₁ (Abamectin) was studied by Corbitt *et al.*, (1989). They found that the relative toxicity decreased from the 3rd to the 4th and 5th larval instars of *S. littoralis* but there was at least a 200-fold increase in toxicity from the 5th to the 6th instar. Injection of Abamectin increased its toxicity (> 20-fold) against 5th larval instar of *S. littoralis* compared with topical application but had little effect on the 6th instar; this suggests that varying rates of penetration may partly account for the observed differential toxicity between instars. Paul and Denis (1990) suggested that differential toxicity of Abamectin is due in part to greater metabolism and reduced penetration in 5th instar of *S. littoralis* than in 6th instar.

Table (1): Susceptibility of the 2nd instar larvae of *S. littoralis* to different concentrations of Methylamine Avermectin (Radical) 0.5% EC after 48h of treatment.

Concentrations * (ppm)	Mean of dead** Larvae \pm SE	Mortality%
0.003	9.33 \pm 3.21	46.7
0.005	8.33 \pm 1.15	43.3
0.008	9.33 \pm 0.58	46.7
0.05	19.67 \pm 0.58	98.3
0.08	19.67 \pm 0.58	98.3
0.1	20 \pm 0.00	100
0	0	0
LC ₅₀	0.005	
Upper - lower limits	0.006-0.004	
Slop \pm SD	1.79 \pm 0.03	

*larvae fed on treated casterleaves

**20 larval instars/three replicates were used

Table (2): Susceptibility of the 4th instar larvae of *S. littoralis* to different concentrations of Methylamine Avermectin (Radical) 0.5% EC after 48h of treatment.

Concentrations * (ppm)	Mean of dead** Larvae \pm SE	Mortality%
0.003	6.0 \pm 1.73	28.3
0.005	8.0 \pm 1.0	40
0.01	14.33	71.7
0.05	16.0 \pm 3.0	80
0.06	17.33	86.7
0.08	18.67 \pm 2.52	93.3
0.1	16 \pm 1.73	80
0.2	20 \pm 0.00	100
0.3	20 \pm 0.00	100
0	0	0
LC ₅₀	0.008	
Upper - lower limits	0.010-.0.006	
Slop \pm SD	1.35 \pm 0.008	

*larvae fed on treated caster leaves

**20 larval instars/three replicates were used

The Avermectins are both insecticides and acaricides which are effective by either contact or ingestion. The target for avermectins is the GABA receptor in the peripheral nervous system. Avermectins stimulate the release of GABA from nerve endings and enhance the binding of GABA on the post-junction membrane of muscle cells of insects and other arthropods. This eventually results in an increased flow of chloride ions into the cell, with consequent hyperpolarisation and elimination of signal transduction, resulting in an inhibition of neurotransmission (Jansson and Dybas 1996).

Laboratory bioassays are quicker and less labor intensive than field experiments, and are thus a useful way of screening a range of insecticides to

determine which should be taken forward to full field evaluation. Also, insecticides that are toxic in laboratory bioassays may be less effective in the field, where insects may not experience the same degree of direct exposure as in laboratory bioassays, and may also be able to avoid residues on the plant. (Fitzgerald 2004).

2-Field Experiment:-

The field efficiency of recommended dose (200 ml /100 liter water) of Radical 0.5 % EC against *S. littoralis* is shown in Table (3). The obtained data indicated that the larval mortality decreased over time. The reduction percent decreased from 89.9 after 2 days to 77.6 after 8 days of application with general mean 84.6%.

Similar results were obtained by Attala (2007) who applied the recommended dose of Radical 5% EC during 2006 and 2007 cotton seasons at Fayoum Governorate. The obtained data showed that the percent of reduction for *S. littoralis* population density reached 89.7 and 87.6% for 2006 and 2007 cotton seasons, respectively.

Table (3): Field efficiency of Methylamine Avermectin (Radical) 0.5%EC on population reduction of *S. littoralis* after treatment by recommended dose during 2008 cotton season.

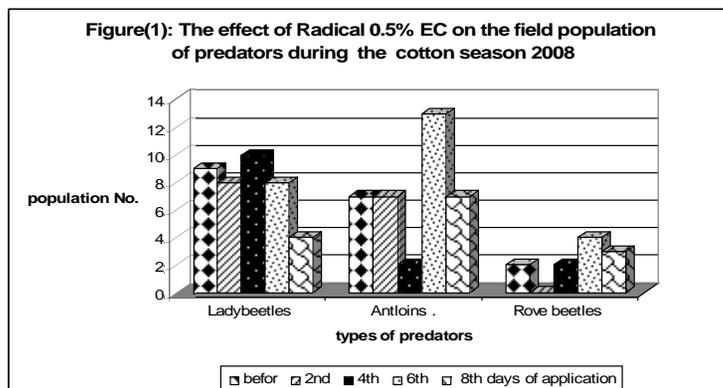
Rate of application	Reduction % after (days)				General mean %
	2	4	6	8	
200ml/ 100 liter water	89.8	87.6	83.3	77.6	84.6

This decline in population in the field may be due to the effects of natural enemies, entomopathogens, or to the physiological condition of the plants (Fitzgerald 2004) or photodegradation of avermectins by the sunlight Mac- Connell *et al.* (1989).

Effect of Radical on field Predators:-

Insecticide should not only suppress the insect pest population, but also be suffer to their natural enemies. Hence, it is imperative to screen the insecticides before incorporating them into Insect Pest Management Programme (Ambrose, 2001).

Since broadcast pesticide applications directed against foliage-feeding insects are most likely to disrupt foraging natural enemies, we compared the toxicity of the field recommended dose of Radical pesticide to a number of beneficial insects that are all important in biological control of cotton pests lady beetles, *Coccinella* spp., aphid lion, *Chrysops* spp. and rove beetle, *Paederus* spp. The obtained data illustrated in Figure (1). It is clear from the figure that the recommended dose of Radical had no adverse effects on any of the tested species after 8 days of application.



Novel insecticides including Avermectins, and Abamectins are believed to be relatively safe to beneficial arthropods (Roberto, *et al.*, 2007 and Fitzgerald 2004)

Hence, beneficial- friendly insecticides must be identified, promoted and incorporated in the Integrated Pest Management Programme. Such bioregional insecticides, including insect growth regulators, chitin synthesis inhibitors, anti-feedants, etc., usually cause lower natural enemy mortality than conventional synthetic insecticides. (Ambrose, 2001).

3-Semi Field Experiment:-

On the other hand, the semifield experiment of the same recommended dose (Table 4) on the 2nd instar larvae showed the same results as the mortality percent ranged between 100 to 51.6 % for the zero time to the 7 days after application, respectively, and the general mean of mortality % was 73.6 %.

Table (4): Corrected mortality % for the 2nd instar larvae of *S. littoralis* after treatment by Methylamine Avermectin (Radical) 0.5% EC during 2008 cotton season.

Rate of application	Corrected Mortality % after (days)								
	0	1	2	3	4	5	6	7	General mean
200 ml/ 100 liter water	100	93.6	81.7	72.8	68.4	62.1	58.5	51.6	73.6

Another semi field experiment was performed by (Attala 2007) with the same recommended dose on the 2nd and 4th larval instar of *S. littoralis* showed that the general mean of mortality percentages for the 2nd and 4th instar larvae were 49 and 17.7%, respectively.

Avermectins are very susceptible to photodegradation (Mac- Connell *et al.* 1989) and due to the protected environment in the semifield experiment slower photodegradation undoubtedly occurred in the laboratory than what would be expected in the field and leaf residue of insecticide could be toxic to the pest larvae longer than that in the field conditions.

4-Biochemical Studies:-

Often, the release of the pesticides pollutes the environment and affects non-target organisms. It is sometimes difficult to measure the effects of this pollution, especially if the poisoning is low-level chronic or intermittent. There may be measurable biochemical changes that are useful as short-term biomarkers (Crane *et al.*, 1995). Thus this work measured some biochemical changes in the 4th instar larvae of *S. littoralis* after 48 hours of treatment with tested bioinsecticide. Because the 4th instar larvae were used in the toxicity bioassay, the enzyme activity assay should use the larvae of the same instar. The effect of the Radical on total amount of carbohydrate and protein of 4th instar larvae shown in (Table 5). The results found that carbohydrate content not significantly changed at all doses except at dose 0.005 ppm; it was significantly decreased (0.20 ± 0.012 mg/ml) than the control (1.53 ± 0.09 mg/ml). A significant increase in proteins content was measured at dose 0.003 ppm (38.44 ± 2.38 mg/ml) comparing with the control (12.1 ± 1.44 mg/ml) but no significant change was observed at LC₅₀ or upper concentration.

The effect of Radical on the activities of trehalase and invertase as well as acid and alkaline phosphatase enzymes of the 4th instar larvae of *S. littoralis* after 48 hours of treatment with LC₅₀, upper and lower concentrations is shown in Table (6). The

data indicate that the specific activity of both trehalase and invertase was increased in all pesticide-treated compared with the parallel control. The highest activities were observed at doses 0.003 and 0.005 ppm, its values were 654.88 and 702, respectively for trehalase and 823 and 873, respectively for invertase. Tested bioinsecticide reduced significantly the activity of alkaline phosphatase at all doses compared to untreated larvae. No changes in acid-phosphatase activities were observed at all treatment doses.

Table (5): The total contents of carbohydrate and protein of 4th instar larvae of *S. littoralis* after 48 hours of treatment with Methylamine Avermectin (Radical) 0.5% EC.

Concentrations * (ppm)	Nutritional Materials (mg/ml)	
	Carbohydrate (mg/ml)	Proteins (mg/ml)
Control	1.53 ± 0.09 ^a	12.1 ± 1.44 ^b
0.003	1.14 ± 0.14 ^a	38.44 ± 2.38 ^a
0.005	0.20 ± 0.012 ^b	13.15 ± 0.76 ^b
0.008	1.17 ± 0.09 ^a	9.21 ± 1.47 ^b

*larvae fed on treated castor leaves

Table (6): Enzyme activities of 4th instar larvae of *S. littoralis* after 48 hours of treatment with Methylamine Avermectin (Radical) 0.5 % EC.

Concentrations (ppm)	Trehalase (µg Glu /g/ min)	Invertase (µg Glu /g/min)	Acid-phosphatase (µg phenol/g/min)	Alkaline phosphatase (µg phenol/g/min)
Control	306.64 ± 8.17 ^c *	318.41 ± 14.61 ^c *	3.28 ± 0.158 ^a *	72.8±13.61 ^a *
0.003	654.88 ± 31.11 ^a	823.63 ±17.89 ^a	3.28 ± 0.19 ^a	30.06±2.49 ^b
0.005	702.76 ± 12.83 ^a	873.26 ± 13.59 ^a	3.55 ± 0.16 ^a	40.01±3.39 ^b
0.008	527.53 ± 26.58 ^b	603.47 ±11.51 ^b	3.04 ± 0.13 ^a	30.12±1.45 ^b

Means have the same letter vertically are no significant difference ($P = 0.001$).

The obtained results indicate that the specific activity of both trehalase and invertase was increased in all pesticide-treated compared with the parallel control. Similar trend was observed with variable insect control agent by Abdel-Hafez *et al.*, (1993), Mohamady (2000) and Fahmy (2005). While, the activity of alkaline phosphatase decreased at all doses, its values were 30, 40 and 30 for 0.003, 0.005 and 0.008 ppm, respectively compared to 72 for control. No changes in acid-phosphatase activities were observed at all treatment doses.

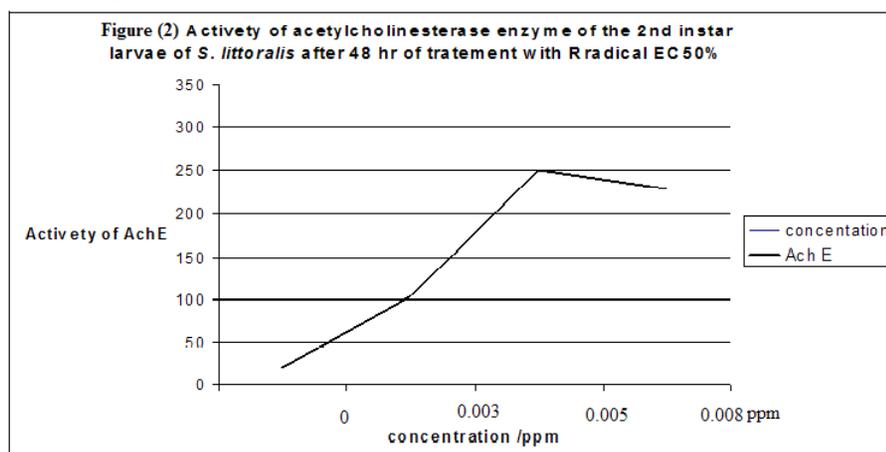
Trehalase is an important enzyme found in hymph and fat bodies (Wigglesworth, 1972) in which insect degrade trehalose to glucose for internal energy supply (Wyatt, 1967), trehalose is activated during moulting to generate production of glucose for chitin build up (Candy and Kilby, 1962). Trehalase and invertase could be used as parameters for assessing the availability of nutrients (Ishaaya and Swirski,

1976). In insects, acid phosphatase known as a lysosomal marker enzyme (Csikos and Sass 1997) is active in the guts (Ferreira and Terra 1980). Alkaline phosphatase is a brush border membrane marker enzyme and is especially active in tissues with active membrane transport, such as intestinal epithelial cells and Malpighian tubules (Ferreira and Terra 1980).

Accordingly, such insect digestive enzymes could be used as a parameter for determining antifeeding activity (Ascher and Ishaaya, 1973 and Ishaaya and Swirski, 1976). The increase of enzymatic activities after larval treatment with the tested bioinsecticide may be attributed to the destructions of midgut epithelial which may lead to intensive release trehalse and invertase. As the insect suffer loss of weight and paralysis in mouth parts muscles causing cessation of feeding, the insect may try to compensate these pathological features by excess production of digestive enzymes for faster growth and development to pass quickly to pupal stage and escape the insecticidal exposure via ingestion. (Fahmy 2005).

5-Acetylcholinesterase activity (AChE):

The role of treatment with Radical with different concentrations (0.003, 0/005 and 0.008 ppm) on AchE activity was estimated and the results of these treatments were illustrated in Fig (2). The obtained results show increase in the activity of this enzyme compared to the control.



An explanation of this increase of AchE activity could be referred to the new mode of action of this newly derived avermectins, which seem to work in a similar manner of other closely related compounds (i.e. metabolites of actinomycetes). This hyperactivity was different from insect control agents which all of them caused either no change or a reduction in AchE activity. It seems as if it works in a reversible manner, producing an extra release of AchE which may prevent principally any message to be sent to the receptor and thus the insect becomes without neural orientation.

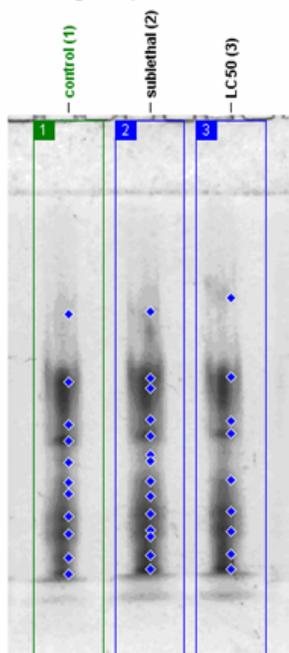
Although the previously used Abamectin was believed to be of non-cholinergic role, it seems that the new derivative used in the present study does. A hypothesis was offered to explain this increase in AchE during the use of a closely related actinomycete-derived compound Spinosad where Salgado (1997) demonstrated that the receptors do so by mimicking the action of Ach at its binding site. Since Ach cannot then also bind, such compounds do not enhance and usually antagonize the response to currently applied Ach. Spinosad, instead of depressing the Ach response, greatly prolongs its duration. The ability of spinosad to prolong the action of AchE indicates that it and Ach can act simultaneously and therefore that

they must act at separate and distinct sites. But Fahmy (2005) demonstrated that AchE activity remained nearly unchanged in case of *S. littoralis* larval treatment with Abamectin compared with the control one, and similar conclusion was achieved by (Radwan 2001) in case of Abamectin treatment. Further studied are needed in more precisely molecular level to strictly detect the mode of action of this newly compound which holds much promise to control insects in a novel mode of action.

6-General Esterase Patterns:

In the present study, esterase isozyme analysis with native polyacrylamide gel electrophoresis revealed that eight common bands No. 1, 2, 4.5, 8, 10, 13 and 14 were detected in the larval body homogenate of *S. littoralis* Fig. (3). Bands No. 3 and 6 were specific to the sublethal concentration treated larvae. On the other hand, esterase bands No. 7, 9 and 12 were found to show by both control and sub lethal concentration treated larvae, while band No. 11 was found to be the only common in both sublethal concentration and LC₅₀ treated larvae and was not detected in the untreated ones.

Figure (3): Polyacrylamide gel zymogram of esterase isozyme patterns in the body tissues of *S. littoralis* stained with α -naphthyl acetate as substrate



Scanning densitometer of esterase patterns in Table (7) revealed that the concentration of bands in most cases varied not only due to treatment but also the concentration of Radical (LC₅₀ and sublethal concentration), For example the concentration of band No.1 in case of sublethal concentration and LC₅₀ treated larval tissue decreased to 87.2 % and 97.98%, respectively, compared to the control one. Similar trend was observed in band No. 5 where both two concentrations decreased compared to the control to 85.29% and 83.66%, respectively. This is not a prevalent trend all over the rest of bands as the treatment showed an enhancement of band concentration in both treated larval tissues as compared to control. This can be seen in bands No. 10 as sublethal concentration and LC₅₀ concentration were 168.81% and

123.75% and in band No. 13 were 140.61% and 108.4% as well as band No. 14 were 156.23% and 166.13%, respectively compared to the check one.

Table (7): Relative concentrations of esterase bands in the body tissues of *S. littoralis* larvae stained with -naphthyl acetate as substrate.

Band No.	Control	Sub lethal Concentration		LC ₅₀	
	Band Conc.	Band Conc.	% relative to control	Band Conc.	% relative to control
1	6.19	4.67	87.2	5.88	97.98
2	23.95	14.05	67.79	34.21	147.19
3	-----	9.92	0	-----	-----
4	6.32	4.56	83.33	6.33	103.31
5	10.77	7.95	85.29	8.74	83.66
6	-----	2.48	0	-----	-----
7	8.03	2.79	40.09	-----	-----
8	6.48	7.27	129.63	5.82	92.48
9	5.49	3.3	69.61	-----	-----
10	9.49	13.87	168.81	11.4	123.75
11	-----	7.96	0	14.21	0
12	12.85	7.9	71.02	-----	-----
13	6.05	7.36	140.61	6.36	108.4
14	4.38	5.92	156.23	7.06	166.13

Conc. = Concentration

On studying the effect of esterases isozymes patterns, there was a difference in number but not in position between untreated and treated larvae although there were variable differences in each band concentration. Radical caused a considerable change also in all band concentrations compared to the control ones, similar findings were offered by Fahmy (2005) whose found that Abamectin caused change in all band concentrations compared to control in haemolymph larvae of *S. littoralis*. Similar quantitative differences were observed also in esterases bands due to many insecticidal treatments including Abamectin were observed by Eid *et al.*, (1979) and Radwan (2001). Such increases in enzyme activities have been showed by Terriere (1984) who described the induction of several detoxication enzymes such as esterases in insects. Such increase in enzyme activities has been shown to protect insects from insecticide poisoning as part of defense mechanism. Saleem *et al.*, (1998) reported that the increased activities of esterase enzymes of *Tribolium castaneum* adults after Cypermethrin treatment may be due to decreased body weight defend against insecticide stress conditions and or increase the energy production.

In conclusion, the results of the study showed that Methylamine Avermectin 0.5 % EC (Radical) is very effective in the control of *S. littoralis*. Therefore, in order to maximize the negative effects of the chemical insecticides on the environment and natural enemies in the management of pests, the bioinsecticide could be integrated into Integrated Pest Management Programmes.

REFERENCES

- Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.
- Abdel-Hafez, M. M.; Abdel-Kawy, A.M.; Mohanna, A. and El-Bishry, M.H. (1993). Effect of IGR / insecticide mixtures on carbohydrate hydrolyzing enzymes of *Spodoptera littoralis* larvae. J. Product. Dev., 1 (2): 165-177.

- Aly, M. M. (1999). Bioactivity of certain plant family Myraceae and other biocides on some pests attacking cotton cultivation. Ph. D. thesis, Institute of Environmental Studies and Research, Ain Shams University.
- Ambrose, P. D. (2001). Friendly insecticides to conserve beneficial insects. *Zoos' Print J.* 16(8): 561-572.
- Ascher, K. R. S. and Ishaaya, I. (1973). Antifeeding and protease and amylase inhibiting activity of fentin acetate in *Spodoptera littoralis* larvae. *Pestic. Biochem. Physiol.*, 3: 326-336.
- Atalla, F. A. (2007). Efficacy of Methylamine avermectin 0.5% EC (Radical), a new type of bioinsecticides on the cotton leafworm, *S. littoralis* (Boisd) and its parasitoid *Microplitis rufiventis* Kok. (Hymenoptera: Braconidae). *Egypt. J. of Biolog. Pest Cont.*, 17(2): 153-157.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle protein dye binding. *Anal. Biochem.* 72:248-254.
- Burg, R. W.; Miller B. M.; Baker E. E.; Birnbaum, J.; Currie S. A.; Hartman, R.; Kong Y. L.; Monaghan, R. L.; Olson G., Putter, I.; Tunac, J. B.; Wallick H.; Stapley, E. O.; Oiwa, R. and Omura, S. (1979). Avermectins, a new family of potent anthelmintic agents producing organisms and fermentations. *Antimicrob. Agents Chemothe.*, 15:361-367.
- Candy, D. J. and Kilby, B. A. (1962). Studies on chitin synthesis in the desert locust. *J. Exp. Boil.*, 39:129-140.
- Corbitt, T. S.; Stgreen, A. J. and Wright, D. J. (1989). Relative potency of abamectin against larval stages of *S. littoralis* (Boisd.), *Heliothis armigera* (Hübner) and *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) *Crop Protection.* 8(2):127-132.
- Crane, M.; Delaney, P.; Watson, S.; Parker, P. and Walker, C. H. (1995). The effect of malathion 60 on *Gammarus pulex* below watercress beds. *Environ. Toxicol. and Chem.* 14: 1181-1188.
- Csikos, G. and Sass, M. (1997). Changes of acid phosphatase content and activity in the fat body and the hemolymph of the flesh fly *Neobellieria* (Sarcophaga) *bullata* during metamorphosis, *Arch. Insect Biochem. Physiol.* 34: 369-390.
- David, K. R.; Namoi, J. T.; and Gray, L. R. (1985). Activity of Avermectin B₁ Against codling Moth (Lepidoptera: Olethreutidae). *J. Econ. Entomol.*, 78: 1067-1071.
- Eid, A. M. H.; Gadallah, A. I.; Abo-Donia, S. A. and Abd El-Lateef, M. F. A. (1979). Effect of Curacron and sumithion on the biochemical system of *S. littoralis* (Boisd) using acrlamid gel electrophoresis. *Proc. Of 3rd pesticide Conf. Tanta Univ.*, 1:55-56.
- El- Baramawy, Z. A.; Elshikh, A. A.; Rashwan, M. H. and Radwan, H. S. A. (1991/1992). Pyrethroids resistance in *S. littoralis* (Boisd.), (Lepidoptera Noctuidae) in lower Egypt. *Bull. Ent. Soc. Egypt. Econ. Ser.*, 19: 41-51.

- El-Guindy, M. A.; Keddis, N. E.; Abd El-Satter, M. M. and Ghonieim, Y.A. (1989). Status of resistance to pesticides in the cotton leaf worm *S.littoralis* (Boisd.), under the present Egyptian cotton pest control programme. Proc. 1st Int. Conf. Ent., (11): 453-462.
- El-Guindy, M. A.; Madi, S. M.; Keddis, M. E; Issa, Y. H. and Abd El-Satter, M. M. (1982). Development of resistance to pyrethroids in field population of the Egyptian cotton leaf worm *S. littoralis* (Boisd.). Int. Pest. Cont. 24: 6-10.
- Ferreira, C. and Terra, W. R. (1980). Intracellular distribution of hydrolases in midgut caeca cells from an insect with emphasis on plasma membrane-bound enzymes, *Comp. Biochem. Physiol.* 66(B): 467– 473.
- Fahmy, N. M. (2005). Comparative toxicity of certain actinomycetes on the cotton leaf worm *S. littoralis* as a microbial insect control agent. Ph. D. thesis, Fac of Sci. Ain Shams Univ., Cairo, Egypt.
- Finney, D. J. (1971). Probit analysis. Cambridge univ., London pp 333.
- Fitzgerald, J. (2004). Laboratory bioassays and field evaluation of insecticides for the control of *Anthonomus rubi*, *Lygus rugulipennis* and *Chaetosiphon fragaefolii*, and effects on beneficial species, in UK strawberry production. *Crop Protec.*, 23:801–809.
- Fritz, L. C., Wang, C. C. and Gorio, A. (1979). Avermectin B a irreversibly blocks postsynaptic potentials at the lobster neuromuscular junction by reducing muscle membrane resistance. Proc. Natl. Acad. Sci. U.S.A. 76: 2062-2066.
- Hafez, M. and Hassan, S. M. (1969). On the corrected identify of Egyptian cotton leafworm *S. littoralis* (Lepidoptera - Noctuidae). Bull. Soc. Ent. Egypt. 35: 107:121.
- Henderson, C.S. and Tilton, E.W. (1955): Tests with acaricides against the brown wheat mite. *J. Econ. Entomol.* 48: 157-161.
- Ibrahim, M. and Tawfik, K. (1975). A report on the survey of natural enemies attacking the American bollworm. Agric. Res. Center, plant Prot. Res. Inst., Giza Egypt (in Arabic).
- Ishaaya, I. and Swirski, E. (1976): Trehalase, Invertase and amylase activities in the black scale *Saissetia oleae*, and their relation to host adaptability. *J. Insect Physiol.* 22:1025-1029.
- Jansson, R. K. and Dybas, R. A. (1996). Avermectins: Biochemical mode of action biological activity and agricultural importance. In *Insecticides with Novel Modes of Action: Mechanisms and Application*; Ishaaya, I., Ed.; Springer-Verlag: New York, NY,
- Lasota, J. A., and Dybas, R. A. (1991). Avermectins, a novel class of compounds: implications for use in arthropod pest control. *Annu. Rev. Entomol.* 36: 91-117.
- Laufer, I.I. and Schin, K.S. (1971). Quantitative studies of hydrolytic enzymes activity in the salivary gland of *Chironomous tentans* (Dipter-Chironomidae) during metamorphosis. *Can. Entomol.* 103:454-457.

- Mac-Connell, J. G.; Demchak, R. J.; Preiser, F.A. and Dybas., R. A. (1989). Relative stability, toxicity, and penetrability of Abamectin and its 8: 9-oxide. *J. Agric. Food Chem.* 37: 1498-1501.
- Mohamady, A. H. (2000). Biochemical and toxicological studies on the effect of some insecticides on the cotton leafworm *S. littoralis* (Biosd). M. Sc. Thesis, Fac. Agric., Zagazig Univ., Egypt.
- Mrozik, H. (1994). Advances in research and development of avermectins. *An. Chem. Soc. symp. Ser.* 551: 54-73.
- Paul, T. C.; Denis W.J. (1990). Activity of Abamectin against larval stages of *S. littoralis* (Boisduval) and *Heliothis armigera* (hübner) (Lepidoptera: Noctuidae) and possible mechanisms determining differential toxicity. *Pest Manag. Sci.*, 29 (1):1 –122.
- Pienkowski, R. L. and Mehring, P. R. (1983). Influence of Avermectin B1 and carbofuran on feeding by alfalfa weevil larvae (Coleoptera-Curculionidae). *J. Econ. Entomol.* 76: 1167-1169.
- Putter, I.; MacConnell, J. G.; Preiser, F. A.; Haidri, A. A.; Ristich, S. S. and Dybas, R. A. (1981). Avermectins: novel insecticides, acaricides, and nematicides from a soil microorganism. *Experientia* 37: 963-964.
- Radwan, E. M. A. (2001). Biological and Biochemical effects of certain insecticides on the spiny bollworm, *Earias insulana* (Biosd.) Ph. D. Thesis, Fac., Sci., Ain Shams Univ.
- Rashwan, M. H.; Elbaramawy, Z. A.; El-Sheikh A. E. and Radwan, H. S. A. (1991/1992). The onset of organophosphate and carbamate resistance among lower Egypt population of the cotton leafworm *S. littoralis* (Boisd). *Bull. Ent. Soc. Egypt, Econc. Ser.* 19 : 211-220.
- Roberto, J. C.; Jeffrey, R. B. and Thomas, P. K. (2007). Susceptibility of two diamondback moth parasitoids, *Diadegma insulare* (Cresson) (Hymenoptera; Ichneumonidae) and *Oomyzus sokolowskii* (Kurdjumov) (Hymenoptera; Eulophidae), to selected commercial insecticides. *Biological Control* 42(1): 48-54.
- Saleem, M.A.; Shakoori, A.R. and Mantle, D. (1998). Macromolecular and enzymatic abnormalities induced by a synthetic pyrethroid, Ripcord (Cypermethrin), in adult beetles of stored grain pest, *Tribolium castaneum* (Herbst) (Coleoptera-Tenebrionidae). *Arch. Ins. Biochem. Physiol.*, 39:144-145.
- Salgado, V. L. (1997). The mode of action of spinosad and other insect control Products. *Down to Earth*, 52(1): 35-34.
- Sell, D. K.; Wilt, G. S., Metcalf, R. L. and Kan Lee, L. P. (1974). Enzymes polymorphism in the corn earworm *Heliothis zea* (Lepidoptera–Noctuidae) hemolymph esterase polymorphism *Can. Entomol.* 106:701-709.
- Simpson, D. R.; Bull, L.D.; and Lidquist, A. D. (1964). A semi-microtechnique for estimation of cholinesterase activity in boll weevil. *Ann. Ent.Soc. Am.* 57(3)367-377.

- Singh, N. B. and Sinh, R. N. (1977). Carbohydrates, lipids and protein in the development stages of *Sitophilus oryzae* and *Sitophilus grannarius*. Ann. Ent.Sos. Am. 107-111.
- Terrier, L. C. (1984). Induction of detoxification enzymes in insects Annu. Rev. Entomol. 29:71-88.
- Wigglesworth, V. B (1972). The principles of insect physiology. 7th Ed., Chapman and Hall Ltd.
- White, S. M.; Dunbar, D. M.; Brown, R.; Cartwright, B.; Cox, D.; Eckel, C.; Jansson, R. K.; Mookerjee, P .K.; Norton, J. A.; Peterson, R. F.; and Starner, V. R., (1997). Emamectin benzoate: a novel avermectin derivative for control of lepidopterous pests in cotton. Proceedings of Beltwide Cotton Conferences, 1078–1082.
- Wright, J.E. (1984). Biological activity of avermectin B1 against the boll weevil (Coleoptera- curculionidae). J. Econ. Entomol.77: 1029-1032.
- Wyatt, G. R. (1967). The biochemistry of sugar and polysaccharides in insects. Adv. Insect Physiol. 4:287-360.

ARABIC SUMMARY

دراسات سمية وكيموحيوية لميثيل أمين أفيرميكتين (الراديكال) كمركب حيوي جديد
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تهدف هذه الدراسة الى إلقاء الضوء على الدور الحيوي الذي تقوم به المبيدات الحيوية المستخلصة من نواتج الأيض الثانوية لعملية تخمر بكتيريا الأكتينومييسيتات. و قياس مدى قدرتها على أحداث إبادة أو أعراض مرضية لحشرة دودة ورق القطن الكبرى (سبوتوبتيرا ليتورالز). فقد أهتمت هذه الدراسة بتأثير المبيد الحيوي الجديد الراديكال (ميثيل أمين أفيرميكتين) على يرقات دودة ورق القطن و ذلك بأجراء تجارب حقلية و نصف حقلية و معملية على الأفة. و قد أظهرت التجارب المعملية مدى حساسية كل من الطور الثاني و الرابع للمبيد الحيوي. حيث كانت (LC₅₀) 48 هي (0.005, 0.008) للطور الثاني و الرابع على التوالي. و بتطبيق الجرعة الموصى باستخدامها في تجربة حقلية و شبه حقلية 84.6% بعد ثمانية أيام من المعاملة بينما أنخفضت الى 73.6% بعد سبعة أيام من المعاملة نصف الحقلية. كذلك أهتمت هذه الدراسة بالتأثير البيوكيميائي على للطور الرابع بعد 48 ساعة من المعاملة و قد لوحظ أنه أدى إلى زيادة نشاط أنزيمات تريهاليز و الأنفرتيز و الأستيلكولين استريز بينما أنخفضت فاعلية أنزيم الألكلين فوسفاتيز بالمقارنة بالحشرات غير المعاملة. كما أظهر التحليل لأنزيم الأستيراز باستخدام خاصية التفريد الكهربائي لهلامة البولي أكريلاميد تأثير الرديكال على الأنزيم حيث ظهرت بعض الأحزمة و أختفت أخرى. كما تمت دراسة فاعلية المبيد في الحقل على بعض الأ الطبيعية المصاحبة للأفة و قد وجد انه لا يوجد للمبيد أي تأثير يذكر على أي من هذه الأعداء.