

TOXICITY AND LATENT EFFECT OF ABAMECTIN AGAINST THE AMERICAN BOLLWORM, *Helicoverpa armigera* (HUBNER)

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ABSTRACT

The susceptibility of the 4th larval instar of the laboratory strain of *Helicoverpa armigera* (Hubner) to Abamectin efficiency was evaluated by using different techniques; i.e., dipping, surface film and immersion methods of technique. Abamectin was bio-assayed after 24, 48 72 and 96 hours from the treatment for each technique. The obtained results revealed that the order of the efficiency of the product used against the tested larvae was the same at both LC₅₀ and LC₉₀ values. The highest efficiency of Abamectin for dipping technique was attained 96 hours. The corresponding LC₅₀ and LC₉₀ values were 51.76 and 93.78 ppm, respectively, while, the lowest efficacy of the product was pronounced at 24 hours, the corresponding LC₅₀ and LC₉₀ values were 93.51 and 236.71ppm, respectively. Whereas the biological activity of the compound against the 4th larval instar fed on treated leaves for 48 and 72 hr. occupied middle situation among its efficiency at 24 and 96 hr. The corresponding LC₅₀ and LC₉₀ values of the tested biocide after 48 hrs., were 81.52 and 214.46 ppm. On the other hand these values after 72 hr. of feeding were 69.92 and 119.15 ppm, respectively. The susceptibility rates of the 4th larval instar to abamectin toxicity at LC₅₀ and LC₉₀ values were 185.93 & 421.34, 135.34 & 378.25, 114.66 & 211.64 and 95.58 & 124.29 ppm for surface film technique and 114.73 & 257.19 & 91.32 & 188.65 & 81.56 & 151.15 and 60.58 & 101.19 ppm for immersion method respectively. On the other hand, the susceptibility index as well as the potency levels at both LC₅₀ and LC₉₀ values increased with increasing the period determination. The latent effects of Abamectin on the pupation as well as the adult emergence was determined. The corresponding EC₅₀ and EC₉₀ values associated to quantal scoring of pupation due to dipping surface film and immersion bioassay were 2.63 & 120.51, 82.02 & 198.12 and 45.45 & 137.42 ppm respectively. Whereas the corresponding IC₅₀ and IC₉₀ values for inhibition of the adult emergence were 20.14 & 91.02, 74.12 & 136.22 and 43.22 & 101.32 ppm respectively.

INTRODUCTION

Cotton occupies a prominent place in Egyptian agriculture and industry. The American bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is one of the most destructive pest in Egypt and other countries. The pest a polyphagous insect can attack more than 181 cultivated and wild species belonging to 45 botanical families (Manjunath, *et al.* 1989). (Khidr 1982) reported that one leave of *H. armigera* may consume as many as 19 cotton squares during its larval life. The author determined the average consumption per larvae as 8 squares, 1 flower and 1 2/3 boll. Thus relatively few larvae per feddan may inflict significant yield losses to cotton yield while large number may cause severe damage. Abamectin benzoate is a novel macrocyclic lactone insecticide derived from natural occurring avermectin isolated by fermentation from the soil microorganism *Streptomyces avermitilis*. The miscellaneous insecticide, Abamectin acts by

stimulating the release of gamma-aminobutyric acid and inhibitory neurotransmitter, thus stomach action. It has limited plant systemic activity, but exhibits translaminar movement Turner and Schaeffer,(1989) . Abamectin benzoate has been reported to be the most active compound against *Grapholitha lobarzewskii* Nowicki, with an LC₅₀ of 0.01 mg/L. Charmillot *et al.* (2007), found that LC₅₀ value for Abamectin benzoate was 2.783 and 1.656ppm against the second larval instar of *Spodoptera littoralis* (Boisd.) and first larval instar of *Pectinophora gossypiella* (Saund.), respectively. The objective of this study was to investigate the relationship between the different methods and the susceptibility of the *H. armigera* as well as to evaluate the latent effects of Abamectin benzoate on pupation and adult emergence resulted from treated larvae under laboratory condition.

MATERIALS AND METHODS

The experiments were conducted under laboratory conditions at 26± 1 C° and 75±5 R.H. at the Bollworms Research Department, Plant Protection Research Institute, Dokki, Agricultural Research Center(ARC).The insect was reared in the laboratory as describe by (khidr 1982)

Tested compound used:

Common name: Avermectin

Trade name: Abamectin 1.9 % EC.

Empirical formula: C₅₆H₈₁NO₁₅ (B_{1a}); C₅₅H₇₉NO₁₅ (B_{1b}) as benzoate salts.
C₄₉H₇₅NO₁₃ (B_{1a}); C₄₈H₇₃NO₁₃ (B_{1b}) as Abamectin.

Bioassay tests:

1- Surface film technique:

Same serial concentration of commercial formulated Abamectin in water as ppm was prepared. Five ml of each concentration were poured in glass Petri-dish (15 cm in diameter), shacked and left till air dryness. Batches of the 4th larval instars were exposed to each concentration. For the untreated; the 4th instars larvae were exposed to the water surface film. The treated and the untreated larvae were fed daily on fresh untreated castor bean leaves till pupation.

2- Immersion technique:

Batches 4th larval instar were immersed in each concentration of Abamectin for 20 seconds, and then transferred and confined daily with fresh untreated castor bean leaves in glass jars covered with muslin till pupation.

3- Dipping technique:

Serial aqueous dilutions of Abamectin benzoate based ppm of commercial formulation of Abamectin benzoate were prepared. Castor bean leaves were dipped in each concentration for 20 seconds then left to dry in the room for one hour. The 4th larval instar were contained with the treated leaves in glass jars covered with muslin for four days. The treated leaves were then removed and fresh untreated leaves were provided for another days till pupation. Bioassay included untreated check in which leaves was dipped in water only.

For each experiment mentioned previously three replicates (each of 20 larvae) were tested for each concentration. Daly inspection was carried

out for all treatments and mortality percentages were recorded till the fourth day after treatments. The average of mortality percentages were corrected using Abbott's formula (1925). The corrected mortality percentage was statistically computed according to Finney (1971). From which the corresponding concentration probit lines (LC-p lines) were estimated. The slope, LC₅₀ and LC₉₀ values were estimated. Toxicity ratio was calculated by dividing the recommended field rate in ppm by LC₅₀ values of each test.

The biocide efficacy and potency levels:

The biocide efficacy, against the 4th instar larval of the pest; the toxicity index method of Sun (1950) is used to determine the degree of toxicity of different insecticides by comparing them with a standard compound. In this study, the equation of Sun(1950) was adopted to find out the degree of susceptibility of the larval instar exposed to or fed on the compound action for different periods as follows:

$$\text{Susceptibility index} = \frac{\text{LC}_{50} \text{ or } \text{LC}_{90} \text{ of the highest susceptible larval instar attained period}}{\text{LC}_{50} \text{ or } \text{LC}_{90} \text{ of the treated larval instars at each period}} \times 100$$

The potency levels (number of folds) were obtained by dividing the LC50 or LC90 for less susceptible larval instars at the period by the corresponding figure for each period.

4- Evaluation of the latent effect:

With the objective of evaluating the latent effect of Abamectin against the 4th larval instar *H. armigera* tested with different techniques, the pupation and adult emergence percentages as well as the abnormalities of the pupae and adults resulted from each test were estimated and recorded. Quintal scoring of pupation expressed as IC₅₀ and IC₉₀ were assessed. The resulted of the present study were statistically analyzed using the analysis of variance.

RESULTS AND DISCUSSION

Susceptibility of the 4th larval instar of *Helicoverpa armigera* to Abamectin:

The susceptibility of the 4th instar larval of the laboratory stain of *H. armigera* to Abamectin was evaluated by using different techniques; i.e., dipping, surface film and immersion methods of technique. Abamectin was bio-assayed after 24, 48 72 and 96 hours from treatment for each technique. Results presented in Table (1) illustrated that the susceptibility of the laboratory strain of *H. armigera* fed continuously on various concentrations of the product until four days from treatment. The obtained data revealed that the order of the efficiency of the product used against the tested larvae was the same at both LC₅₀ and LC₉₀ values. The highest efficiency of Abamectin was attained 96 hours. The corresponding LC₅₀ and LC₉₀ values were 51.76 and 93.78 ppm, respectively, while, the lowest efficacy of the product was pronounced at 24 hours where the LC₅₀ and LC₉₀ values were 93.51 and 236.71ppm, respectively. Whereas the biological activity of the compound

against the 4th instar larval fed on treated leaves for 48 and 72 hr. occupied middle situation among its efficiency at 24 and 96 hr. The corresponding LC₅₀ and LC₉₀ values of the tested biocide after 48hrs. were 81.52 and 214.46 ppm. On the other hand these values after 72 hr. of feeding were 69.92 and 119.15 ppm, respectively. Regarding the slope values, the steepest value was attained 96 hr. post-treatment giving 2.56, whereas the flattest one was noticed at 24 hours from the biocide treatment where the corresponding slope value was 2.08.

Data presented in (Table, 1) recorded that the susceptibility index at LC₅₀ level against the 4th larval instar to Abamectin at 24, 48, and 72 and 96 hr. respectively, were 55.35, 63.49, 74.03 % , respectively, and these values at LC₉₀ were 39.62, 43.73, 78.72% as the susceptibility at 96 hrs , respectively .The potency levels value at 48, 72 and 96 hrs for LC₅₀ were 1.15, 1.34 and 1.81 times as the susceptibility of the larvae at 24 hrs , respectively an at LC₉₀ levels, these values of the potency levels were 1.10 , 1.99 and 2.52 times as the susceptibility at 24 hours; respectively .

Table (1) Susceptibility of the 4th larval instar of the American bollworm *H. armigera* laboratory strain fed continuously on castor bean leaves (dipping) treated with Abamectin formulation.

Period after treatment (hr.)	Slope ± S.D.	LC ₅₀	LC ₉₀	Toxicity ratio	Toxicity Index at		Potency levels at	
					LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
24	2.08	93.51	236.71	8.56	55.53	39.62	1.00	1.00
48	2.16	81.52	214.46	9.81	63.49	43.73	1.15	1.10
72	2.21	69.92	119.15	11.44	74.03	78.72	1.34	1.99
96	2.56	51.76	93.78	15.46	100	100	1.81	2.52

Effect of surface film (contact action):

The obtained data resulted in Table (2) revealed that, the susceptibility of the laboratory strain of *H. armigera* to the contact action of Abamectin for 24, 48, 72 and 96 hr. The data indicated that there was similarity in the trend of the susceptibility in the pest exposed four days to the contact action of the product used at both LC₅₀ and LC₉₀ values. The highest toxicity of Abamectin was noticed after 96 hours of exposure, the corresponding LC₅₀ and LC₉₀ levels were 95.58 and 124.29 ppm, respectively ;while the lowest toxicity was attained 24 hours of exposure, the corresponding LC₅₀ and LC₉₀ levels were 185.93 and 421.34 ppm , respectively. On the other hand, the efficiency of the product used against the 4th larval instar exposed to the surface film of the product for 48 and 72 hours occupied the middle situation among its toxicity at 24 and 96 hours. The LC₅₀ and LC₉₀ values were 135.73 & 378.25 ppm at 48 hours respectively, and 114.66 & 211.64 ppm , respectively at 72 hours.

Concerning the susceptibility index exposed to the contact action of Abamectin benzoate for 24 – 48 and 72 hours at LC50 levels were 51.41, 79.90 and 83.36 % as susceptibility index to the product for 96 hours, whereas these values at LC90 were 29.60, 32.86 and 58.73 % respectively. On the other hand , the potency levels of the larval susceptibility that exposed to the contact action of Abamectin for 48 , 72 and 96 hours at LC₅₀ were 1.36 , 1.62 and 1.95 whereas these values at LC₉₀ were 1.11 , 1.99 and 3.39

times as the susceptibility to the contact action of the product at 24 hours respectively. The toxicity ratio of the product against the 4th larval instar exposed continuously for 24, 48, 72 and 96 hours were 4.30, 5.89, 6.98 and 8.37 indicating moderately effect of the product against the 4th instar larvae.

Table (2) Susceptibility of the 4th larval instar of the American bollworm *H. armigera* laboratory strain exposed continuously to the surface film of Abamectin.

Period after treatment (hr.)	Slope ± S.D.LC ₅₀	LC ₅₀	LC ₉₀	Toxicity ratio	Toxicity Index at		Potency levels at	
					LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
24	1.76	185.93	421.34	4.30	51.41	29.60	1.00	1.00
48	1.91	135.73	378.25	5.89	69.90	32.86	1.36	1.11
72	2.01	114.66	211.64	6.98	83.36	58.73	1.62	1.99
96	2.26	95.58	124.29	9.37	100	100	1.95	3.39

Data presented in Table (3) recorded the biocide toxicity at LC₅₀ and LC₉₀ against the 4th larval instar for different periods, of 24, 48, 72 and 96 hrs. of exposure via immersed technique. The LC₅₀ values high decreased from 114.73 after 24hr. from treatment to 60.58 ppm after four days post treatment. At LC₉₀ values the descending orders of the product toxicity were 257.19, 188.65, 151.15 and 101.19 ppm for 24, 48, 72 and 96 hours respectively.

According to the larval susceptibility, to the efficacy of the compound for 24, 48 and 72 hours at LC₅₀ level were 52.8, 66.5 and 74.28 as the susceptibility to product action for 96 hours respectively, while these values of susceptibility at LC₉₀ level were 39.34, 53.64 and 66.95 % respectively. Whereas the potency levels of the larval susceptibility immersed in the product for 48, 72 and 96 hours at LC₅₀ values were 1.26, 1.41 and 1.89, whereas is these values at LC₉₀ levels were 1.36, 1.70 and 2.54 times as the larval susceptibility after 24 hours; respectively.

Table (3) Susceptibility of the 4th larval instar of the American bollworm *H. armigera* laboratory strain immersed in serial concentrations of Abamectin formulation.

Period after treatment (hr.)	Slope ± S.D.	LC ₅₀	LC ₉₀	Toxicity ratio	Toxicity Index at		Potency levels at	
					LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
24	1.97	114.73	257.19	6.97	52.80	39.34	1.00	1.00
48	2.15	91.32	188.65	8.76	66.34	53.64	1.26	1.36
72	2.22	81.56	151.15	9.81	74.28	66.95	1.41	1.70
96	2.47	60.58	101.19	13.21	100	100	1.89	2.54

Evaluation of the latent effect of Abamectin on basis of quantal scoring of pupation and the inhibition of the adult emergence.

According to this method of assessment the quantal scoring of pupation included the larval stage and the percent of deformed pupae. On the other hand, the inhibition of the adult emergence percentages is based on recorded larval mortality, deformed pupae and remaining number of pupae that failed to produce normal emerged adults. Both quantal scoring as well as

inhibition of the adult emergence percentages was assessed as related to the original number of treated larvae. These percentages were corrected for natural mortality and abnormality in the control by the use of Abbott's formula (1925).

The obtained results are summarized in Table (4) showed that the EC₅₀ and EC₉₀ values of the quantal scoring as well as the IC₅₀ and LC₉₀ values of the inhibition of the adults emergence very clearly illustrate the superiority of dipping technique for the 4th larval instar to the biological action of Abamectin on both pupae and adults of *H. armigera*. However, on basis of these values the efficacy of the bioassay techniques could be descending arranged as follows dipping, immersion and surface film .The corresponding EC₅₀ values of the biocide associated to these techniques were 36.15,45.45 and 82.05 ppm; whereas the corresponding EC₉₀ values were 120.51, 137.42 and 198.12 ppm ; while the corresponding IC₅₀ values associated with the inhibition of the adult emergence were 20.14 , 43.22 and 74.12 ppm and the IC90 values were 91.02 , 101.32 and 136.22 ppm, respectively. It could be concluded that Abamectin had moderately toxicity against the 4th larval instar of *H. armigera* on the basis of toxicity ratio

The present results are in accordance with those published by Pena (1990) he revealed that the surface residues of Abamectin increased the mortality of *Anastrepha suspense* adults when bio-assayed one day after treatment. Also he found that emergence of *suspense* and *toxtrypana curvicauda* was reduced when the fruits were bio-assayed 7-24 and 25 days after Abamectin treatment, respectively.

Table (4) Latent effect of Abamectin on the quantal scaring of pupae and the inhibition of the adults' emergence resulted from 4th larval instar of the American bollworm *H. armigera* laboratory strain treated with Abamectin formulation.

Methods of treatment (hr.)	Quantal scaring of Slope	Quantal scaring at		Inhibition of the adults emergence at	
		EC ₅₀	EC ₉₀	IC ₅₀	IC ₉₀
Surface film	2.04	82.02	198.12	74.12	136.22
Immersion	1.99	45.45	137.42	43.22	101.32
Dipping	2.34	36.15	120.51	20.14	91.02

In this field study Christie and Wright (1990) suggested that the Susceptibility of 5th larval instar of *S. littoralis* was more than the 6th one to Abamectin is due in Part to greater metabolism of 5th instar than 6th one. The Present conclusion was in harmony With (Hua *et al.* 2003) who determined the LC50 and LT50 of Abamectin benzoate to 1st, 3rd, 4th, and 5th larval instars of *S. exigua* in the laboratory. The LC50 values were 28.8-131.7 µg/ml for 1st and 5th larval instars after 48 hours of feeding. The toxicity of Abamectin benzoate was quite different for different instar larvae. The value of LC50 to 5th larval instar after 72 hours of feeding was 8.3 times higher than that of 1st-instar larvae. There was a difference of 7 times in the toxicity of the different concentrations of the insecticide. Other studies, investigative considered that spinosad was relatively slow acting with the

maximum toxicity noted at 72 hours to house fly (Scott, 1998). Mascarenhas and Boethel (1997) cited that spinosad had lower LC50 at 72 hours against the field strain of the soybean, pseudoplusia includes collected from hamburg Louisiana than that of the susceptible USDA strain . Mascarenhas *et al* . (1998) indicated that all field strains of *spodoptera exigua* responded similarly to the laboratory strain for spinosad bioassays , except the strain which collected from Tallulah and Louisiana that had significant higher LC50 .

El-aw (2003) found that the toxicity of spinosad persisted for 5 days o the 2nd and the 4th larval instar of *spodoptera littoralis* under laboratory conditions. Schmandke (2001) stated that spinosad is environmentally degraded by photolysis, oxidation and bacteria. Its half-life in sunlight is 1 day in soil and water and about 2 days on plants. Abdel-maged (2005) reported that the highest efficiency of spinosad against *spodoptera littoralis* was obtained after 72 hours from treatment. he added that degradation of spinosad in the environment occurs mainly by photodegradation. Also, Kang *et al*. (2008): controlled beet armyworm, using Abamectin benzoate in the laboratory. The tested insecticides were taken very high mortalities to 1st to 3rd larva of beet armyworm. Otherwise, there were decreased the death rate from 4th to 6th larva. On the other hand, their value of control effects were relatively good against welsh onion beet armyworm in the field between 87.2 and 90.5% on 10 days after insecticide application. In this field of study khidr *et al* (2012) reposted that the spinosad efficiency of the bioassay reflecting the larval susceptibility of *H.ammigesa* could be descendingly arranged as follows: second, third, fourth, fifth, and sixth larval instars.

Wang *et al* (2013) supported the results obtained in this study they investigated the susceptibility of two populations of *b. dorsalis* to abamectin, deltamethrin and malathion insecticides. Bioassay results demonstrated that Abamectin was more effective insecticide than deltamethrin and malathion. Akel (2014) found that Abamectin was effective against the pupae and has no effect against the prepupae of peach fruit fly, *Bactrocera zonata*. Also Abamectin had no latent effect on the adults emerged from treated prepupae.

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التأثير السام والمتأخر لمركب أباميكيتين على دودة اللوز الامريكيه *armigera Helicoverpa* (Hubner)

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درست حساسية العمر اليرقي الرابع لدودة الامريكيه *H. armigera* لفعل مبيد أباميكيتين كما درس التأثير المتأخر للمبيد المختبر علي كل من العذاري وخروج الفراشات الناتجة من معاملة اليرقات . أستخدم في هذه الدراسة ثلاث طرق من الأختبارات البيولوجية . الطريقة الأولى هي غمر أوراق الخروج في تركيزات مختلفة من المبيد وتغذية العمر اليرقي الرابع علي الأوراق المعاملة لمدة أربع أيام متتالية وتسمي بطريقة **Dipping technique** ، والطريقة الثانية تم غمر يرقات العمر الرابع في تركيزات مختلفة من المركب لمدة ١٥ ثانية وتسمي بطريقة **technique Immersion**، وتشمل الطريقة الثالثة تعريض يرقات العمر الرابع لفيلم من تركيزات مختلفة من المبيد لمدة أربع أيام وتسمي **Surface film technique** وقدرت حساسية اليرقات المعاملة بعد ٢٤ & ٤٨ & ٧٢ & ٩٦ ساعة . وقد أوضحت النتائج أعلي تأثير للمبيد المختبر في جميع الأختبارات البيولوجية الثلاث كان بعد ٩٦ ساعة كما أشارت النتائج أن طريقة **Dipping technique** أعلي تأثير علي العمر اليرقي الرابع . بصفة عامة أمكن ترتيب فعالية طرق الأختبارات الثلاث من حيث درجة سميتها ضد العمر اليرقي الرابع كما يلي طريقة **Dipping technique** ، طريقة **Immersion technique** ثم طريقة **Surface film technique** وكانت قيم **LC₅₀ & LC₉₀** لهذه الطرق ضد اليرقات المختبرة بعد ٩٦ ساعة هي ٣٦.١٥ & ١٢٠.٥١ ، ٤٥.٤٥ & ١٣٧.٩٢ ، ٨٢.٠٢ & ١٩٨.١٢ جزء في المليون علي الترتيب . كما أشارت النتائج أن حساسية العمر اليرقي الرابع للمبيد عند إجراء الأختبارات البيولوجية سالفة الذكر كانت متوسطة أستنادا ألي قيمة **Toxicity ratio** . عند ألقاء الضوء علي التأثير المتأخر للمركب علي عمليات التطور لتكوين العذاري وخروج الفراشات الناتجة من معاملة اليرقات أظهرت النتائج أن قيم **EC₅₀ ، EC₉₀** المطابقة لتكوين العذاري عند إجراء الأختبارات البيولوجية **Dipping technique** ، طريقة **Immersion technique** ثم طريقة **Surface film technique** وبلغت ٣٦.١٥ & ١٢٠.٥١ ، ٤٥.٤٥ & ١٣٧.٩٢ ، ٨٢.١٢ & ١٩٨.١٢ جزء في المليون علي الترتيب ، بينما كانت قيم **IC₅₀ ، IC₉₀** المطابقة لمعدلات تثبيط خروج الفراشات هي ٢٠.١٤ & ٩١.٠٢ ، ٤٣.٢٢ & ١٠١.٣٢ ، ٧٤.١٢ & ١٣٦.٢٢ جزء في المليون علي الترتيب .