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EFFICIENCY OF THE BIOCIDE, EMAMECTIN BENZOATE AND THE HEXAFLUMURON (IGR) IN CONTROLLING THE AMERICAN COTTON BOLLWORM, *Helicoverpa armigera* (HÜBNER) IN LABORATORY

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ABSTRACT

The American cotton bollworm has been found to develop resistance against conventional insecticides. This study is an attempt to investigate the efficiency of emamectin benzoate and hexaflumuron, (biorational insecticides) on the eggs and the 1st instar larvae of *Helicoverpa armigera* (Hübner), under the laboratory conditions. The Hexaflumuron was ineffective insecticide when eggs were treated by field rate compared to emamectin benzoate and alpha cypermethrin that induced 100% mortality only when the recommended field rate was applied. The LC_{50} values of emamectin benzoate, hexaflumuron and alpha cypermethrin on the 1st instar larvae were 0.0041, 2.442 and 11.362 ppm, respectively based on formulated materials. The results showed that emamectin benzoate, hexaflumuron were more effective insecticides compared to alpha cypermethrin. The mean larval duration periods were12.0, 13.7, 15.0 days when treated with the LC_{25} values of emamectin benzoate, hexaflumuron and alpha cypermethrin compared to 16.0 days recorded for control. It is obvious that the three tested insecticides induced highly significant increase in larval and pupal mortality. The longevity of adult moths attained 8.0, 9.0, 11.0 and 15.5 days for female; 8.0, 7.5, 11.0 and 14.0 days for male resulting from the 1st instar larvae treated with emamectin benzoate, hexaflumuron, alpha cypermethrin and control, respectively. Emamectin benzoate and hexaflumuron showed severe reduction in female moth fecundity, eggs fertility and hatchability. Therefore, the two compounds could be recommended to control this pest, especially they are environment friends.

Key words: *H. armegira*, longevity, biocide, IGR, fecundity, fertility, emamectin benzoate, hexaflumuron.

INTRODUCTION

The American cotton bollworm, *Helicoverpa armigera* (Hübner) is a polyphagous multivoltine and economically important pest of cotton, tomato and other crops (Nair *et al*., 2010). It causes severe damage and loss to a wide range of foods, fiber, oil, fodder, vegetable, horticultural, ornamental, aromatic and medicinal plants (Nadda *et al*., 2012). This pest exhibited resistance to the conventional chemicals such as endosulfan, pyrethroids, organophosphates and carbamates (Ahmad *et al*., 2003). Potential non-conventional alternatives are biorational insecticides (spynosins, oxadiazines, avermectins, *etc*.) which have no cross resistance with pyrethroids, are more specific, require lower rates, degrade more rapidly in the environment, and have lower mammalian and aquatic toxicity (Holloway *et al*., 1999). Therefore, new and environmentally friendly approaches are urgently needed for controlling this pest. Using new types of insecticides, originated from natural agents or products that disrupt the physiological processes of the target pest, could be useful as an alternative for the integrated management approach (Smagghe *et al*., 2003). From this stand point, the present work was designed to study the efficiency of two compounds of newer generation of insecticides, *i.e.*, emamectin and

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hexaflumuron in comparison with the traditional compound alpha cypermethrin against the American cotton bollworm.

MATERIALS AND METHODS

The present investigation was carried out in the laboratories of Plant Protection Dept., Fac. Agric, Zagazig Univ. and Plant Prot. Res. Institute, (Sharkia Branch), Dokki, Giza, Egypt during 2012-2014.

Materials

Insect culture

A laboratory strain of the cotton bollworm, *H. armigera* originated from cotton fields of Mansoura region was used. Insects were reared on an artificial diet under laboratory conditions of 26 ± 1°C, 80 ± 5% RH and a photoperiod of 16 : 8 hr., (L: D) (Saber *et al*., 2012). The artificial diet consists of (215 g) kidney beans boiled in water, (32 g) dried active yeast, (2.25 g) ascorbic acid and (11.50 g) agar to which 500 ml distilled water was added (Abd El-Hafez *et al.,* 1982). To prevent cannibalism, the first instar larvae were transferred into individual glass vials (2 × 8 cm) and maintained until pupation. On emergence, 20 pairs of adult moths were released into 20 × 30 cm plastic containers with 1:1 sex ratio for mating and egg-laying. The adults were fed on a 10% bee honey solution.

Insecticides used

Emamectin benzoate a semi synthetic bioinsecticide compound isolated from fermentation of *Streptomyces avermitilis* (Burg *et al*., 1979). Hexaflumuron a newer class of IGR ingested and systemic insecticide. Alpha cypermethrin a pyrethroid compound.

Methods

Toxicity to eggs

The dipping technique for egg stage was applied. Series of insecticide dilutions in water were prepared from the stock solution (ten to fourteen dilutions). Three replicates were used for each concentration and each replicate contained 25 of 1-day old eggs of American bollworm.

Toxicity to larvae

The larval diet was sprayed with the prepared dilutions of the tested insecticides using 1 liter hand sprayer (atomizer). Diet of the control was sprayed with water only. Each concentration was replicated four times. Each replicate was represented by a patch of 25 newly hatched larvae (0.0-6.0 hr. old) that were transfered to the treated diet in 8 cm diameter petri dishes 30 minutes post treatment. Treated and control petri dishes were covered with tissue papers below the glass cover to prevent larval escaping then placed in an incubator running at constant conditions of 26 ± 1°C and 80 ± 5% RH. After 24 hr., of exposure and feeding dead and alive larvae were counted and the mortality percentages were calculated and corrected according to Abbott's formula (1925). Concentration-mortality regression lines were calculated and drawn according to the method described by Finney (1971). Toxicity index (T.I.) and relative toxicity were determined by using Sun's equation (1950) as follows:

$$\text{Toxicity index (T.I.)} = \frac{LC_{50} \text{ or } LC_{90} \text{ of compound (A)}}{LC_{50} \text{ or } LC_{90} \text{ of compound (B)}} \times 100$$

Where:

A is the most effective compound.

B is the other tested compound.

Relative potency (RP) values were measured according to the method describe by Zidan and Abd El-Megeed (1988) as follows .

$$\text{Relative potency (RP)} = \frac{LC_{50} \text{ of the lowest toxic insecticide}}{LC_{50} \text{ of the tested insecticide}}$$

Latent effect

Three replicates of 25 newly hatched larvae each were considered for each insecticide. The LC_{25} only was applied by spraying on the artificial diet that was poured in petri dishes. The dishes were covered as mentioned above, then incubated under the same previously mentioned conditions. After 24 hr., larval mortality was recorded, and the survived larvae of each treatment were separated individually into glass tubes (2x7cm) containing 5 g of untreated diet, covered with a piece of absorbent cotton and kept under the same conditions. The latent effect of the tested insecticides on certain biological aspects of *H. armigera* was studied by the following survived larvae.

Larvae were examined daily to record the pupal weight, the date of pupation and date of adult emergence. The larvae were left to pupate in the vials. Pupae were tansferred individually to other clean vials and incubated till moth emergence. The adults were sexed by the difference in color of male and female. From these records, the following biological aspects were measured: duration of larval and pupal stages; (%) larval and pupal mortality; (%) pupation, pupal weight and pupal deformation; (%) adult emergence and longevity. Sex ratio (as % of females); female fecundity (No. of eggs/ female); (%) eggs fertility and (%) Hatchability of eggs.

The results were analyzed by one way analysis of variance (ANOVA) using costat statistical software. When the ANOVA statistics were significant ($P<0.05$), means were compared by the Duncan`s multiple range test.

RESULTS AND DISCUSSION

Toxicity of Emamectin Benzoate, Hexaflumuron and Alpha Cypermethrin to Eggs and the 1st Instar Larvae

Ovicidal action

Obtained results clear that the IGR hexaflumuron was no effective against *H. armigera* eggs when applied by dipping technique, as the percentage of mortality in exposed eggs did not exceed 3%. On the other hand, emamectin benzoate and alpha cypermethrin induced 100% kill when the recommended field rate was applied in laboratory. However, decreasing the concentration was associated with decreased ovicidal action. For instance, toxicity data of emamectin benzoate and alpha cypermethrin cleared that the LC_{50} recorded 0.935 and 3.792 ppm after 24 hr., respectively (Table 1 and Fig. 1).

Toxic effect on the 1st instar larvae

Toxicity data of emamectin benzoate, hexaflumuron and alpha cypermethrin clear that the LC_{50} recorded 0.0041, 2.442 and 11.362 ppm after 24 hr., respectively (Table 2 and Fig. 2). Emamectin benzoate and hexaflumuron were more toxic to the 1st instar larvae of *H. armigera* compared to alpha cypermethrin however, emamectin benzoate proved to be the most potent insecticide.

Toxicity index revealed that emamectin benzoate was the standard (100.00) at LC_{25}, LC_{50} and LC_{90} meanwhile, relative potency of alpha cypermethrin was the least active one and was being the standard (1.00). In this respect, Parsaeyan *et al.* (2013) investigated the lethal and sublethal effects of emamectin benzoate and alpha cypermethrin on the third instar larvae of *H. armigera* by residue contact methods at 26 ± 1°C, 70 ± 5% RH and a photoperiod of 16:8 hr. (L: D) under laboratory conditions. LC_{50} values on larval stage of the pest of the two compounds were 1.75 and 127.74 µg a.i./ml, respectively. Data of the present work indicate clearly that emamectin benzoate and alpha cypermethrin were more potent than in the results obtained by Parsaeyan *et al*. (2013) on the same insect. This variation may be attributed to the instar treated and the conditions of application. Dastjerdi *et al.* (2008) recorded an intermediate data as they found that the LC_{25} of hexaflumuron to *H. armigera* attained 0.31 µg a.i./ml.

Latent Effect of Emamectin Benzoate, Hexaflumuron and Alpha Cypermethrin on some Biological Aspects

The first instar larvae of *H. armigera* were fed on artificial diet treated with the LC_{25} of the tested insecticides once, then on untreated diet. The effect of such treatment on some biological aspects was studied. Data obtained (Tables 3,4 and Figs. 3 , 4) are as follows:

Larval duration

Data in Table 3 clear that the mean larval duration recorded 12.0, 13.7, 15.0 days for emamectin benzoate, hexaflumuron and alpha cypermethrin, respectively compared to 16.0 days for control larvae. All the tested compounds shortened the larval duration. However, emamectin benzoate caused the severest decrease. Analysis of variance revealed highly significant differences in the larval duration of the treated larvae and control ones.

Table 1. Toxicity of emamectin benzoate, hexaflumuron and alpha cypermethrin to *H. armigera* eggs under laboratory conditions

Insecticides	Slope	Lethal concentrations (µg a.i./ml)			Toxicity index			Relative potency (fold)		
		LC_{25} ppm	LC_{50} ppm	LC_{90} ppm	LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}
Emamectin benzoate	0.954	0.184	0.935	20.5	100.00	100.00	100.00	4.472	4.055	3.368
Hexaflumuron	0	0	0	0	0	0	0	0	0	0
Alpha cypermethrin	1.017	0.823	3.792	69.059	22.35	24.65	29.68	1.00	1.00	1.00

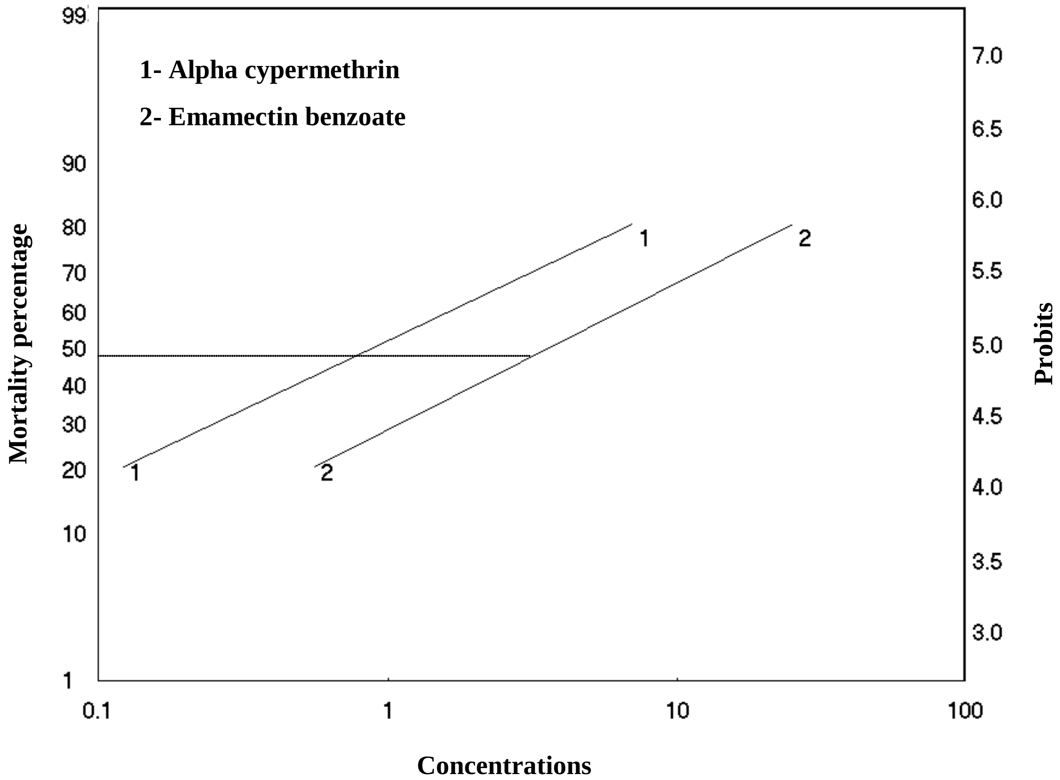

Fig. 1. Mortality regression lines of emamectin benzoate and alfha cypermethrin against eggs of *H. armigera* after 24 hr. exposure

Table 2. Toxicity of emamectin benzoate, hexaflumuron and alpha cypermethrin to the 1st instar larvae of *H. armigera* under laboratory conditions

Insecticides	Slope	Lethal concentrations (µg a.i/ml)			Toxicity index			Relative potency (fold)		
		LC_{25} ppm	LC_{50} ppm	LC_{90} ppm	LC_{25}	LC_{50}	LC_{90}	LC_{25}	LC_{50}	LC_{90}
Emamectin benzoate	1.718	0.0017	0.0041	0.023	100.00	100.00	100.00	2751.17	2771.21	2667.52
Hexaflumuron	1.886	1.072	2.442	11.675	0.15858	0.16789	0.19700	4.36287	4.6527	25.1277
Alpha cypermethrin	1.75	4.677	11.362	61.353	0.03634	3.60851	0.03748	1.00	1.00	1.00

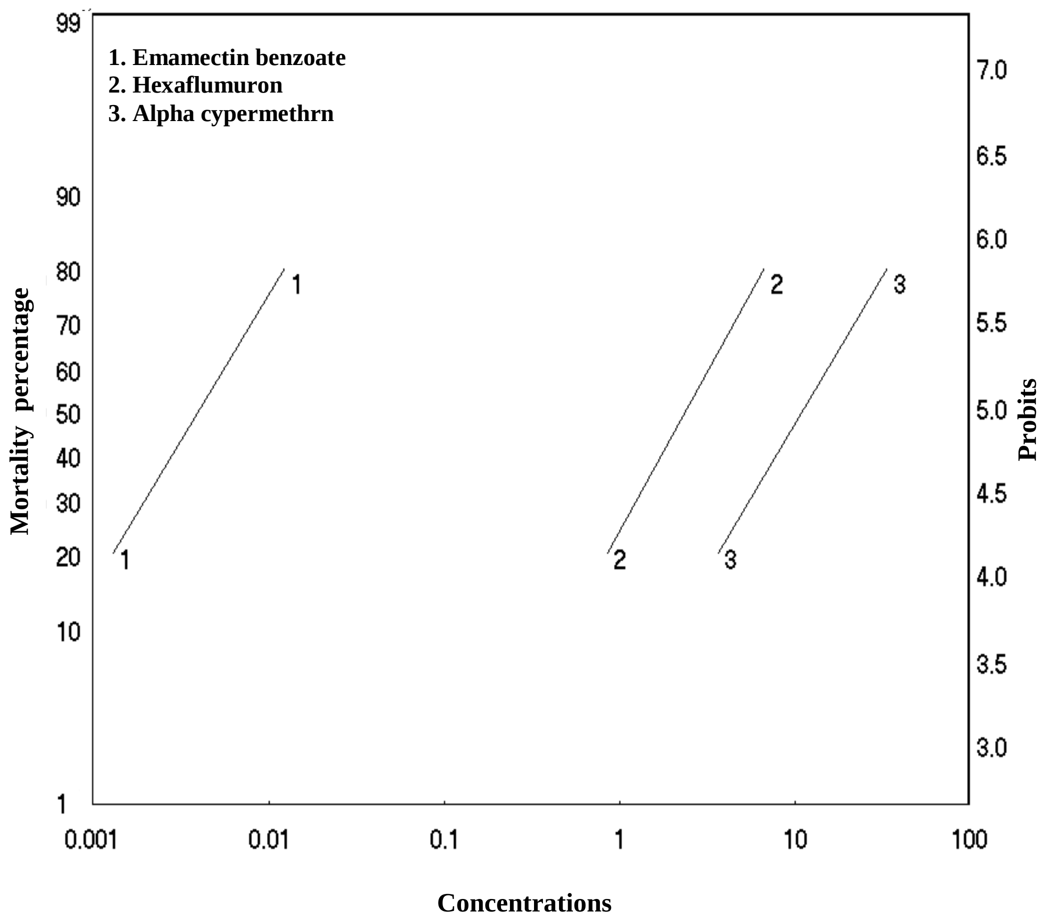

Fig. 2. Mortality regression lines of emamectin benzoate, hexaflumuron and alpha cypermethrin against the 1st instar larvae of *H. armigera* after 24 hr., exposure

Table 3. Effect of LC_{25} of emamectin benzoate, hexaflumuron and alpha cypermethrin on some biological aspects when the 1st instar larvae of *H. armigera* were fed on treated diet for 24 hr.

Insecticides	Larval duration (day)	Larval mortality (%)	Pupation (%)	Pupal weight (mg)	Pupal mortality (%)	Pupal duration (day)	Adult emergence (%)	Sex ratio of females (%)	Deformation of pupal stage (%)
Emamectin benzoate	12.0^{d}	67.0^{a}	33.0^{c}	0.2100^{bc}	31.5^{b}	15.0^{b}	71.0^{b}	44.8^{a}	30.0^{a}
Hexaflumuron	13.7^{c}	56.0^{a}	44.0^{c}	0.1889^{c}	54.0^{a}	16.5^{a}	45.8^{c}	39.0^{a}	25.0^{a}
Alpha cypermethrin	15.0^{b}	41.0^{b}	58.7^{bc}	0.2317^{b}	36.0^{b}	15.0^{b}	64.0^{b}	45.5^{a}	15.0^{b}
Control	16.0^{a}	3.5^{c}	96.5^{a}	0.3815^{a}	4.5^{c}	13.0^{c}	95.5^{a}	50.8^{a}	5.8^{c}
LSD	0.74^{**}	12.05^{**}	12.05^{**}	0.041^{**}	15.95^{**}	0.69^{**}	15.61^{**}	13.49^{NS}	7.65^{**}

NS = Not significant * Significant at 0.05 level of probability. ** Highly significant at 0.01 level of probability.

Table 4. Effect of LC_{25} of emamectin benzoate,hexaflumuron and alpha cypermethrin on the reproductive characteristics and adult longevity of *H. armigera* after diet treatment of the 1st instar larvae for 24 hr.

Insecticides	Preovi position (day)	Ovi position (day)	Post oviposition (day)	Female longevity (day)	Male longevity (day)	Fecundity (No. of eggs/female)	Fecundity of control (%)	Fertility (%)	Hatchability (%)	Deformation of adult (%)
Emamectin benzoate	4.25a	2.25b	1.50c	8.00c	8.00bc	153.00c	19.69	59.00c	36.75c	32.50a
Hexaflumuron	3.25bc	4.00b	2.00c	9.00bc	7.50c	262.00bc	33.72	45.50c	34.50c	28.25a
Alpha cypermethrin	3.75ab	4.25b	3.00ab	11.00b	11.00b	383.00b	49.29	70.00b	53.25b	20.25b
Control	2.87c	8.63a	4.00a	15.50a	14.00a	777.00a	100.0	90.00a	93.50a	4.50c
LSD	0.08^{**}	2.11^{**}	1.15^{**}	1.87^{**}	2.38^{**}	164.63^{**}		9.89^{**}	9.30^{**}	5.87^{**}

Fig. 3. Deformation of pupal stage

[(1, 3, 4, 6 and 7 deformed pupae), (2 and 5 normal pupae)]

(1) (2) (3)

Fig. 4. Deformations of adult stage [(1 and 3 deformed adults), (2 normal adult)]

Larval mortality percentage

As shown in Table 3, the percentages of larval mortality attained 67.0, 56.0, 41.0% in the larvae treated with the LC_{25} of emamectin benzoate, hexaflumuron and alpha cypermethrin, respectively compared to 3.5% mortality in control larvae. It is obvious that the three tested insecticides induced highly significant increase in percent larval mortality.

Pupation percentage

Data in Table 3 indicated that pupation percentage of treated larvae with emamectin benzoate, hexaflumuron and alpha cypermethrin recorded 33.0, 44.0, 58.7%, respectively, compared to 96.5% pupation in control larvae. The differences between the tested insecticides were mostly highly significant.

Pupal weight

Data in Table 3 demonstrated that the mean pupal weight of pupae arised from the larvae of the American bollworm treated with LC_{25} of emamectin benzoate, hexaflumuron and alpha cypermethrin recorded 0.2100, 0.1889 and 0.2317 mg, respectively compared to 0.3815 mg for control larvae. Analysis of data revealed highly significant differences between the means.

Pupal mortality percentage

The percentages of pupal mortality of *H. armigera* arised from the larvae treated with the LC_{25} of emamectin benzoate, hexaflumuron and alpha cypermethrin attained 31.5, 54.0 and 36.0% compared to 4.5% for control pupae (Table 3). The three tested insecticides induced highly significant increase in percent pupal mortality.

Pupal duration

Obtained results indicated that the mean duration of pupal stage attained 15.0, 16.5 and 15.0 days for pupae resulted from *H. armigera* larvae treated with emamectin benzoate, hexaflumuron and alpha cypermethrin, respectively compared to 13.0 days recorded for control larvae. Statistical analysis revealed highly significant differences between the tested insecticides and the control (Table 3).

Percentage of adults emergence

Obtained results cleared that the mean percentages of adults emergence attained 71.0, 45.8 and 64.0% for moths arised from larvae exposed to emamectin benzoate, hexaflumuron and alpha cypermethrin, respectively as compared to 95.5% emergence in control larvae (Table 3). The differences between treatments are highly significant.

Sex ratio

Data presented in Table 3 reveal that sex ratio (as females %) recorded 44.8, 39.0, 45.5 and 50.8% for moths resulted from larvae treated with the three tested insecticides as well as the control ones, respectively .The differences between the tested insecticides and control were insignificant.

Deformation of pupal and adult stages

Data in Table 3 and Fig. 3 indicated that deformation percent of pupal stage recorded 30.0, 25.0 and 15.0% for emamectin benzoate, hexaflumuron and alpha cypermethrin treatments, respectively compared to 5.8% in control. The differences between treatments are highly significant.

Data in Table 4 and Fig. 4 indicated that deformation percent of adult stage recorded 32.50, 28.25 and 20.25% for emamectin benzoate, hexaflumuron and alpha cypermethrin treatments, respectively compared to 4.5% in control. The differences between treatments are highly significant.

Female longevity

Preoviposition period

The mean preoviposition recorded 4.25, 3.25 and 3.75 days for emamectin benzoate, hexaflumuron and alpha cypermethrin, respectively as compared to 2.87 days for control moths. It is clear that the tested insecticides induced highly significant elongation in this period (Table 4).

Oviposition period

Data in Table 4 indicated that the mean period of oviposition attained 2.25, 4.00, 4.25 and 8.63 days for the three compounds and the control moths, respectively. It is obvious that emamectin benzoate shortened this period to be about 1/4 as that of the control.

Postoviposition period

As shown in Table 4, the mean of post-oviposition period attained 1.50 , 2.00 , 3.00 and 4.00 days for emamectin benzoate, hexaflumuron, alpha cypermethrin and control treatments, highly respectively. The differences are highly significant.

As a result, the longevity of female moths attained 8.00, 9.00, 11.00 and 15.50 days for the three compounds and the control, respectively. Generally, control moths lived the longest period, meanwhile those treated with emamectin benzoate manifested the shortest one. The differencse were significant except that between emamectin benzoate and hexaflumuron treated moths.

Male longevity

The mean longevity of male moth recorded 8.00, 7.50 and 11 days while the control one recorded 14.00 days (Table 4). Insecticidal treatment caused significant decrease in male moths longevity (Table 4).

Female fecundity (No. of eggs/female)

Obtained results cleared that the mean number of eggs laid per female moth reached 153.00, 262.00 and 383.00 eggs/female moth fed during its 1st larval instar on artificial diet with LC_{25} of emamectin benzoate, hexaflumuron and alpha cypermethrin, respectively. Control moths laid a mean of 777,00 eggs/female. It is obvious that the application of the three insecticides caused severe reduction in female fecundity where the number of eggs/ moth treated with emamectin benzoate, hexaflumuron and alpha cypermethrin become as 19.69, 33.72 and 49.29% as that of the control. In this respect, Lopez *et al.* (2010) recorded significant reduction in fecundity observed in *H. zea* that exposed to a sublethal concentration of emamectin benzoate. In contrast, Hari and Mahal (2011) stated that eggs laying by female adults of *H. armigera* on the fruiting bodies of cotton was higher in case of cypermethrin application at LC_{50} than control.

Fertility (%)

Obtained results clear that the percentage of firtilized eggs laid per female moth recorded 59.00, 45.50 and 70.08% for moth fed during its 1stlarval instar on artificial diet polluted with LC_{25} of emamectin benzoate, hexaflumuron and alpha cypermethrin, respectively, meanwhile, the fertility of control eggs recorded 90.00%.

Hatchability

The mean percentage of hatchability of fertilized eggs laid per female moth treated with emamectin benzoate, hexaflumuron and alpha cypermethrin recorded 36.75, 34.50 and 53.25%, respectively while this value was 93.50% for the control female (Table 4).

In conclusion, data of the present work clear that emamectin benzoate, hexaflumuron and alpha cypermethrin could be classified as highly toxic insecticides to *H. armegira* larvae even feeding the larvae on sublethal dose (LC_{25}) of the two compounds caused clear harmful effects on the biology of the pest represented by elongation pupal duration and highly significant increase in larval and pupal mortality and decrease (%) of adult emergence and shortening larval duration period and female and male moth longevity. Severe reduction of eggs fertility and hatchability (%).

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فاعليـــة المبيد الحيوى ايمـامكتيـــن بنـــزوات ومنظم النموهيكسـافلـــوميـرون فـى مكـافحـــة دودة اللـــوز الأمـــريكيـــة في المعمــــل

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نظراً لاكتساب دودة اللوز الأمريكية مقاومة للمبيدات التقليدية فقد صُمم هذا البحث لتقييم فعالية اثنين من مبيدات الجيل الجديد هما hexaflumuron وemamectin benzoate على البيض والعمر اليرقى الأول لهذه الآفة مقارنة بالمبيد البيرثرويدى التقليدى alpha cypermethrin، وقد أظهرت النتائج أن hexaflumuron لا يؤثر على بيض دودة اللوزالامريكية عند معاملته بالتركيز الحقلي الموصى به مقارنة بسمية عالية وصلت لـ ١٠٠% عند نفس التركيز لكـــل من emamectin benzoate و alpha cypermethrin، كما لوحظت الفعالية الشديدة عند معاملة العمر اليــرقــى الأول بـ hexaflumuron و emamectin benzoate حيث بلغت قيمة LC_{50} ٠.٠٠٤١ و ٢.٤٤٢ جزء فى المليون مقارنة بـ ١١.٣٦٢ جزء فى المليون للمركب alpha cypermethrin، هذا وقد سبب التركيز تحت القاتل (LC_{25}) للمركبين بعض التغيرات البيولوجية في الحشرة حيث قصرت مدة الطور اليرقى إلى ١٣.٧ و ١٢ يوماً للمركبين على الترتيب مقارنة بـ ١٥ يوماً للمبيد التقليدى وبلغت فى اليرقات غير المعاملة ١٦ يوماً، علاوة على ذلك فقد أحدثت المبيدات الثلاثة نسبة قتل عالية فى الطور اليرقى والعذراء، كما قصر معنوياً عمر الفراشات الإناث والذكور وانخفضت بشدة إنتاجية الإناث من البيض ونسبة خصوبة وفقس البيض مما يؤكد إمكانية استخدام المركبين فى المجال التطبيقى لمكافحة هذه الآفة خصوصا أنهما من المركبات الصديقة للبيئة.

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