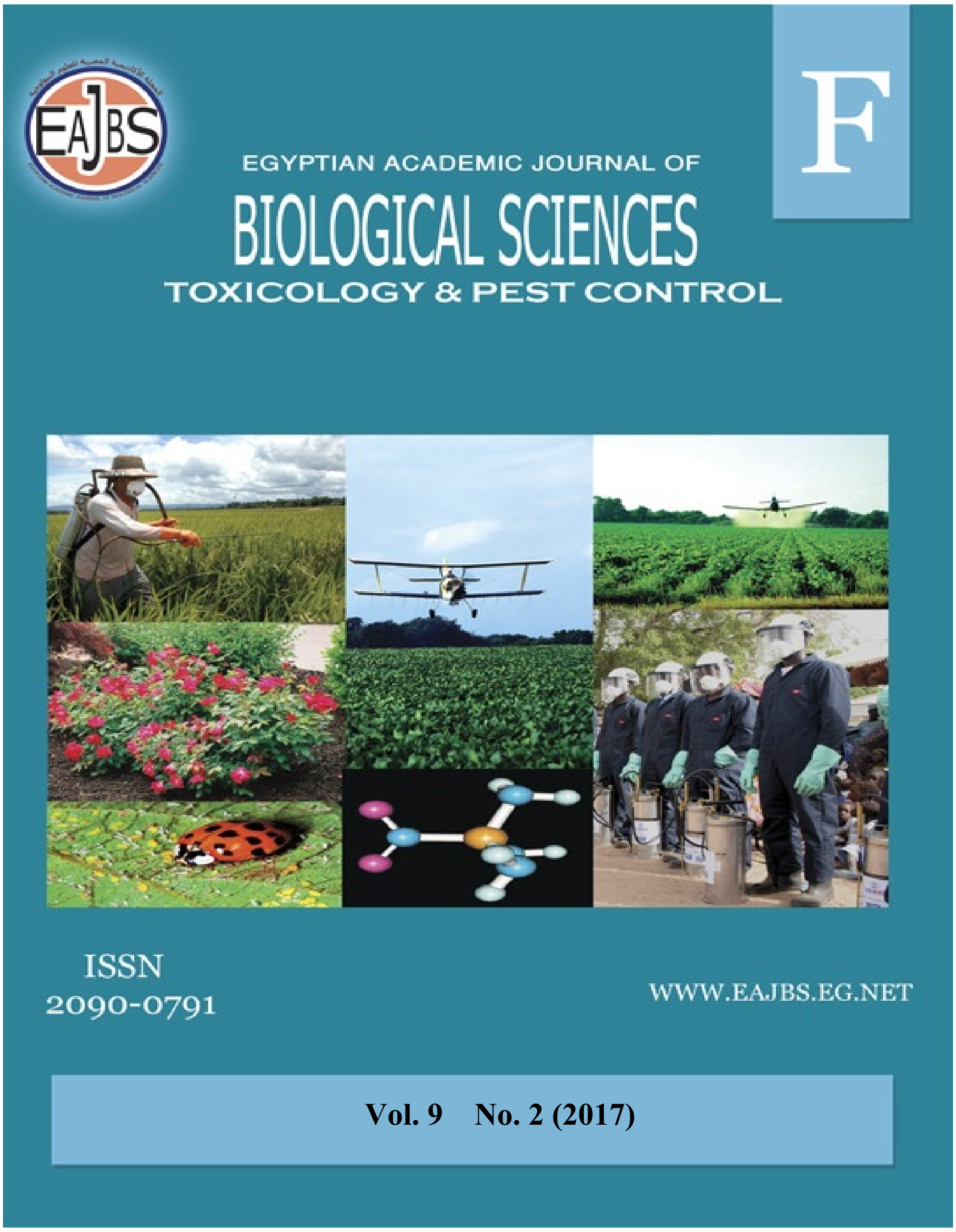

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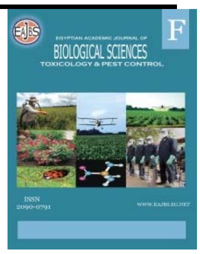

Laboratory studies to evaluate the difference in the toxic effects of three biopesticides on the two ladybird predators *Coccinellaundecimpunctata* L. and *Hippodamiaconvergens*Guer.(Coleoptera : Coccinellidae), reared on an artificial diet.

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Biopesticides, toxic effects, ladybird predators, *Coccinella undecimpunctata*, *Hippodamia convergens*, artificial diet, laboratory conditions.

ABSTRACT

Laboratory studies were conducted to evaluate the toxic effects of three different treatments: the bioinsecticide Biovar (containing the fungus *Beauveria bassiana*), Andros (an Abamectin derivative) and Radiant (a Spinosad derivative) on the two ladybird beetles; *Coccinella undecimpunctata* and *Hippodamia convergens*, that were reared on a laboratory artificial diet. The LC_{50} values obtained for the ladybird beetle *C. undecimpunctata* were; 2.9608×10^5 viable spores, 0.8003% and 0.3142%. The corresponding LC_{50} values obtained for the ladybird beetle *H. convergens* were; 3.8659×10^5 viable spores, 0.8065% and 0.3987%, for the three biopesticides Biovar, Andros and Radiant, respectively. The mortality percentages were higher in case of the ladybird beetle, *C. undecimpunctata* comparing with the ladybird beetle, *H. convergens.* As, the concentrations of each treated compounds increased, the mortality percentages were also increased. Therefore, using the fungal biofungicide; Biovar and the natural pesticide Andros may be recommended to be applied, for giving more safety on the two previous predatory species especially by using them at low concentrations rates. They could be used side by side with other available safe control methods, within Integrated Pest Management (IPM) strategies, to protect man and natural enemies including predators and also his surrounding environment from pollution.

INTRODUCTION

The advanced developed researches in the field of biological control have diverted their efforts to encourage the role of natural enemies including predators, by maintaining proper habitats in the agro-ecosystem attain their efficiency. However, predators belonging to family Coccinellidae comprise a group of the most active predatory species that feed on different sucking pests including aphids, whiteflies, jassids and mites as well as other small insects. This family gained an interested role as important group of predators in the biological control of insect pests attacking different crop plants (El-Heneidy *et al*., 2008).

Limited information is available on the relative toxicity of different pesticides on natural enemies and this makes the selection of these pesticides suitable for building Integrated Pest Management (IPM) difficult (Stevenson and Walters, 1983). Where, the recent IPM strategies developed for pest control are mainly concentrated with the use of suitable safe control method (Kares*et al*., 2012).

The repeated usage of harmful pesticides in many cultivated areas formed an adverse effect on many beneficial insect populations (El-Sebae, 1981 and Farag, 2008).therefore,the use of biological control methods has received much crucial attention worldwide and revealed significant impact as possibly safe mean for insect control (Sabbour & Abbas, 2007). Microbial pesticides (containing pathogenic microorganisms) represented one of the important components of biological control techniques (Moussa *et al*., 2014). Successful attempts of using microbial pesticides in IPM strategies have been made. One of the promising microorganisms that have attracted attention was the fungal bioinsecticides (Hosny*et al*., 2009). The fungus *Beauveria bassiana* (Balsamo) Vuillemin was one of the species of fungi that have been evaluated to measure their virulence against numerous pests (Wan, 2003) and also on many natural enemies found associated with common agricultural pests (Sabra *et al*., 2005).

In addition, a new approach, which has captured worldwide attention, is the use of the natural products of Abamectin derivatives. Where, Abamectins are produced by the soil actinomycete *Streptomyces avermitilies* (Burg *et al*., 1979). However, many Spinosad derivatives had also been developed and had got a great attention in the field of biological control (El Arnaouty *et al*., 2010).

The present study was conducted under laboratory conditions and aimed to evaluate the toxic effects of three different biopesticides; the microbial insecticide (Biovar, containing the fungus *B. bassiana*), Andros (an Abamectin derivative) and Radiant (a Spinosad derivative), on the two ladybird beetles; *C. undecimpunctata* and *H. convergens*, that were reared on a laboratory artificial diet, in order to evaluate the possibility of their future uses in the field of biological control without making any harm to useful biocontrol agents.

MATERIALS AND METHODS

The laboratory studies were carried out in the Biological Control Research Department, Plant Protection Research Institute, Agricultural Research Center (ARC), at Giza, Egypt.

Tested predators:

The two ladybird predators; *C. undecimpunctata* and *H. convergens* adults were collected during April, from pesticide free infested clover fields in Qalubia Governorate. As for the two predators; *C. undecimpunctata* and *H. convergens* adults, they were fed on an artificial diet (consisted of: died yolk of eggs, dry powdered aphids, fresh powdered aphids, sucrose, powdered yeast, pollen grains, maize oil, royal jelly as capsules, multi vitamins and sterptophenicol), as previously described by Bahy El-Din (2014), in plastic jars until they started their activity and laying predatory eggs. The newly deposited eggs were collected and put in other clean plastic jars until their hatching and subsequently development on the same artificial diet. Formed emerged adult predators of both two species, were collected and used in the experimental treatments, under the laboratory conditions of 25 ± 2˚C and 65 ±5% R.H.

Selected biopesticides:

The three biopesticides compounds were:

A- Biovar (10%W.P.) which is a fungal insecticide,the active componentis *B. Bassiana* containing 32×10^6 viable spores/gm. It was recommended to be applied against the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), with rate of application 200g/100 L water.

B- Andros (5%W.P.), which is an Abamectin derivative, manufactured by Pesticide and Chemical Limited Ltd China. It was recommended to be applied against cotton boll borers and *S. littoralis* with rate of application 80gm/feddan.

C- Radiant (12%SC), the common name is Spinetram. It was recommended to be applied against *S. littoralis*, with rate of application 30cm³/feddan.

Treatments:

Five dilutions for each were prepared in distilled water by serial dilutions to obtain a constant volume of 100 ml., while the control was only treated with water.

A- The fungal biopesticide Biovar.

For the treatment of both the two predators; *C. undecimpunctata* and *H. convergens* adults, weights of 0.157, 0.313, 0.625, 1.25 and 2.50 grams of Biovar were diluted in distilled water to obtain a constant volume of 100 ml. (total volume), to represent the dilutions of 0.5×10^5, 1×10^5, 2×10^5, 4×10^5 and 8×10^5 viable spores, respectively.

B- The biopesticide Andros.

In case of both the two predators; *C. undecimpunctata* and *H. convergens* adults, five dilutions of 0.25, 0.50, 0.75, 1.00 and 1.25%, were prepared by diluting the weights of5,10, 15, 20 and 25gm. of the pesticide in distilled water, to obtain a constant volume of 100 ml. (total volume), respectively.

C- The biopesticide Radiant.

Firstly, a stock solution of 6% was prepared for making the desired different dilutions. However, for the two predators; *C. undecimpunctata and H. convergens* adults, five concentrations; 0.13, 0.25, 0.50, 1.00 and 2.00%, were prepared in distilled water by using 2.09, 4.17, 8.33, 16.67 and 33.33ml of the pesticide to obtain a constant volume of 100 ml. (total volume), respectively.

Experiments:

Fresh equal weights (one gram in each replicate) of the artificial diet were put as food. They were put on filter papers that were dipped for one minute in the different dilutions of the three treatments used. Where, these filter papers were then left for 10 minutes for air dryness at room temperature. Ten healthy adults (replicates) of either *C. undecimpunctata* or *H. convergens*, were kept individually in plastic cups (7.5×4 cm.), with perforated plastic covers. Where, they were allowed to feed for 24 hours on the artificial diet on treated filter papers (by each of the five different concentrations of the three tested treatments). Adults of the two predatory species; *C. undecimpunctata* or *H. convergens* were starved for 6 hours for allowing them to move on the treated filter papers searching for the untreated food. After 24 hours were transferred to other clean plastic cups containing artificial diet on untreated filter papers until no mortalities were observed. As for the untreated control test was conducted using the same source of food, but the filter papers were only dipped in water and harbored an equivalent numbers of the two predators. The adults of both predatory species were daily examined. As a result, the mortality percentages were recorded after 1, 2, 3 and 4 successive days post treatments, (where, the cumulative mortality percentages of both *C. undecimpunctata* and *H. convergens* adults were calculated).

Statistical analysis:

The effectiveness of different treatments was expressed in terms of

LC50 values at 95 fiducially limits slopes of regression lines. Statistical analysis of the obtained data was made based on the analysis of variance and linear regression analysis (Finney, 1971) and also by using Costat computerized program (Cohort 6).

RESULTS

Effect of the fungal bioinsecticide Biovar:

Daily mortality percentages among treated *C. undecimpunctata* and *H. convergens* adults are shown in Table (1). The mortality percentages after 4 and 4 days (at which LC_{50} values were estimated for both two predators), ranged between (20.00 & 80.00%) and (10.00 & 70.00%), at concentrations ranged between $0.50\times10^5$and 8.00×10^5 viable spores, for *C. undecimpunctata* and *H. convergens*, respectively.

In addition, obtained data in Table (1) revealed that, the mortality percentages after treatmentwere positively correlated with increasing the applied concentrations of the fungal bioinsecticide Biovar, for *C. undecimpunctata* and *H. convergens* adults (the r-values were 0.9446 & 0.9799, respectively). Also, there was a highly significant difference between the cumulative mortalities of the two predatory species (the r-value was 0.9817). The obtained LC_{50} values were; 2.9608 and 3.8659×10^5 viable spores, for the two tested predators for *C. undecimpunctata* and *H. convergens* adults, respectively.

Table (1):Cumulative mortality percentages of *C. undecimpunctata* and *H. convergens* adults, fed on the artificial diet put on treated filter papers with the fungal bioinsecticide Biovar, under the laboratory conditions.

Predators	Con. (viable spores)	Cumulative mortality % after (days) of treatments				
		1	2	3	4	
C. undecimpunctata	0.00	0.00	0.00	0.00	0.00	Remaining adults of both two predators survived after that
	0.50×10^5	0.00	10.00	20.00	20.00	
	1.00×10^5	0.00	20.00	30.00	30.00	
	2.00×10^5	10.00	30.00	40.00	40.00	
	4.00×10^5	30.00	40.00	50.00	60.00	
	8.00×10^5	50.00	60.00	60.00	80.00	
H. Convergens	0.00	0.00	0.00	0.00	0.00	
	0.50×10^5	0.00	0.00	10.00	10.00	
	1.00×10^5	0.00	0.00	20.00	20.00	
	2.00×10^5	0.00	10.00	30.00	30.00	
	4.00×10^5	20.00	30.00	40.00	50.00	
	8.00×10^5	30.00	40.00	50.00	70.00	

- Statistical analysis:
LC50 value for*C. undecimpunctata* 2.9608×10^5Slope 2.2529 ± 0.2596
LC50 value for*H. convergens*3.8659×10^5 Slope2.5936 ± 0.2936
% cumulative mortality of*C. undecimpunctata* × concentrations of tested treatments r value =0.9446**
% cumulative mortality of *H. convergens* × concentrations of tested treatments r value =0.9799***
% cumulative mortality of *C. undecimpunctata* × % cumulative mortality of *H. convergens* r value =0.9817***

** = Moderate significant ***= Highly significant r = Correlation coefficient

Effect of the biopesticide Andros:

After one and 2 days of the pesticide Andros treatments, the percentages of cumulative mortality (at which LC50 values were estimated) for both *C. undecimpunctata* and *H. convergens*adults, ranged from (10.00 to 80.00%) and (10.00 to 80.00%), respectively, in concentrations ranged from (0.25 to 1.25 %) for the two predatory species (Table, 2).

Table (2):Cumulative mortality percentages of *C. undecimpunctata* and *H. convergens* adults, fed on artificial diet put on treated filter papers with Andros, under the laboratory conditions.

Predators	Con. (%)	Cumulative mortality % after (days) of treatments				
		1	2	3	4	
C. undecimpunctata	0.00	0.00	0.00	0.00	0.00	Remaining adults of both two predators survived after that
	0.25	10.00	10.00	20.00	20.00	
	0.50	20.00	30.00	40.00	40.00	
	0.75	40.00	50.00	60.00	60.00	
	1.00	50.00	70.00	80.00	80.00	
	1.25	80.00	90.00	100.00		
H. Convergens	0.00	0.00	0.00	0.00	0.00	
	0.25	0.00	10.00	10.00	10.00	
	0.50	10.00	20.00	30.00	30.00	
	0.75	30.00	40.00	50.00	50.00	
	1.00	50.00	60.00	60.00	70.00	
	1.25	70.00	80.00	90.00	100.00	

- Statistical analysis:
LC50 value for*C. undecimpunctata* 0.8003 Slope 0.6827 ± 0.0689
LC50 value for*H. convergens*0.8065Slope 0.7753 ± 0.0755
% cumulative mortality of*C. undecimpunctata* × concentrations of tested treatments r value =1.0000***
% cumulative mortality of *H. convergens* × concentrations of tested treatments r value =0.9906***
% cumulative mortality of *C. undecimpunctata* × % cumulative mortality of *H. convergens* r value =0.9906***

***= Highly significant r = Correlation coefficient

Obtained data in Table (2) revealed also that, the cumulative mortality percentages increased by increasing the tested concentrations of the pesticide against adults of *C. undecimpunctata* and *H. convergens* adults (the r-values were 1.0000 & 0.9906, respectively). Also, there was a highly significant difference between the cumulative mortalities of the two predatory species (the r-value was 0.9906). The obtained LC50 values were; 0.8003 and 0.8065 %, for *C. undecimpunctata* and *H. convergens* adults, respectively.

Effect of the biopesticide Radiant:

Data in Table (3) show the daily cumulative mortality percentages after treatments by the pesticide Radianton *C. undecimpunctata* and *H. convergens* adults. The mortality percentages 24 hours after treatments (at which LC50 values were estimated) ranged from (20.00 to 90.00%) and from (10.00 to90.00 %), respectively, in at concentrations of (0.13 to 2.00%) for both the two previous predatory species.

Table (3): Cumulative mortality percentages of *C. undecimpunctata* and *H. convergens* adults fed on artificial diet put on treated filter papers with the biopesticide Radiant, under laboratory conditions.

Predators	Con. (%)	Cumulative mortality % after (days) of treatments				
		1	2	3	4	
C. undecimpunctata	0.00	0.00	0.00	0.00	0.00	Remaining adults of both two predators survived after that
	0.13	20.00	30.00	40.00	40.00	
	0.25	30.00	40.00	50.00	50.00	
	0.50	50.00	60.00	70.00	70.00	
	1.00	70.00	80.00	80.00	90.00	
	2.00	90.00	100.00			
H. convergens	0.00	0.00	0.00	0.00	0.00	
	0.13	10.00	20.00	30.00	30.00	
	0.25	20.00	30.00	40.00	40.00	
	0.50	30.00	50.00	60.00	60.00	
	1.00	60.00	70.00	70.00	80.00	
	2.00	90.00	90.00	100.00		

- Statistical analysis:

LC50 value for*C. undecimpunctata* 0.3142 Slope 0.3503 ± 0.0337

LC50 value for*H. convergens* 0.3987Slope 0.4302 ± 0.0399

% cumulative mortality of*C. undecimpunctata* × concentrations of tested treatments r value =0.8476**

% cumulative mortality of *H. convergens* × concentrations of tested treatments r value =0.9128**

% cumulative mortality of *C. undecimpunctata* × % cumulative mortality of *H. convergens* r value =0.9899***

= Moderate significant *= Highly significant r = Correlation coefficient

Moreover, it is evident from Table (3), that the percentages of cumulative mortality increased because of increasing the concentrations of pesticide (the r-values were; 0.8476 & 0.9128, respectively). Also, there was a highly significant difference between the cumulative mortalities of the two predatory species (the r-value was 0.9899). The obtained LC50 values were; 0.3142 and 0.3987 %, for the two ladybird beetles *C. undecimpunctata* and *H. convergens* adults, respectively.

Generally, it could be concluded that, there were significant differences between the cumulative mortality percentages between *C. undecimpunctata* (had higher cumulative mortality percentages)and *H. convergens* adults, in case of using all the three tested biopesticides (Biovar, Andros and Radiant), (Tables, 1, 2 &3).

DISCUSSION

Effect of the fungal bioinsecticide Biovar:

The fungal bioinsecticide Biovar was found to be less effective on*H. convergens* adults than *C. undecimpunctata* ones. The important use of the fungal bioinsecticides as a biological control component wasrecommended by many authors. For example, Sabra *et al.* (2005)investigated the effect of Biovar in onion field on the common predators associated with onion thrips *Thripstabaci*. They found that the biopesticide showed low toxic effects on the predators.Also,Ormond *et al*. (2007) reported that the fungus *Beauveria* was relatively injurious to the ladybird beetle *Coccinella septempunctata*.

Effect of the biopesticide Andros:

The biopesticide Andros was found to be of lower effect on the ladybird beetle*H. convergens* adults comparing with *C. undecimpunctata* ones. Similar findings were found by Michaud (2002), when exposing the two ladybeetles; *Cycloneda sanguinea* (L.) and *Harmonia axyridis* Pallas to leaf residues of five acaricide formulation commonly used in citrus production. He recorded that, three of these compounds including; Abamectin + petroleum oil, Diflubenzuron and Pyridaben were toxic to both larval predatory species. While, Fenbutation was only toxic to *C. sanguinea* larvae and Dicofol was the only compound demonstrating no toxicity to both larval predatory species of the coccinellids *C. sanguinea* and *H. axyridis*.

Effect of the biopesticide Radiant:

The obtained results are in agreement with those of Tillman and Mulrooney (2000) who recorded that, Spinosad generally did not affect the number of the two ladybird predators *H. convergens* and *Coleomegilla maculata* in the cotton field. Moreover, ELArnaouty *et al.* (2010) showed that, the field treatments with low concentrations by Spinosad (Tracer), on cotton plants could preserve natural enemies.Radiant was recorded in this study to be less effective onthe ladybird beetle*H. convergens* adults than *C. undecimpunctata* ones. Therefore, the pesticide Radiant showed the highest toxic effect on the two predators and the biopesticide Andros has a moderate one, while, the fungal bioinsecticide Biovar had the lowest toxicity. Similar results were recorded by Bahy El-Din *et al.* (2016), when testing the same previous treatments at concentrations ranged from: (1.00 to 16.00×10^5 viable spores in case of Biovar treatment), (0.50 to 1.50% in case of Andros) and (0.50 to 2.50% in case of Radiant),on the two clover pests; the Egyptian alfalfa weevil *Hyperabrunneipennis* and the terrestrial snail, *Monachaobstructa*.

Generally, as a result of the obtained data the lowest concentrations of the two treatments; Biovar and Andros were relatively safe to the predator's adults, while the highest ones were injurious. So, both of the two previous biopesticides are recommended to be applied in IPM programs, with low applied concentrations. Results are in agreement of those reported by Farag (2008) who mentioned that, the lowest concentrations of Biovar (0.2 and 0.4g/L) were relatively safe to the predatory adults of *C. undecimpunctata*. Also, Starb and Jentsch (2005) reported that, low rate of the chemical insecticides applications provided adequate suppression of apple aphid, while preserving their associated predators.

CONCLUSION

- It could be concluded from the present study that:-

1-The cumulative mortality percentages for the two ladybird beetles predators; *C. undecimpunctata* and *H. convergens* adults increased, as the concentrations of the three tested biopesticides used were increased.

2-The ladybird beetle, *H. convergens* was less susceptible to all of the three tested treatments than*C. undecimpunctata* adults. The lowest concentrations of the two treatments; the fungal biopesticide Biovar and the natural biopesticide Andros were relatively safe to the predator's adults, while the highest ones were injurious. Therefore, these two compounds can be applied in IPM strategies for minimizing the extensive use of the harmful chemical pesticides and thus minimizing the harmful effects on man and his surrounding environment including predators. More safety will be attained in agricultural crops that will help in increasing the Egyptian economic income.

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ARABIC SUMMERY

دراسات معملية لتقييم الاختلاف في التأثير السمي لثلاثة مبيدات حيويةعلى مفترسي أبى العيد **Coccinellaundecimpunctata L.** و ***Hippodamiaconvergens*Guer.** (Coleoptera: Coccinellidae) ، المرباة على بيئة صناعية.

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أجريت دراسة معملية لتقدير التأثيرات السمية المختلفة لثلاثة معاملات تشمل المبيدات الحيوية :Biovar (يحتوى على الفطر *Beauveriabassiana*)، المبيد Andros (أحد مشتقات Abamectin) والمبيد Radiant (أحد مشتقات Spinosad) على مفترسين رئيسيين من مفترسات عائلة Coccinellidae هما: أبو العيد ١١ نقطة *Coccinella undecimpunctata*وأبو العيد ١٣ نقطة *Hippodamia convergens*. وأوضحت النتائج أن نسب الموت كانت الأعلى في حالة المعاملة لأبى العيد ١١ نقطة *C.undecimpunctata* مقارنةبأبى العيد ١٣ نقطة *H. convergens* ، وذلك بالتغذية على بيئة صناعية (غير معاملة) موضوعة على سطح معامل بالتركيزات المختلفة للمعاملات الثلاثة السابقة. وقد تم تقدير التركيزات القاتلة ل ٥٠% من الحشرات الكاملة للمفترسين بالمعاملات الثلاثة السابقة حيث بلغت : (2.9608×10^5جراثيم حية و 0.8003% و0.3142 %) و (3.8659×10^5جراثيم حيةو 0.8065 % و 0.3987 %) ، لأبى العيد ١١ نقطة *C. undecimpunctata*وأبى العيد ١٣ نقطة*H. convergens* ، على التوالي.

ومن نتائج هذه الدراسة، يمكن التوصية باستخدام المبيد الفطري Biovar والمبيد الطبيعي Androsلإعطاء أمان أكبر على مفترسي أبى العيد فى عمليات الإطلاق خاصة عند استخدام تلك المبيدات بتركيزات منخفضة.

ويجب العمل على أن يكون هذا الاستخدام جنباً إلى جنب مع باقي الوسائل الأخرى الآمنة والمتاحة وذلك ضمن منظومة إستراتيجيةمكافحة الآفات(IPM) Management Integrated Pest حفاظاًعلى الإنسان والأعداء الحيوية المتواجدة والبيئة المحيطة خالية من التلوث.وبالتالي ، يؤدى ذلك إلى إنتاج محاصيل ترفع من الكفاءة التصديرية للمنتجات الزراعية فتزيد من العملات الصعبة ، سواء كانت منتجة من الحقول أو الأنواع المختلفة من الصوب الزراعية.

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