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Bioassay of three biopesticides against *Hypera brunneipennis* (Boheman) (Coleoptera: Curculionidae) and *Monacha obstructa* Ferussac. (Moullusca: Helicidae) in the laboratory.

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Abstract

Laboratory studies were conducted to evaluate the toxic effects of three different treatments: the bioinsecticide Biovar (*Beauveria bassiana*), Andros (Abamectin derivative) and Radiant (Spinosad derivative) on the Egyptian alfalfa weevil *Hypera brunneipennis* and the terrestrial snail, *Monacha obstructa*. The LC50 values obtained for the alfalfa weevil, *H. brunneipennis* were; 7.7641×10^5 viable spores, 0.9703% and 1.5276% (computed at 6, 2 and one days after treatment). The corresponding LC50 values obtained for the terrestrial snail, *M. obstructa* were; 5.4995×10^5 viable spores, 0.5822% and 0.5956% (5, 2 and one days after treatment), for the three tested biopesticides, respectively. The mortality percentages were higher in case of the terrestrial snail than the Egyptian alfalfa weevil. Therefore, using the fungal bioinsecticide Biovar and Andros may be recommended. They could be used side by side with other available safe control methods, when planning for Integrated Pest Management (IPM) strategies against the two tested pests in clover fields.

Key Words: Bioinsecticides, *Hypera brunneipennis*, *Monacha obstructa*, Bioassay

Introduction

In Egypt, the Egyptian clover (*Trifolium alexandrium* L.), is the main fodder crop for animal feeding (Shoeb *et al*., 2008). It is subjected to attack by many agricultural pests, that cause considerable and severe damage to the crop. From these pests, the Egyptian alfalfa weevil, *Hypera brunneipennis* (Boheman) (Coleoptera: Curculionidae) (El-Sufty *et al*., 1993) and the terrestrial snail, *Monacha obstructa* Ferussac. (Moullusca: Helicidae) (Hendawy *et al*., 2015).

Because of the direct and latent hazards of pesticides applications for the control of the mentioned pests in clover to animals which feed on this fodder crop, in addition to their bad effect on the ecosystem, it is always advised to use safe biopesticides which are of minor effect on the clover feeding animals and on the environment. Where, the recent Integrated Pest Management (IPM) strategies developed for pest control are mainly concentrated with the use of suitable safe control method (El-Metwally *et al*., 2010 and Kares *et al*., 2012), for reaching minimum pesticides residues in food (Vu *et al*., 2007).

The use of biological control has received much crucial attention worldwide and revealed significant impact as possibly safe mean for insect control (Sabbour&Abbas, 2007). Microbial insecticides (containing pathogenic microorganisms) represented one important component of biological control techniques (Sayed & Abolmaaty, 2013 and 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 (Hosney *et al*., 2009). It had been used against a wide range of agricultural pests (Eilenberg *et al*., 2001), causing mortality in the populations of these pests (El-Husseini *et al*., 2010). 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).

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).

The present study was conducted under laboratory conditions and aimed to evaluate the toxic effect of a commercial microbial insecticide (Biovar, containing the fungus *B. bassiana*) Andros (Abamectin derivative) and Radiant (Spinosad derivative), against the Egyptian alfalfa weevil, *H. brunneipennis* adults, as well as the terrestrial snail, *M. obstructa* adults.

Material 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.

I- Tested pests:

The two pests *H. brunneipennis* and *M. obstructa* adults, were collected during April, from pesticide free infested clover fields in Qalubia Governorate. As for *H. brunneipennis* adults, those were fed on lettuce leaves in plastic jars until they started their activity. While, for *M. obstructa* adults, they were kept in plastic jars (85±5% moisture) on clean clay. Adults of healthy snails as well as weevils were put individually for two weeks (to acclimatize to laboratory conditions), before they were used in treatment experiments. Snails were allowed also to feed on lettuce or cabbage leaves like adult weevils, under the laboratory conditions of 25 ± 2˚C and 65 ±5% R.H.

Π- Materials used:

1-The bioinsecticide Biovar (10%W.P.), is a fungal insecticide, the active component is *B. bassiana* containing 32×10^6 viable spores/gm. It was recommended to be applied against the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), at a rate of 200g/100 L water.
2-The biopesticide Andros (5%W.P.), is an Abamectin derivative, manufactured by Pesticide and Chemical Limited Ltd China. It was recommended to be applied against boll borers and *S. littoralis*, at a rate of 80gm/feddan.
3-The biopesticide Radiant (12%SC), the common name is spinetram. It was recommended to be applied against *S. littoralis*, at a rate of 30 cm³/feddan.

III- Treatment:

1- The fungul bioinsecticide Biovar

Weights of 0.313, 0.625, 1.25, 2.50 and 5.00 grams of Biovar were diluted in distilled water to obtain a constant volume of 100 ml. (total volume), to represent the dilutions of 1×10^5, 2×10^5,4×10^5, 8×10^5,16×10^5 viable spores, respectively.

2- The biopesticide Andros

In case of *H. brunneipennis* adults, five dilutions of 0.50, 0.75, 1.00, 1.25 and 1.50%, were prepared by diluting the weights of 10, 15, 20, 25 and 30gm. of the pesticide in distilled water, to obtain a constant volume of 100 ml. (total volume), respectively. However, for *M. obstructa*, five concentrations of 0.25, 0.50, 0.75, 1.00 and 1.25%, were prepared 5, 10, 15, 20 and 25gm. of the same pesticide in distilled water, to obtain a constant volume of 100 ml. (total volume), respectively.

3- The biopesticide Radiant

Firstly, a stock solution of 6% was prepared for making the desired different dilutions. In case of *H. brunneipennis* adults, five concentrations 0.50, 1.00, 1.50, 2.00 and 2.50%, were prepared 8.33, 16.67, 25.00, 33.33 and 41.67ml. of the pesticide in distilled water, to obtain a constant volume of 100 ml. (total volume), respectively. However, for *M. obstructa* adults, five concentrations; 0.25, 0.50, 0.75, 1.00 and 1.25%, were prepared in distilled water 4.17, 8.33, 12.50, 16.67 and 20.83 ml. in distilled water of the pesticide to obtain a constant volume of 100 ml. (total volume), respectively.

- The following procedures were followed:

1- Ten (replicates) of either *H. brunneipennis* and *M. obstructa* healthy adults were tested.
2- Fresh equal size of lettuce leaves were dipped for one minute in the different dilutions of the three treatments used.
3- The treated leaves were then left for 10 minutes for air dryness at room temperature.
4- Each of ten adults of *H. brunneipennis* or *M. obstructa*, were kept in plastic cups (7.5×4 cm.), with perforated plastic covers. Those were allowed to feed for 24 hours on treated lettuce leaves treated by each of the five different concentrations of the three tested treatments.
5- Surviving adults of both the two previous pest species were transferred to other clean plastic cups containing untreated lettuce leaves until no mortalities were observed.
6- The untreated control test was conducted using the same source of food, but dipped only in water and harbored an equivalent numbers of the two pests.
7- Before exposing the adults of the two tested pests to treated food, those were starved for 6 hours in order to obtain rapid feeding of the contaminated food.
8- Experiments were carried out under the laboratory conditions of 25 ± 2˚C and 65 ±5% R.H.
9- The adults of both pest species were daily examined and the mortality percentages were recorded after 1, 2, 3, 4, 5, 6 and 7successive days post treatments, where, the cumulative mortality percentages of both *H. brunneipennis* and *M. obstructa* adults were calculated.

IV- 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).

Results and discussion

I- Effect of the fungal bioinsecticide Biovar:

Daily mortality percentages among treated *H. brunneipennis* and *M. obstructa* adults, are shown in Table (1). The mortality percentages after 5 and 6 days post treatments with the fungal bioinsecticide Biovar (at which LC50 values were estimated), ranged between (10.00 & 70.00%) and (20.00 & 80.00%), at concentrations between 1×10^5 and 16×10^5 viable spores, for *H. brunneipennis* and *M. obstructa*. respectively. Results agreed with findings of Abdel-Raheem *et al*. (2009) and Saruhan *et al.* (2014). Obtained data also indicated that, the mortality reached 100% after 6days post treatments in case of *M. obstructa* adults.

In addition, obtained data revealed that the mortality percentages after treatments were positively correlated with increasing the applied concentrations of the fungal bioinsecticide Biovar, for *H. brunneipennis* and *M. obstructa* adults (the p-values were; 0.813 & 0.774, respectively, Table, 4). These results agreed with those of Merghem (2011) and Soliman *et al*. (2014). The LC50 values were; 7.7641 and 5.4995×10^5 viable spores, for the two tested pests, respectively (Table, 5). In general, from Tables (1, 4&5), it could be concluded that, Biovar was less effective on *H. brunneipennis* adults than *M. obstructa* ones. The use of the fungal bioinsecticides as a biological control components was recommonded by many authors. El-Sufty *et al*. (1993) showed that, the entomopathogenic fungus *B. bassiana* has been successfully used as a bio-control agent for management of a number of coleopteran insects. While, Hendawy *et al*. (2015) stated that, control of the terrestrial gastropods using microbial agents such as fungi is alternative control method to pesticides.

Table 1. Cumulative mortality percentages of *H. brunneipennis* and *M. obstructa* adults, fed on cabbage leaves treated with the fungal bioinsecticide Biovar, under laboratory conditions.

Pest species	Concent-rations (viable spores)	Cumulative mortality % after (days) of treatments							
		1	2	3	4	5	6	7	
H. brunneipennis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	Remaining adults of both two pests survived after that
	1×10^5	0.00	0.00	0.00	0.00	0.00	10.00	10.00	
	2×10^5	0.00	0.00	0.00	10.00	10.00	20.00	20.00	
	4×10^5	0.00	10.00	10.00	20.00	20.00	30.00	30.00	
	8×10^5	10.00	20.00	20.00	30.00	40.00	50.00	50.00	
	16×10^5	20.00	30.00	30.00	50.00	60.00	70.00	70.00	
M. obstructa	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	1×10^5	0.00	0.00	10.00	10.00	20.00	30.00	30.00	
	2×10^5	10.00	10.00	20.00	20.00	30.00	40.00	40.00	
	4×10^5	20.00	20.00	30.00	30.00	40.00	60.00	60.00	
	8×10^5	30.00	30.00	40.00	50.00	60.00	80.00	80.00	
	16×10^5	40.00	40.00	50.00	70.00	80.00	100.00	100.00	

Π- Effect of the biopesticide Andros:

After 2 days, the pesticide Andros treatments, the percentages of cumulative mortality (at which LC50 values were estimated) for both *H. brunneipennis* and *M. obstructa* adults, ranged from (20.00 to 90.00%) and (20.00 to 90.00%), in concentrations ranged from (0.50 to 1.50%) and (0.25 to 1.25%) (Table, 2).

Table 2. Cumulative mortality percentages of H. *brunneipennis* and *M. obstructa* adults, fed on cabbage leaves treated with Andros, under laboratory conditions.

Pest species	Concentrations (%)	Cumulative mortality % after (days) of treatments					
		1	2	3	4	5	
H. brunneipennis	0.00	0.00	0.00	0.00	0.00	0.00	Remaining adults of both two pests survived after that
	0.50	0.00	20.00	20.00	30.00	30.00	
	0.75	0.00	30.00	30.00	40.00	40.00	
	1.00	10.00	40.00	50.00	50.00	60.00	
	1.25	30.00	60.00	70.00	70.00	80.00	
	1.50	60.00	90.00	90.00	100.00	100.00	
M. obstructa	0.00	0.00	0.00	0.00	0.00	0.00	
	0.25	10.00	20.00	20.00	30.00	30.00	
	0.50	20.00	30.00	30.00	40.00	40.00	
	0.75	40.00	50.00	60.00	60.00	70.00	
	1.00	60.00	80.00	80.00	80.00	90.00	
	1.25	80.00	90.00	100.00	100.00	100.00	

Obtained data in Table (4) revealed also that, the cumulative mortality percentages increased by increasing the tested concentrations of the pesticide against adults of both pest species (the p-values were; 0.855 & 0.949, respectively, Table, 4). Similar findings were found by Abd El Aziz (2005) and Mahmoud (2011). In addition, Attala (2007) showed that, Radical (Methyl amineavermectin), proved as a potent control compoud against a broad range of economically important lepidopterous pests. The mortality reached 100% and 80-100%, in case of the second and the fourth larval instars of the cotton leafworm, *S. littoralis* respectively, after 48 hours post treatment. While, Mahmoud (2011) indicated that, the maximum mortality by Abamectin occurred within 3-4days.The obtained LC50 values were; 0.9703 and 0.582%, for *H. brunneipennis* and *M. obstructa* adults, respectively (Table, 5). Generally, from Tables (2, 4&5), it could be concluded that, Andros was of lower effect on *H. brunneipennis* adults comparing with *M. obstructa* ones.

Ш- Effect of the biopesticide Radiant:

Data in Table (3) show the daily cumulative mortality percentages after treatments by the pesticide Radiant on *H. brunneipennis* and *M. obstructa* adults. The mortality percentages 24 hours after treatments (at which LC50 values were estimated), ranged from (10.00 to 90.00%) at concentrations of (0.50 to 2.50%) and from (20.00 to 90.00%) at concentrations of (0.25 to 1.25%), for the two previous pest species, respectively. Moreover, it is evident from Table (3), that the percentages of cumulative mortality increased because of increasing the concentrations of pesticide (the p-values were; 0.981 & 0.957, respectively; Table, 4). The obtained results are in agreement with those of Genena (2008) and Abd El-wahed (2014). The LC50 values were; 1.5276 and 0.5956%, for *H. brunneipennis* and *M. obstructa* adults, respectively (Table, 4). In general, from Tables (3, 4&5), it could be concluded that, Radiant was less effective on *H. brunneipennis* adults than *M. obstructa* ones.

Generally, it could be concluded that, there were significant differences between the cumulative mortality percentages between *H. brunneipennis* (had higher cumulative mortality percentages) and *M. obstructa* adults, in case of using Biovar, Andros and Radiant. Where, the p-values were; 0.968, 0.963 & 0.964, respectively (Table, 4).

Table 3. Cumulative mortality percentages of H. *brunneipennis* and *M. obstructa* adults, fed on cabbage leaves treated with the biopesticide Radiant, under laboratory conditions.

Pest species	Concentrations (%)	Cumulative mortality % after (days) of treatments			
		1	2	3	
H. brunneipennis	0.00	0.00	0.00	0.00	Remaining adults of both two pests survived after that
	0.50	10.00	20.00	20.00	
	1.00	20.00	30.00	30.00	
	1.50	40.00	50.00	50.00	
	2.00	60.00	70.00	70.00	
	2.50	90.00	90.00	100.00	
M. obstructa	0.00	0.00	0.00	0.00	
	0.25	20.00	30.00	40.00	
	0.50	40.00	50.00	60.00	
	0.75	50.00	60.00	70.00	
	1.00	70.00	80.00	80.00	
	1.25	90.00	100.00	100.00	

Table 4. Correlations (Pearson correlation; P-value) between mortality percentages and dilutions of different tested treatments and also between the cumulative mortality percentages of the two pests; *H. brunneipennis* and *M. obstructa* adults.

Tested factors	Biovar	Andros	Radiant
% cumulative mortality of *H. brunneipennis* × % cumulative mortality of *M. obstructa*	0.968 **	0.963 **	0.964 **
% cumulative mortality of *H. brunneipennis* × concentrations of tested treatments	0.813 **	0.855 **	0.981 **
% cumulative mortality of *M. obstructa* × concentrations of tested treatments	0.774 **	0.949 **	0.957 **

** Highly significant

Table 5. Comparative toxicity to *H. brunneipennis* and *M. obstructa* adults (computed from mortality data: (6&5 days after treatments with Biovar, respectively), (two days for both after treatments with Andros) and (one day for both species after treatments with Radiant).

Treatments	*H. brunneipennis*				*M. obstructa*			
	LC50	Slope	r-value	Regression equation	LC50	Slope	r-value	Regression equation
Biovar (viable/spores)	7.7641 $\times 10^5$	1.492± 0.1551	0.814**	Y= 2.41+ 2.99X	5.4995 $\times 10^5$	1.189 ± 0.1999	0.774**	Y= 12.50+ 3.94X
Andros (%)	0.9703	3.843± 0.3868	0.854**	Y= -10.0+ 24.00X	0.5822	3.203± 0.2823	0.949**	Y= -9.23+ 32.90X
Radiant (%)	1.5276	3.458± 0.3151	0.981**	Y= -3.81+ 36.40X	0.5956	2.668± 0.2635	0.957**	Y= 11.00+ 61.00X

****** Highly significant.

Conclusion

It could be concluded from the present study that:-

1-The cumulative mortality percentages for the Egyptian alfalfa Weevil, *H. brunneipennis* and the terrestrial snail, *M. obstructa* adults, increased as the concentrations of the three tested pesticides used were increased.

2-The Egyptian alfalfa weevil, *H. brunneipennis* was less susceptible to the three tested treatments than the terrestrial snail, *M. obstructa* adults.

3-The pesticide Radiant showed the highest toxic effect on the two pests and the biopesticide Andros has a moderate one, while, the fungal bioinsecticide Biovar had the lowest toxicity.

4- Using both the fungal bioinsecticide Biovar and the natural biopesticide Andros may provide better alternatives to than traditional chemical insecticides against the two pests in fields that are subjected to their attacks. Their use in conjunction with good agricultural practices may reduce the use of chemical pesticides and provide a safe element with any applied IPM system. Therefore, this can help in minimizing the extensive use of the harmful chemical pesticides and thus, will minimize the harmful effects on man and his surrounding environment.

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اختبارات حيوية على ثلاث مبيدات بيولوجية على سوسة ورق البرسيم وقوقع البرسيم

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قسم بحوث المكافحة الحيوية – معهد بحوث وقاية النباتات – مركز البحوث الزراعية

أجريت دراسة معملية لتقدير التأثيرات المختلفة لثلاثة معاملات تشمل المبيدات الحيوية Biovar (يحتوى على الفطر *Beauveria bassiana*) ، المبيد Andros (أحد مشتقات Abamectin) والمبيد Radiant (أحد مشتقات Spinosad) على آفتين رئيسيتين من آفات البرسيم هما: سوسة البرسيم (*Hypera brunneipennis*) وقوقع البرسيم (*Monacha obstructa*).

أظهرت النتائج أن نسب الموت كانت الأعلى في حالة المعاملة لقوقع البرسيم (*M. obstructa*) مقارنة بسوسة ورق البرسيم (*H. brunneipennis*) ، وذلك بالتغذية على غذاء معامل بالمعاملات الثلاثة السابقة. وقد تم تقدير التركيزات القاتلة ل 50% للآفتين بالمعاملات الثلاثة السابقة ، حيث بلغت (7.7641×10^5 جراثيم حية و 0.9703 و 1.5276%) و (5.4995×10^5 جراثيم حية و 0.5822 و 0.5956 %) ، لسوسة ورق البرسيم وقوقع البرسيم ، على التوالي.

من نتائج هذه الدراسة ، يمكن التوصية باستخدام المبيد الفطري Biovar والمبيد الطبيعي Andros لمكافحة سوسة ورق البرسيم وقوقع البرسيم، في حقول البرسيم أو حقول المحاصيل الأخرى التي تصاب بهما. ويكون هذا الاستخدام جنبا إلى جنب مع باقي الوسائل الأخرى الآمنة والمتاحة، وذلك ضمن منظومة إستراتيجية مكافحة الآفتين ، حفاظاً على الإنسان وبيئته المحيطة من التلوث.