EFFECT OF FEEDING TWO PREDACIOUS INSECTS ON APHIDS TREATED WITH SOME ENTOMOPATHOGENS

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Abstract

Chrysoperla carnea (Stephens) and *Orius albidipennis* (Rueter) are a biological agents commonly used to control insect pests. Three concentrations ($10^{7,}$ 10^8 and 10^9 spores/ml) of entomopathogenic agents *Beauveria bassiana* (Balsamo), *Metarhizium anisopliae* (Metsch) and *Bacillus thuringiensis* var. *kurstaki* (Berliner) were evaluated against the 2nd instar larvae of *C. carnea* and 2nd instar nymph of *O. albidipennis.* Aphids and its containers were sprayed with each concentration of spores suspension by small sprayer. The treated aphids were offers to predators as prey under laboratory conditions (27 ± 2 ºC, 65 ± 10% RH). Mortality was recorded daily from the 2nd day to the 6th day of treatment. The results revealed that , the values of the a cumulative percentage mortalities of *C. carnea* and *O. albidipennis* increased gradually from the 2nd day to the 6th day after exposure and increased by increae concentrations of *B. bassiana,* and *M. anisopliae*. While the third agent, (Btk.) had no effect on two tested predators till the 5th day and had slight effect on the 6th day. The LT_{50} values of *C. carnea* treated with *B. bassiana* and *M. anisopliae* were 11.22 and 12.61day at concentration of 10^7 spores /ml and were 10.05and 11.01day at concentration 10^8 spores /ml and were 8.004 and 10.73day at concentration 10^9, respectively. But not detected LT_{50} values for (Btk) against *C. carnea* or *O. albidipennes* at concentrations 10^7, 10^8 and 10^9 spores/ml. Whereas, The LT_{50} values of *O. albidipennes* treated with *B. bassiana* and *M. anisopliae* were 6.3 and 5.82day at concentration 10^7 spores /ml and were 6.023 and 5.59day at concentration 10^8 spores /ml and were 3.56 and 4.32day at concentration 10^9, respectively.

INTRODUCTION

Entomopathogenic fungi and bacteria have great potential control agents against insects and it considers as one component within integrated pest management systems. The entomopathogenic fungi are being developed worldwide for the control of many pests of agricultural importance (Ferron, 1985). Previous research reported that among 41 isolates of entomopathogenic fungi *Beauveria bassiana* and *M. anisopliae* were virulent against *Myzus persicae* (Sulzer) *Thrips tabaci* (Lindeman) (Thungrabead *et al*., 2006). The entomopathogenic bacteria varieties of *B. thuringiensis* which is based on Cry toxins (also known as δ- endotoxins) are now

commercially available for use against a wide variety of insect pests including species of Lepidoptera, Coleoptera and Diptera (Gill *et al*., 1992).

However, the success of entomopathogens as biological control agents depends not only on high efficacy against insect pests, but also on low or no effect against non-target insects. Moreover, use of such biological control agents might have effect on beneficial insects, such as natural enemies of insect pests (Ahmadzadeh & Hatami1, 2006).

Larvae of *C. carnea* are efficient predator as bio control tool predators against many key pests including aphids, whiteflies, young larvae and eggs of Lepidoptera, spider mites and other soft bodied arthropods (Cardoso and Lazzari, 2003). It can be mass reared in the laboratory and released against pests in field and greenhouses (Mirnoayedi, 2001).

Species in the genus *Orius* (Hemiptera: Anthocoridae) are generalist predators that attack eggs and immature stages of various arthropods, or small soft-bodied adult arthropods, including numerous important agricultural pest species (Butler and O'Neil 2007). Although, *Orius* spp. are polyphagous and show a preference for attacking larval and adult thrips (Thysanoptera) over other available prey (Xu and Enkegaard 2009). Consequently, they are considered promising and effective as biological control agents and have been used successfully in biological control programs in greenhouse and open-field cropping systems against various thysanopteran pests.

The present work aimed to study the effect of entomopathogenic agents *B. bassiana*, *M. anisopliae* and (Btk) on *C. carnea* and *O. albidipennis* in the laboratory to clarify the possibility of the introduction of these elements together in integrated pest management systems and the interaction between them.

MATERIALS AND METHODS

A-Culture of *B. bassiana* and *M. anisopliae* :

Two entomopathogenic fungi, *B. bassiana* and *M. anisopliae* were isolated in Bio- insecticide Production Unit, Plant Protection Research Institute-Agriculture Research Center. The first fungus was isolated from the white fly in the Sharkia Governorate and the second fungus was isolated from the red palm weevil in Ismailia Governorate. (Ibrahim, 2006).

Fungal cultures of *B. bassiana* and *M. anisopliae* were grown at 25±2ºC, in dark, on Sabouraud dextrose agar (SDA), consisted of peptone 10 g/L, glucose 20 g/L, and agar-agar 20 g/L. Spores were harvested from 15 days old plates by scraping

into sterile 0.05% Tween 80 . The suspension was vortexed for 2 min. Dilutions were prepared to give range of concentrations 10^7, 10^8 and 10^9 spores/ml.

B- Culture of *Bacillus thuringiensis* :

Bacillus thuringiensis subspecies *kurstaki* (Btk) was isolated in Bio- insecticide Production Unit, Plant Protection Research Institute.

Culture of Btk were carried out according to Attathom *et al.* (1995) as follows: T3 medium was prepared which composed of tryptone 3.0g, tryptose 2.0g, yeast extract 1.5g, $MnCl_2$ 0.005g and NaH_2PO_4. H_2O 8.9g, adjusted pH to 6.8 and the final volume was made up to 1 liter with distilled water. The sterilized medium was inoculated and incubated on a shaker (142 rpm) at 28 °C for 72 hr.

The number of CFU/ml of the suspension, which resulted from the previously technique of production, was determined by plate count method, Concentrations of spores suspension were prepared (107, 10^8 and 10^9 spores/ml).

C-Rearing technique of *Chrysoperla carnea* and *Orius albidipennis*:

Stock of *C. carnea* larvae and *Orius albidipennis* nymph were obtained from " Aphid lion, *Chrysopa* mass rearing unit" at the faculty of Agriculture, Cairo University, Egypt.

D-Rearing of *Aphis craccivora*

Rearing of cowpea aphid *A. craccivora* was carried out according to the technique described by Abdel-Samad (1996). Pure culture of the aphid was maintained in the laboratory for this study.

E-Bioassay :

Spores suspension of *B. bassiana, M. anisopliae* and (Btk) were prepared in sterile 0.05% Tween 80 water solution. The mortality effects of the entomopathogenic fungi and bacteria on two insect predators, *C. carnea* and *O. albidipennis* were performed by testing three concentrations of spores suspension from each agent (1×10^7, 1×10^8 and 1×10^9 spores / ml).

Cowpea aphids were transferred into the plastic containers (12 cm diameter and 23cm height). The containers and aphids were sprayed with each concentration of agent and distilled water as control. The experiment was replicated 3 times. Each replicate was provided with ten 2^{nd} instar larvae of *C. Carnea* and ten 2^{nd} nymph of *O. albidipennis*, then were covered with muslin cloth for aeration. The containers were maintained in an incubator at 25 $\pm$ 2, 60-70% RH. Dead larvae and nymphs were counted daily and mortality was calculated. Also, the median lethal time (LT_{50}) of mortality was calculated.

F-Statistical Analysis

The mortality percentage reduction of larvae and nymphs was calculated and corrected according to Abbott's formula (1925). For determination of the lethal times LT_{50}, according to Finney (1971), data were analyzed using Ldp line software developed by Dr. Ehab Bakr, Plant Protection Research Institute http://www.Ehabsoft.com.

RESULTS AND DISCUSSION

The effects of three entomopathogenic agents *B. bassiana, M. anisopliae* and *B. thuringiensis* were evaluated at three concentrations ($10^{7,}10^8$ and 10^9) spores/ml against the 2nd instar larvae of *C. carnea* and 2nd instar nymph of *O. albidipennis.* Suspension were sprayed by small sprayer on aphids (*A. craccivora*) , the aphids were offers to predators as prey under laboratory conditions at 27 ± 2 ºC, 65 ± 10% RH and 16h photophase. Mortality was recorded daily from the 2nd day till the 6th day of treatment.

Data in Table (1) showed cumulative mortalities of the second instar larvae of *C. carnea* fed on aphids treated with *B. bassiana, M. anisopliae* and *B. thuringiensis.* The results revealed that the values of the cumulative mortalities increased gradually from the 2nd day after exposure until the 6th day. Also, as concentration increased for *B. bassiana, M. anisopliae*. While the third agent, *B. thuringiensis* had no effect on *C. carnea* until the 5th day but had a slight effect at the 6th day.

The effect of pathogen, *B. bassiana* was evident by the 2nd day of treatment in the three concentrations ($10^{7,}$ 10^8 and 10^9) spores /ml with recorded mortality (0, 7.95 and 12.44) respectively. However, mortality percentages of *C. carnea* larvae treated with *B. bassiana* at 6th day post treatment reached 18.92% at the concentration 10^7 spores/ml and increased to 40.53% at the highest concentration i.e. 10^9 spores/ml.

Also, Cumulative percent mortality of *C. carnea* larvae fed in the second instar with aphids treated with *M. anisopliae* illustrated in Table (1). The results showed that the cumulative percent mortalities were 1.71, 1.52 and 2.32% at the second day post treatment and increased to reach 19.47, 21.84 and 24.47% at the 6th day after treatment for concentrations ($10^{7,}$ 10^8 and 10^9) spores/ml respectively. While the respective values of mortality percentages of *C. carnea* larvae fed in the second instar with aphids treated with (Btk) were zero at 2nd, 3rd, 4th and 5th day after treatment for all concentrations and reached to 4.70% , 6.80% and 6.80% at the 6th day for concentrations of 10^7 , 10^8 and 10^9 spores/ ml, respectively.

Cumulative percent mortalities of *O. albidipennes* nymphs contaminated with *B. bassiana, M. anisopliae* and fed in the second instar on aphids treated with (Btk*)* are show in Table (2).The results confirmed that the values of the cumulative mortalities increased gradually from the 2nd day after exposure until the 6th day and increased with increasing concentrations of *B. bassiana* and *M. anisopliae*, while the third agent, *B. thuringiensis* had no effect on predator, *O. albidipennes* until the 6th day. Mortality percentage at the second day 0, and11.60 with concentrations $10^{7,}$ 10^{8} spores/ml respectively, and 28.46% at concentration 10^{9} spores/ml for treated with *B. bassiana*. However, mortality percentages of *O. albidipennes* nymphs treated with *B. bassiana* at 6th day post treatment reached 47.14% at the concentration of 10^{7} spores/ml and increased to 69.64% at the highest concentration i.e. 10^{9} spores/ml.

. As for the fungus *M. anisopliae*, mortality percentages in *O. albidipennes* nymphs at 2nd day post treatment were 0, 14.66 and 21.50 % at the concentrations ($10^{7,}$ 10^{8} and 10^{9} spores/ ml) respectively, and increased at 6th day after treatment to reach 55.01, 52.86 and 63.10 % at concentrations ($10^{7,}$ 10^{8} and 10^{9} spores/ ml) respectively. Where the respective values of mortality percentages of *O. albidipennes* nymphs fed in the second instar with aphids treated with *B. thuringiensis* were zero at 2nd, 3rd, 4th and 5th day after treatment for all concentrations and reached to 6.5, 8.49 and 8.49 at the 6th day for concentrations ($10^{7,}10^{8}$ and 10^{9} spores/ ml) respectively.

Table 1. A Cumulative percent mortality of *C. carnea* larvae treated with *B. bassiana, M. anisopliae* and fed in the second instar on aphids treated with *B. thuringiensis* var. *kurstaki.*

Entomopathogenic agents	Con.(spores/ml)	Corrected mortality (%)				
		Days				
		2	3	4	5	6
Beauveria bassiana	1×10^{7}	0	2.85	6.8	12.14	18.29
	1×10^{8}	7.95	14.58	21.08	27.13	32.64
	1×10^{9}	12.44	20.71	28.2	34.77	40.53
Metarhizium anisopliae	1×10^{7}	1.71	4.82	9.17	14.19	19.47
	1×10^{8}	1.52	4.81	9.74	15.60	21.84
	1×10^{9}	2.32	6.49	12.03	18.19	24.47
Bacillus thuringiensis	1×10^{7}	0	0	0	0	4.70
	1×10^{8}	0	0	0	0	6.80
	1×10^{9}	0	0	0	0	6.80

Table 2. A Cumulative percent mortality of *O. albidipennes* nymphs treated with *B. bassiana, M. anisopliae* and fed in the second instar on aphids treated with *B. thuringiensis*

Entomopathogenic agents	Con.(spores/ml)	Corrected mortality (%)				
		Days				
		2	3	4	5	6
Beauveria bassiana	1×10^7	0	14.01	25.42	36.81	47.14
	1×10^8	11.60	22.49	32.86	42.00	49.84
	1×10^9	28.46	43.27	54.55	63.09	69.64
Metarhizium anisopliae	1×10^7	0	0.37	6.49	27.01	55.01
	1×10^8	14.66	26.21	36.59	45.43	52.86
	1×10^9	21.50	35.40	46.81	55.88	63.10
Bacillus thuringiensis	1×10^7	0	0	0	0	6.50
	1×10^8	0	0	0	0	8.49
	1×10^9	0	0	0	0	8.49

Table 3. Median Lethal Time of *C. carnea* and *O. albidipennes* after treated with *B. bassiana, M. anisopliae and (BtK)*..

Entomopathogenic agents	Median Lethal Time (Days)					
	C. carnea			*O. albidipennes*		
	1×10^7	1×10^8	1×10^9	1×10^7	1×10^8	1×10^9
B.bassiana	11.22	10.05	8.00	6.30	6.02	3.56
M. anisopliae	12.61	11.01	10.73	5.82	5.59	4.32
B. thuringiensis	Not detect					

The LT_{50} values of *C. carnea* larvae of *C. carnea* treated with *B. bassiana* in Table (3) were 11.22 days , 10.05 days and8.00 days at concentrations of 10^7, 10^8 and10^9 spores/ml , respectively. They were 12.61 days , 11.01 days and 10.73 days at concentrations of 10^7, 10^8 and10^9 spores/ml , respectively for treated with *M. anisopliae*. Whereas, the LT_{50} values of *O. albidipennes* nymphs treated with *B. bassiana* and *M. anisopliae* were 6.30 days and 5.82 days at concentration of 10^7 spores /ml, respectively. LT_{50} values were 3.56 days and 4.32 days at concentration of 10^9 spores /ml with *B. bassiana* and *M. anisopliae*, respectively But, LT_{50} values had not detected for *(Btk)* against *C. carnea* or *O. albidipennes* at all concentrations.

Our results indicate that beneficial organisms are susceptible to infection by *B.bassiana* and *M. anisopliae* by means of both direct and indirect exposure to entomopathogenic fungi under laboratory. The results reveal that different genera or species of fungi had different pathogenicity and virulence. Entomopathogenic fungus could be quite specific and might infect only certain type of host. These results were

supported by Magalhães *et al.* (1988). Who noted that *B. bassiana* caused mycosis in 60% of adult *Coleomegilla maculata* lengi Timberlake (Col., Coccinellidae) and in 35% of adult *Eriopis connexa* (Col., Coccinellidae), when conidia were applied directly to the insects. Todorova and colleagues (1994) remarked that different strains of *B. bassiana* showed different efficacies on larvae of *C. maculata lengi*. The different ecological host ranges of different entomopathogenic fungus isolates, e.g. co-evolution between hosts and pathogens could partially explain the different susceptibilities found in this study and in previously report. That in agreement to demonstration of James and Lighthart (1994) mentioned that, *B. bassiana* and *M. anisopliae* caused up to 97% and 95% mortality, respectively, to 1st instars larvae of the Coccinellid, *Hippodamia convergens*, a common predator of aphids. Scott and Ronald (2001) evaluated the direct and indirect effect of a commercial formulation of *Beauveria bassiana* strain JW-1 against *Orius insidiosus* (Say), *Phytoseiulus persimilis* Athias-Henriot*, Encarsia formosa* Gahan, and *Aphidius colemani* Viereck were under greenhouse cage and laboratory conditions. They found that the natural enemies were highly susceptible to infection under laboratory conditions, and lower infection rates were observed in the greenhouse trials. It is common for entomopathogenic fungi to infect hosts in laboratory which are never infected in the field (Butt and Goettel, 2000). Furthermore, insect species may be easily infected in laboratory by fungi which are not known to attack them in nature. Also there are different terms between the natural hosts from which the pathogen has been isolated, under laboratory conditions (Hajek and Goettel, 2000).

The experiment in which first instars were treated revealed no negative effects on development time or survivorship of immature *O. sauteri*, or on their longevity as adults, regardless of the concentrations of *B. assiana* formulation used (Gao *et al.* 2012). In contrast, Thungrabeab and S. Tongma (2007) showed that *B. bassiana* was found to be non-pathogenic to *C. carnea* and *Dicyphus tamaninii* and beneficial soil insect with highest fungal concentration (10^8 conidia/ml), while *M. anisopliae* had pathogenicity to natural enemies, *C. carnea* and *D. tamaninii*. Our study revealed also that no significant differences in mortality occurred when *C. carnea* and *O. albidipennes* larvae were treated with Bt and untreated aphids. These results agree with Al-Deeb *et al.* (2001), they suggested that Bt corn and *B. thuringiensis* subsp. *kurstak*i had no significant effect on the predator *O. insidiosus*. Varieties of Bt., the insecticidal activity of which is based on Cry toxins (also known as δ-endotoxins), are now commercially available for use against a wide variety of insect

pests including species of Lepidoptera, Coleoptera and Diptera. The individual Cry toxins are for the most part active against single orders of insect pests and may affect one to several families within an order. The specificity of toxins is determined by the molecular configuration of the toxin and the physiology of the host midgut and presence of toxin receptors on the midgut epithelium (Gill *et al*. 1992).

CONCLUSIONS

We can conclude that both isolates of *B. bassiana*, and *M. anisopliae* are not recommended to be applied at the same time with predators *C. carnea* and *O. albidipennes* as bio control agent in an IPM programs. This isolates of *B. bassiana, M. anisopliae* could be used for rabid, short-term suppression and *C. carnea* and *O. albidipennes* could be used for longer-term suppression of pest. While, we can suggest that use of *B. thuringiensis* var. *kurstaki* can be compatible with *C. carnea* and *O. albidipennes* or other insect predators, which is an important component of an IPM system.

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تأثير تغذية مفترسين حشريين على المن المعامل ببعض الممرضات الحشرية

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فى هذه الدراسة تم تقييم تأثير ثلاث تركيزات (١٠^{٧}، ١٠^{٨}، ١٠^{٩} جرثومة/مل) لكل من *Bacillus thuringiensis* var. *kurstaki* (Btk), و *Beauveria bassiana* و *Metarhizium anisopliae* على العمر اليرقى الثانى لمفترس أسد المن *Chrysoperla carnea* و العمر الثانى لحوريات مفترس بقة الأوريس *Orius albidipennis*. تم رش من الفول بالتركيزات السابقة ثم تقديمه لكلآ المفترسين تحت ظروف المعمل. وتم متابعة وأخذ النتائج من اليوم الثانى حتى اليوم السادس من المعاملة. أظهرت النتائج زيادة تدريجية فى نسب الموت لكلا المفترسين مع زيادة فترة المعاملة من اليوم الثانى حتي اليوم السادس وأيضا مع زيادة التركيزات لكل من فطرى ال *Beauveria bassiana* و و*Metarhizium anisopliae*. بينما لم تظهر بكتريا Btk أى تأثير حتى اليوم الخامس من المعاملة مع ظهور تأثير ضعيف جدا فى اليوم السادس لكل التركيزات مع كلا المفترسين. كانت قيم الوقت اللازم لموت ٥٠% من يرقات مفترس أسد المن المعاملة ب *Beauveria bassiana* و و*Metarhizium anisopliae* هى ١١.٢٢ و١٢.٦ يوم مع تركيز ١٠^{٧} جرثومة /مل وكانت ١٠.٥ و١١.٠١ يوم مع تركيز ١٠^{٨} جرثومة/مل وكانت ٨.٠٠٤ و١٠.٧٣ مع تركيز ١٠^{٩} جرثومة/مل، على الترتيب. بينما لم يكن هناك أى قيم للوقت اللازم لموت ٥٠% من يرقات مفترس أسد المن وحوريات مفترس بقة الأوريس المعاملة بال Btk عند جميع التركيزات المعاملة. فى حين كانت قيم الوقت اللازم لموت ٥٠% من حوريات مفترس بقة الأوريس المعاملة ب *Beauveria bassiana* و *Metarhizium anisopliae* هى ٦.٣ و ٥.٨٢ يوم مع تركيز ١٠^{٧} جرثومة/مل و كانت ٦.٠٢٣ و ٥.٥٩ يوم مع تركيز ١٠^{٨} جرثومة /مل و كانت ٣.٥٦ و ٤.٣٢ يوم مع تركيز ١٠^{٩} جرثومة/مل، على الترتيب.