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Effect of Entomopathogenic Fungi as Biocides Against House Fly, *Musca domestica* L. (Diptera: Muscidae)

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ABSTRACT

The biological and toxicological effects of the formulations of three entomopathogenic fungi, *Beauveria bassiana* , Biovar, *Paecilomyces fumosoroseus*, Apopka Strain 97and *Metarhizium anisopliae*, Bioranza, on house fly, *Musca domestica* L. were evaluated under laboratory conditions. Bioassays experiments were done to determine the effective concentration of fungi, which could be further formulated as a commercial biopesticide against domestic insects. The obtained results indicated that all tested fungi, applied at 1 % concentrations gave the lowest mortality percentages of house fly larvae ranging between 15 - 31.3 %. *Metarhizium anisopliae* 4 % gave the highest mortality percentages of house fly larvae (54 %). The lowest numbers of pupated larvae were recorded with the treatments of *B. bassiana*, *P. fumosoroseus* and *M. anisopliae* at 4 % concentrations. In addition, results indicated that there were significant differences in the numbers of emerged adults between all treatments and control, where the least numbers were recorded with the treatments of the tested fungi at 4 % concentration. The least percentages of emerged adults were recorded with the treatments of *B. bassiana*, *P. fumosoroseus,* and *M. anisopliae* at the treatments of 4 % concentration resulting 37.1, 38.5, and 35.7 %, respectively, in comparison with control treatment which resulted 90%.

Keywords: Biological control; House fly; entomopathogenic fungi; *Beauveria bassiana; Paecilomyces fumosoroseus*; *Metarhizium anisopliae.*

INTRODUCTION

The housefly, *Musca domestica* L. (Diptera: Muscidae) consider one of the most major domestic, medical, and veterinary pest which transmit many medical and veterinary pathogenic organisms (Sukontason *et al*., 2000, Forester *et al*., 2009). Housefly act as a carrier and transmitter of the etiological agents of typhus fever, dysentery, hematic cholera, carbuncle, conjunctivitis and poliomyelitis, bovine mastitis, helminth eggs and protozoan cysts, (Howard 2001; Barin *et al*., 2010), in addition to its nuisance to human and his domestic animals causing egg and milk reduction in both poultry and dairy farms specially with high densities. Most of strategies for control housefly depend on pesticide application, although it can quickly develop resistance to the pesticides (Shono and Scott 2003; Acevedo *et al*., 2009). Srinivasan *et al*., (2008) indicated that houseflies had developed tolerance to the insecticide dichlorvos in the tsunami-hit coastal villages of southern India, thence integrated pest management programs (IPM) including Natural enemies, Bacteria, Viruses, Nematodes and Fungi are highly recommended for effective results and to avoid the emergence of housefly resistance strains (Iqbal *et al*., 2014).

The use of microbial insecticides as a biological control agent of insect pests is a part of (IPM) programs and alternative method to chemical control, it has been noticed that fungal agents consider one of the most hopeful biological control group for pest management. When fungus spores contact with the insect cuticle, they grow and penetrate throw insect cuticle to inside the body and then the fungus reproduce and spread throughout the insect body and parasitize on its entrails which leads to the death.

Apply Deuteromycetes fungi under laboratory and field condition led to decrease in the fly population (Barson *et al*., 1994; Reithinger *et al.,* 1997). Some of the most real entomopathogenic fungi of dipteran insects are *Paecilomyces fumosoroseus* (Wize) (Brown and Smith)*, Beauveria bassiana* and *Metarhizium anisopilae* (Steinkraus *et al*., 1990; Kuramoto and Shimaku 1992; Samson *et al*. 1994; Watson *et al*., 1995). Such entomopathogenic fungi could be used to decrease housefly population without causing mammalian toxicity (Shah and Pell 2003; Goettel *et al*., 2005). *B. bassiana* (Balsamo) and *Entomophthora muscae* (Cohn) were used to manage the house fly larvae and adults in calf hutches on New York dairy farms (Watson *et al.,* 1996). Complete mortality of the housefly population could be achieved within 1-2 weeks, also the isolates of entomopathogenic fungi from the local environment makes them more adaptable and more efficient in the pest control and could thus be a strong competitor for chemical pesticides (Steinkrauss, *et al*., 1990; Geden *et al*., 1995; Watson, *et al*., 1995; Carswell *et al*., 1998, Lecuona, *et al*., 2005; Gisbert Zimmermann, 2008).

Mahmoud (2009) detected that oral application caused higher mortality than contact application, furthermore, the effect of *B. bassiana* and *M. anisopilae* were less virulence than *Lecanicillium lecanii* in both oral and contact trials. Zimmermann (2007) reported that through many experiments in different countries, the entomopathogenic fungus *M. anisopliae* (Metsch.) proved highly efficient against many hosts in different countries, in addition, demonstrated safety to human, animals, birds, fishes and plant, also it has been noticed that the effect of the *M. anisopliae* under laboratory conditions usually more than in the field.

In addition, *P. fumosoroseus*, is considered a very promising biological pesticide for many insect species due to its extensive host range which includes insects in over 25 different families, including the diamondback moth (*Plutella xyllostella*), Russian wheat aphid (Diuraphis noxia), silver leaf whitefly (*Bemisia argentifolii*) and a wide range of mites (Spotted Spider Mite, Red Mite, Brown Mite, and Rust Mite). *P. fumosoroses* Wize (Brown and Smith) was assessed on whitefly nymphs infesting wide range of host plants and it has succeeded in achieving high rates of whitefly mortality (Wraight *et al*.,2000 ;James 2003; Avery *et al*., 2008). Moreover, Gayathri et al., (2010) explained that *P. fumosoroseus* has good effects against third instar larvae of *Culex quinquefasciatus*.

The objective of the present study is to investigate the toxic effect of three formulations of the entomopathogenic fungi, *Beauveria bassiana* Biovar, *Paecilomyces fumosoroseus* Apopka Strain 97, and *Metarhizium anisopliae* Bioranza, on the development of the housefly stages under laboratory bioassays.

MATERIALS AND METHODS

House fly rearing:

The larvae of domestic house fly (Egyptian strain), *Musca domestica* L., were collected from manure piles at the poultry farms of the Faculty of Agriculture, Menoufia University, Egypt. The house fly larvae were provided by nutrient compound to fed and complete its life cycle on it. The nutrient compound was introduced in plastic cups, 10 cm diameter and 10 cm deep, the nutrient compound consisted of 9 g powder milk and 5 g yeast dissolved in 100 ml water, then was added to 100 g fine bran according to (Wilkins and Khalequzzaman, 1993). The mixture was then thoroughly stirred and put into the cups leaving 3 cm from the top. The cups were transferred to an entomological glass cages (60 × 35 × 40 cm) which used for rearing house fly under laboratory conditions (25 ± 2˚C & 60 ± 5 % RH). These cages were covered with a mesh screen with cloth sleeve opening at top and provided with electric lamps 20 watt to control temperature in cages during winter months. When adult house fly emerged in cages, full fat fresh milk, granulated sugar and milk soaked cotton wool balls were provided in Petri dishes as food for house fly adults. After two days of housefly emergency, the beakers containing larval food were placed in the glass cages for egg laying process, and then beakers were removed from cages after 2 - 3 days when the eggs were visible and attached to food along the sides of beakers. The food was changed every 2 - 4 days depending upon the numbers of larvae per beaker. The beakers were kept in separate cages for fly emergency according to (Ahmed and Irfanullah, 2007).

Application of fungal formulations on the second instar larvae of house fly, *M. domestica*:

Three fungal formulations of *Beauveria bassiana* Biovar, *Isaria (Paecilomyces*) *fumosoroseus* Apopka Strain 97 and *Metarhizium anisopliae* Bioranza fungi were obtained from Ministry of Agriculture as biocides WP ($32×10^7$). The three formulations were diluted in distilled water to prepare three concentrations (1.0, 2.0, 4.0 %) of each. One ml of each concentration of fungus formulations was mixed with 80 g of the artificial diet. Each treatment consisted of four plastic cups (10 cm diameter & 10 cm deep) as replicates where each cup contains 80 g of house fly artificial diet mixed with 1 ml of each concentration as well as 25 house fly larvae were putted in , while check treatment was provided only with 80 g of house fly artificial diet mixed with 1 ml water.

Control treatment was provided by 1 ml of the chemical pesticide , Diazinon 60% at the concentration of 1000 ppm.

Cups were maintained in a laboratory under 25 ± 2˚C & 60 ± 5 % RH . Dead larvae were recorded 24 h, 48, h, and 72 h after treatments. Mortality percentages were calculated and modified by Abbott's formula (1925).

Biological and toxicological effects of tested fungi on house fly, *M. domestica*:

As mentioned before, the three fungus formulations were diluted in distilled water to prepare three concentrations (1.0, 2.0, 4.0 %) of each. One ml of each concentration of fungus formulations was mixed with 80 g of the artificial diet. Each treatment consisted of four plastic cups (10 cm diameter & 10 cm deep) as replicates where each cup contains 80 g of house fly artificial diet mixed with 1 ml of each concentration as well as 25 house fly larvae were putted in , while check treatment was provided only with 80 g of house fly artificial diet mixed with 1 ml water.

Control treatment was provided by 1 ml of the chemical pesticide, Diazinon 60% at the concentration of 1000 ppm.

Cups were maintained in a laboratory under 25 ± 2˚C & 60 ± 5 % RH. Treatments were examined daily till the adult's emergency. Number of pupated larvae, weight of pupae, pupation percentages, number of emerged adults, and emergency percentages were counted.

Statistical analysis:

The data were subjected to analysis of variance (ANOVA) using computer and the means were compared by L.S.D. test at 0.05 level, using SAS program (SAS Institute, 2003).

Abbott formula (1925) was used to determine the reduction percentages for different treatments. Reduction % = Control – treatment / control X 100.

RESULTS AND DISCUSSION

Effect of fungi concentrations on house fly larvae:

The obtained results in Table (1) show the effect of three concentrations (1%, 2% , 4 %) of the three fungi : *Beauveria bassiana* , Biovar , *Isaria (Paecilomyces) fumosoroseus* , Apopka Strain 97 , and *Metarhizium anisopliae*, Bioranza on the mortality percentages of house fly larvae, *Musca domestica* after 24 , 48, and 72 hours of applications.

Statistical analysis of the data in Table (1) revealed that there were significant differences in the average mortality of house fly larvae among the tested fungus concentrations. The highest overall reduction percentage was recorded with *M. anisopliae* at 4 % concentration giving 54 % mortality, followed by the treatments of *P. fumosoroseus* at 4 % concentration resulted 48.7 % and *M. anisopliae* at 2 % concentration 47.3 %, in comparison with the pesticide, Diazinon 60% treatment which killed 94 % of larvae. It was noticed that all tested fungus applied at 1 % concentrations gave the lowest mortality percentages of house fly larvae ranging between 15 – 31.3 %.

Table 1. Mortality percentages of house fly larvae, 24, 48, and 72 hours after application of different fungal concentrations

Fungus concentration		House fly larvae mortality % 24 h	48 h	72 h	overall mortality
Beauveria bassiana Biovar	1 %	5	15	25	15 f
	2 %	7	25	40	24 e
	4 %	10	35	65	33.3 d
Paecilomyces fumosoroseus Apopka Strain 97	1 %	6	20	35	20.3 e
	2 %	7	38	54	33 d
	4 %	11	60	75	48.7 c
Metarhizium anisopliae Bioranza	1 %	8	30	56	31.3 d
	2 %	10	54	78	47.3 c
	4 %	12	65	85	54 b
Diazinon 60%		87	95	100	94 a
Control		0.0	0.0	0.0	0.0 g
LSD 5%		-	-	-	4.1

Means in last column followed by the same letter (s) are not significantly different at 5% level.

Effect of fungi concentrations on different stages of house fly , *M. domestica*:

Data presented in Table (2) show the effect of three concentrations (1% , 2% , 4 %) of the three fungi: *B. bassiana* Biovar , *P. fumosoroseus* , Apopka Strain 97 , and *M. anisopliae* Bioranza on the pupation process percentages of house fly larvae, *M. domestica* after 24 , 48, and 72 hours of applications , under laboratory conditions (25 ± 2 C°& 60 ± 5 % RH).

Statistical analysis of the data in Table (2) indicated that there were significant differences among all tested concentrations of the three fungi, in the numbers of the pupated larvae, as well as the average numbers of the weights of pupae.

The lowest numbers of pupated larvae were recorded with the treatments of *B. bassiana*, *P. fumosoroseus* and *M. anisopliae* at 4 % concentration.

Regarding to the effect of different concentrations of the tested fungi on the weights of resulted pupae, results in Table (2) indicated that there were significant variations among tested concentrations of all applied fungi, where the least weights of pupae were recorded with the 4 % concentration of the tested fungi. Statistical analysis of the data Table (2) indicated that there were significant differences in the numbers of pupated larvae among tested concentrations where the least pupation numbers were recorded with the 4 % concentration of all tested fungi resulting 65 to 70 % pupation. In addition, there were significant differences in the numbers of pupated larvae between pesticide treatment and all other treatments.

Regarding to the percentages of emerged adults as influenced by the application of the three fungi isolates in comparison with a pesticide, results in Table (2) indicated that there were significant differences in the numbers of emerged adults between all treatments in comparison with control, where the least numbers were recorded with the treatments of the tested fungi at 4 % concentration. The least percentages of emerged adults were recorded with the treatments of *B. bassiana*, *P. fumosoroseus,* and *M. anisopliae* at the treatments of 4 % concentration resulting 37.1, 38.5, and 35.7 %, respectively, in comparison with control treatment which resulted 90%.

It could be concluded that, the best results in the control of house fly stages, *M. domestica* using fungi were recorded with the treatment of *B. bassiana, P. fumosoroseus*, and *M. anisopliae* at 4 % concentration.

Table 2. Effect of different concentrations of fungus formulations on different stages of house fly

Fungus formulations and concentration		No. treated larvae	No. pupated larvae	weight of pupae (g)	% pupation	No. emerged adults	% emerged adults
Beauveria bassiana Biovar	1 %	100	85 b	0.346 b	85 b	41 b	48.2 b
	2 %	100	80 bc	0.291 c	80 bc	35 d	43.8 bcd
	4 %	100	70 de	0.239 c	70 de	26 g	37.1 ef
Paecilomyces fumosoroseus Apopka Strain 97	1 %	100	75 cd	0.399 b	75 cd	31 ef	41.3 cde
	2 %	100	70 de	0.296 c	70 de	28 fg	40 def
	4 %	100	65 e	0.264 c	65 e	25 g	38.5 ef
Metarhizium anisopliae Bioranza	1 %	100	85 b	0.353 b	85 b	39 bc	45.9 b
	2 %	100	80 bc	0.329 b	80 bc	36 cd	45 bc
	4 %	100	70 de	0.245 c	70 de	25 g	35.7 f
Diazinon 60%		100	0.0 f	0.0 d	0.0 f	0.0 h	0.0 g
Control		100	100 a	0.417 a	100 a	90 a	90 a
LSD 5 %		-	6.3	0.131	6.3	3.9	4.4

Means in each column followed by the same letter (s) are not significantly different at 5 % level.

The obtained results agree with those conducted by Zimmermann (2008) who studied biology, and ecology of the entomopathogenic fungi, *Isaria farinosa* (formerly *Paecilomyces farinosus*) and the *Isaria fumosorosea* species complex (formerly *Paecilomyces fumosoroseus*), in addition to the use in biological control . In addition, Sapna *et al*., 2011, Sharififard *et al*., 2012 successively applied entomopathogenic fungi against various species of insects.

It could be concluded that the three tested fungi *Beauveria bassiana*, *Paecilomyces fumosoroseus,* and *Metarhizium anisopliae* at the treatments of 4 % concentration recorded the highest reduction percentages of house fly larvae , pupae and emerged adults with significant differences, and may be play an important role as a biological agents against domestic insects.

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تأثير الفطريات الممرضة للحشرات كمبيدات حيوية فى مكافحة الذبابة المنزلية

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تعتبر الذبابة المنزلية واحدة من الآفات ذات الأهمية الطبية والبيطرية التي تسبب العديد من الأمراض للإنسان و الحيوان و تنقل المسببات المرضية إلى الكائنات الحية. وتعتبر الفطريات الممرضة للحشرات من الوسائل الآمنة بيئيا والتى يمكن استخدامها فى مكافحة الحشرات المنزلية . تم تقييم التأثير البيولوجى والمميت لثلاثة أنواع من الفطريات الممرضة للحشرات (البيوفاريا باسيانا ، والباسيليومايسيس فيموسوروسيس ، والميتورزيم انيسوبلى) على أطوارالذبابة المنزلية تحت الظروف المعملية وذلك بتطبيق ثلاثة تركيزات من كل فطر ١ ، ٢ ، ٤٪ . أظهرت النتائج عدم كفاءة الفطريات المختبرة بتركيز ١٪ ضد يرقات الذباب المنزلى بينما أعطى الفطر ميتورزيم انيسوبلى بتركيز ٤ ٪ أعلى نسبة موت لليرقات (٥٤ ٪) مقارنة بالمعاملات الاخرى. أعطت الفطريات المختبرة بتركيز ٤ ٪ أقل عدد من العذارى مقارنة بالكنترول . كما أظهرت النتائج أن هناك فرق معنوى فى عدد الحشرات الكاملة والتى أكملت دورة الحياة بين الفطريات المختبرة والكنترول حيث كانت نسب خروج الحشرات الكاملة ٣٧.١ ، ٣٨.٥ ، ٣٥.٧ ٪ للفطريات المختبرة (البيوفاريا باسيانا ، الباسيليومايسيس فيموسوروسيس ، الميتورزيم انيسوبلى) بتركيز ٤ ٪ مقارنة بنسبة خروج الحشرات الكاملة فى الكنترول والتى قدرت ب ٩٠ ٪ .