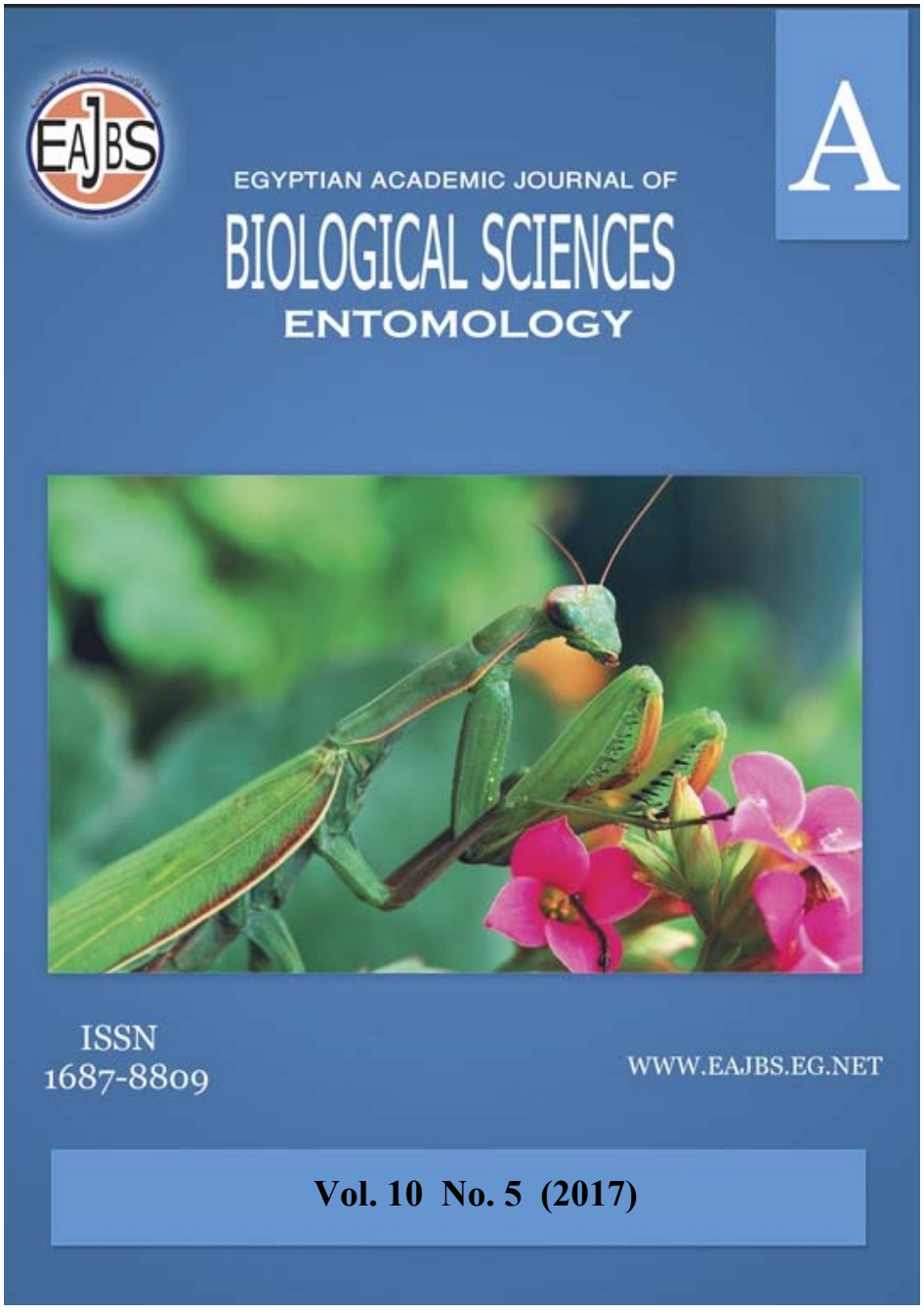

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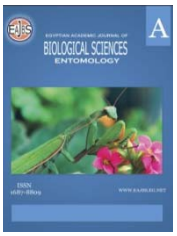

Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* on the adult housefly, *Musca domestica* L.

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B. bassiana, *M. anisopliae, M. domestica*, SEM, pathogenicity.

ABSTRACT

The effects of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on the adult housefly, *Musca domestica* were studied to evaluate their pathogenicity. Scanning electron microscopy allowed observing fungal developmental phases on the *M. domestica* adult. The results obtained revealed that not all areas of the insect cuticle were equally vulnerable to penetration by propagules of entomopathogenic fungi, the penetration pegs of *B. bassiana* were observed at the base of seta, conidia were also observed between the ommatidia of the compound eye and at the articulating membrane of legs. *M. anisopliae* conidia were observed in the regions of the host insect as *B. bassiana,* but in smaller concentrations. In conclusion, the present study established the pathogenicity of entomopathogenic fungi, *B. bassiana* and *M. anisopliae* on the housefly, *M. domestica* as promising biological control agents. Biological control with pathogenic fungi is promising alternative to chemical control against insect pests.

INTRODUCTION

The housefly, *Musca domestica* is a major domestic, medical and veterinary pest that cause irritation, spoils food and acts as a vector of many medical and veterinary pathogenic organisms (Sukontason *et al*. 2000; Forester *et al.* 2009). It is responsible for transmission of over 100 different pathogens (Pospischil *et al.* 1996). It has been found to carry the etiological agents of typhus fever, dysentery, cholera, hematic carbuncle, bovine mastitis, conjunctivitis and poliomyelitis, protozoan cysts and helminthes eggs (Howard, 2001; Barin *et al* .2010).

The most common method for control of houseflies is through the use of insecticide. Over the past seventy years, a variety of chemicals have been used to control houseflies including chlorinated hydrocarbons, organophosphates, carbamates and pyrethroid (Shono *et al*. 2004). Today, commercial housefly control is limited to a few organophosphate, carbamate, pyrethrins and pyrethroids. Resistance to organophosphates, carbamates and pyrethroid insecticide (Boxler and Campbell, 1983; Plapp, 1984; Scott *et al*. 1989; Kaufman *et al*. 2001; Butler *et al*. 2007; Kozaki *et al*. 2009; Memmi, 2010) was noticed and resistance against new promising insecticides, spinosad was documented (Shono and Scott, 2003; Deacutis

et al. 2006). Hence, efforts to control flies using biological control agents are increasingly important.

There are 90 genera and more than 700 species of entomopathogenic fungi are considered as insect infecting fungi including Orthopteran, Lepidopteran, Dipteran and Homoptera that represent about all the major classes of fungi known, the infection process of insect starts by contact of the spores to the host cuticle. Sometimes, conidium attaches to the cuticle or secret mucus for adhesion during its germination and swelling (Hajek & Leger, 1994). Some structures and general process are involved in the penetration of host cuticle and the mechanism of each fungus may also differ. After the penetration of germ tube through the cuticle and insect epidermis, the fungus multiplies into the body cavity of insect.

This study aimed to evaluate the pathogenicity of two entomopathogenic fungi named, *Beauveria bassiana* and *Metarhizium anisopliae* and to study the mode of action of them by using Scanning Electron Microscope.

MATERIALS AND METHODS

Rearing of *Musca domestica* colony.

The housefly, *M. domestica* adults were obtained from Medical Entomology Research Center and then transferred to the rearing room at Bio-insecticide Production Unit, Plant Protection Research Institute, Dokki, Giza. Adults reared at 27±2 ^{o}C and 70±5% relative humidity and photoperiod 12:12 (Light: Dark). Adult houseflies were maintained in (40×40×40) wooden cages covered by gauze. The emerged flies were fed on dry diet, milk powder and sucrose solution (cotton pads soaked in 10 % sucrose solution). Eggs were being collected from paper strips or from cotton pads of feeding. Larvae were reared on an artificial diet wheat bran, milk, powder, and yeast (200:100:5 gm/200 ml distilled water) according the method described by Busvin (1962).

Entomopathogenic fungi:

Entomopathogenic fungi used in this study were *Beauveria bassiana* and *Metarhizium anisopliae.* The first fungus was isolated from the red palm weevil, *Rhynochophorus ferruginens* in Ismailia governorate, while the second one was isolated from the white fly *Bemisia tabaci* in Sharkiah governorate (Ibrahim, 2006).

Mass production of entomopathogenic fungi:

Conidia obtained from fungal culture of *B. bassiana* and *M. anisopliae* were grown at 25 ^{o}C, in dark, on sabouraud dextrose agar (SDA), consisted of peptone 10 g/L, glucose 20g/L, agar-agar 20 g/L., (constant volume of 15 ml) in standard Petri-dishes (90 mm diameter). Conidia were harvested from 15 days old plates by scraping into sterile Triton-X. The suspension was vortexed for 2 minutes and agitated for 90 mins. on a flask shaker at room temperature before filtering through four layers of sterile muslin. The conidial concentrations of the resulting stock suspension were estimated using an improved Neubaure bright line hemocytometer (Reichart) under a Leitz Dialu×40 EB microscope (400×magnification).

Scanning Electron Microscopy preparation:

The samples were fixed by immersion for 6 hours in 4% glutaraldehyde with a 0.2M sodium cacodilate PH 7.2 buffer. Specimens were then fixed in 1% Osmium tetroxide (OsO_4) in a 0.1M pH 7.2 sodium cacodilate buffers for 1 hour. Fixation and dehydration were performed in plastic Eppendroff tubes. After fixation, specimens were dehydrated in a 10, 20, 30, 50, 70, 90 and 100% ethanol series using automatic

tissue processor Leica Electron Microscopy tissue processing (EMTP). The samples were finally washed three times in 100% ethanol solution. Then the samples were dried using CO_2 critical point drier. The samples were then mounted on stubs and coated by gold sputter coater (SPI-Module). Three samples were prepared for each sampling time. Then the samples were investigated using scanning electron microscopy (JEOL-JSM-5500 LV) by high vaccum mode at the Regional Center of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Duration of the different phases of the infection process was estimated from SEM observations.

RESULTS

Beauveria bassiana:

Scanning electron microscopy of *Musca domestica* adults treated with the LC_{50} of the fungus, *B. bassiana* clearly revealed adhesion of conidia to the ommatidia 6 and 12 hrs post infection to the fly abdomen (Figures 1 & 2). Signs of hyphal penetration of the insect cuticle as well as proliferation of the cuticle were also seen 24 hrs post infection (Figures 3 & 4). A network of mycelium was clearly seen on the compound eyes of the fly 48 hrs post infection (Figure 5). Also, germ tubes in-between ommatidia were clearly seen 48 hrs post infection (Figure 6). A network of mycelium covering the abdomen was appeared 72 hrs post infection (Figure 7). Moreover, the massive sporulation of conidia on the abdomen of the adult housefly was clearly seen (Figure 8).

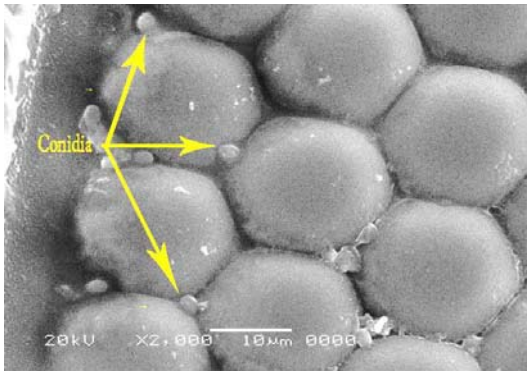

Fig. (1): SEM of *B. bassiana* on adult housefly, *M. domestica*: Showing conidia adhering to the ommatidia of a fly (X2000), 6 hrs post infection.

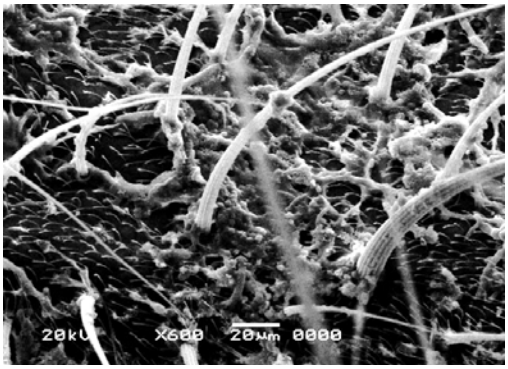

Fig. (2): SEM of *B. bassiana* on adult housefly, *M. domestica*: Showing conidia adhering to the abdomen of the fly (X680), 12 hrs post infection

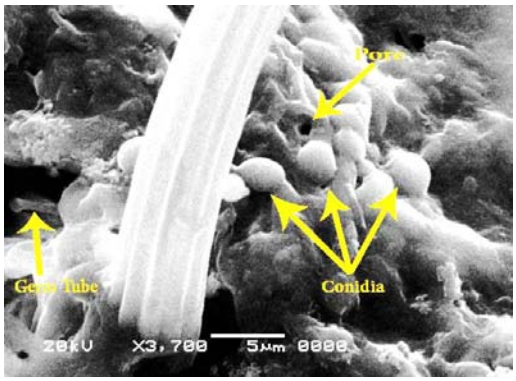

Fig. (3): SEM of *B. bassiana* on adult housefly, *M. domestica*: Showing penetration of a germ tube at the base of a seta (X3700), 24 hrs post infection.

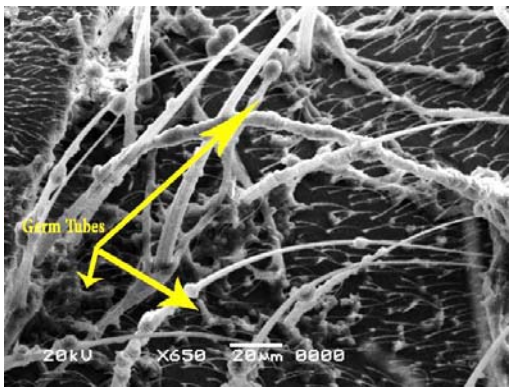

Fig. (4): SEM of *B. bassiana* on adult housefly, *M. domestica*: Showing penetration of a germ tubes in the abdomen of a fly (X650), 24 hrs post infection.

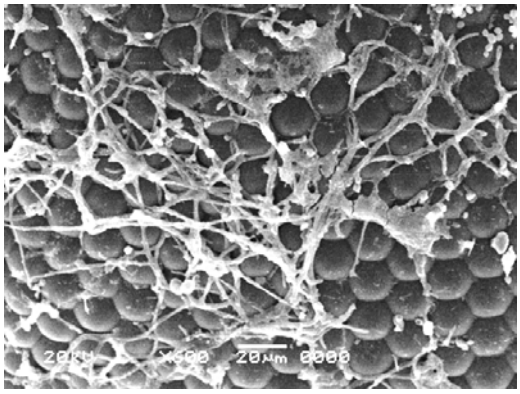

Fig. (5): SEM of *B. bassiana* on adult housefly, *M. domestica:* Showing the network mycelium on the compound eyes (X600), 48 hrs post infection.

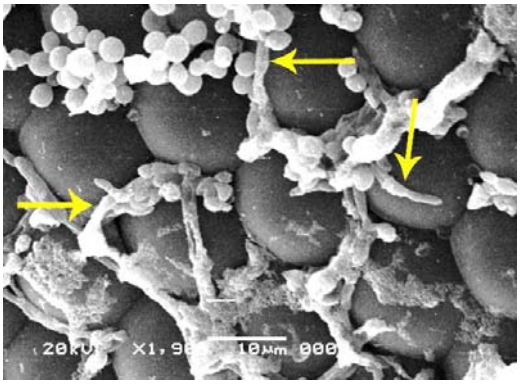

Fig. (6): SEM of *B. bassiana* on adult housefly, *M. domestica*: Showing the germ tube in-between ommatidia (X600), 48 hrs post infection.

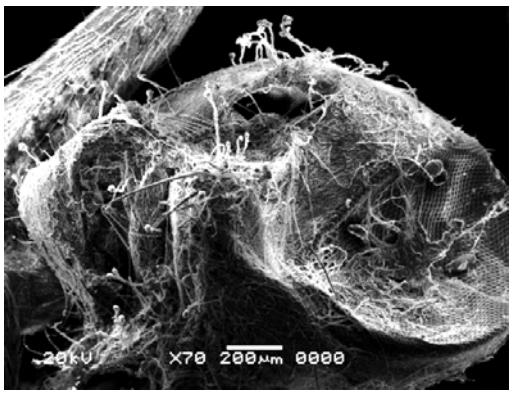

Fig. (7): SEM of *B. bassiana* on adult housefly, *M. domestica*: Showing the network mycelium on the abdomen (X70), 72 hrs post infection.

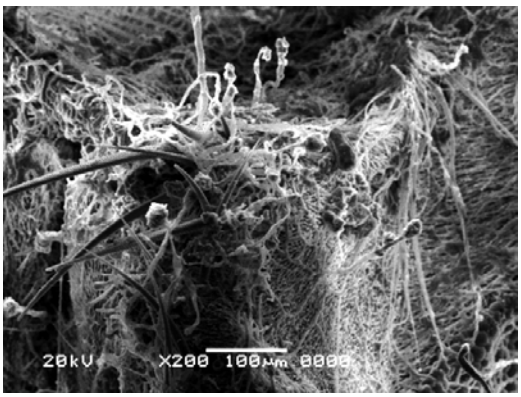

Fig. (8): SEM of *B. bassiana* on adult housefly, *M. domestica*: Showing the massive sporulation of conidia (X200), 72 hrs post infection.

***Metarihizium anisopliae*:**

SEM of *M. domestica* adults treated with the LC_{50} of the fungus, *M. anisopliae* showed conidial adhesion to the ommatidia 12 hrs post infection (Figure 9). Germination and penetration were seen (Figures 10 & 11) 24 hrs post infection. Conidiogenesis on the abdomen of the fly was appeared 120 hrs post infection (Figure 12). A dense network of mycelium on the fly abdomen was also seen 144 hrs post infection (Figure 13).

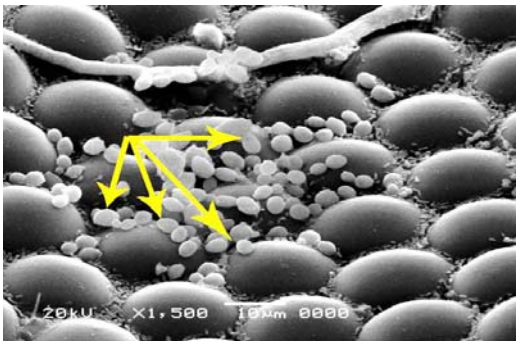

Fig. (9): SEM of *M. anisopliae* on adult housefly, *M. domestica*: Showing conidia adhering to the ommatidia (X1500), 12 hrs post infection.

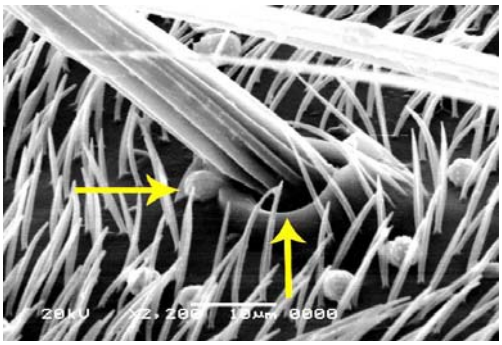

Fig. (10): SEM of *M. anisopliae* on adult housefly, *M. domestica*: Showing germination at the base of the seta (X2200), 24 hrs post infection.

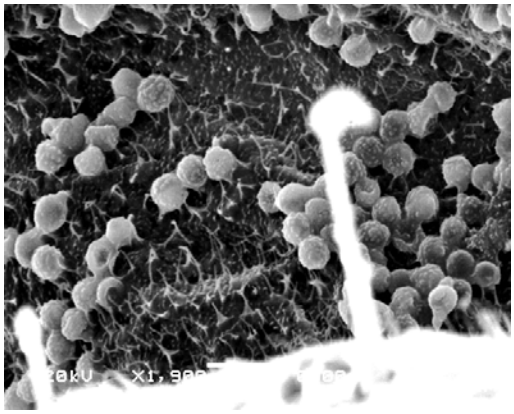

Fig. (11): SEM of *M. anisopliae* on adult housefly, *M. domestica*: Showing germination and penetration, (X1900), 24 hrs post infection.

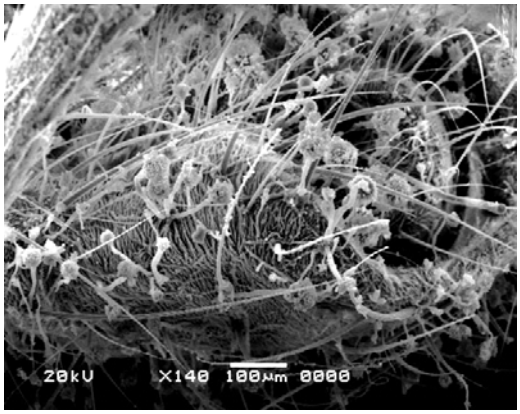

Fig. (12): SEM of *M. anisopliae* on adult housefly, *M. domestica*: Showing conidiogenesis on the abdomen (X140), 120 hrs post infection.

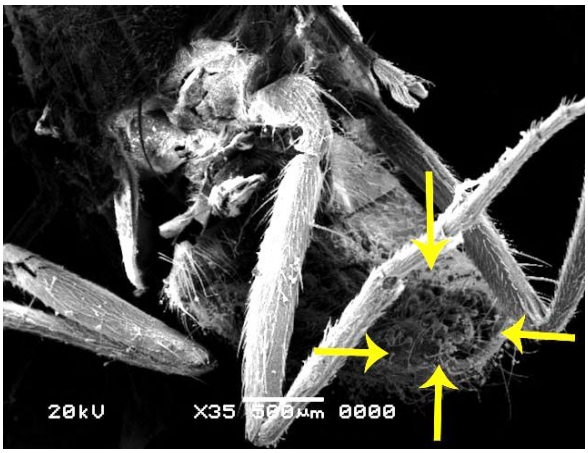

Fig. (13): SEM of *M. anisopliae* on adult housefly, *M. domestica*: Showing dense network mycelium on the cuticle of abdomen (X35), 144 hrs post infection.

Data given in (Table 1) Compare the developmental periods of the two fungi, *B. bassiana* and *M. anisopliae* on the adult housefly, *M. domestica.* The developmental phases of *B. bassiana* were faster than those of *M. anisopliae*. Conidial adhesion occurred 6 and 12 hrs post infection for *B. bassiana* and *M. anisopliae*; respectively. Germination appeared 12 and (12-24) hrs post infection for *B. bassiana* and *M. anisopliae*; respectively. Penetration was seen 24 hrs post infection. Colonization was occurred (36-48 hrs) post infection for *B. bassiana*, while for *M. anisopliae* it was found 120 hrs post infection. Conidiogenesis of *B. bassiana* was seen 72 hrs post infection, while it occurred (120-144 hrs) in the case of *M. anisopliae*. These observations indicated that the development of the fungus, *B. bassiana* in housefly was faster than *M. anisopliae*. The aforementioned results suggested that *B. bassiana* is highly effective biocontrol agent against the housefly, *M. domestica*.

Table (1): Duration of different developmental phases of the fungi; *B. bassiana* and *M. anisopliae* on the adult housefly, *M. domestica*.

Phase	Inoculation time (hrs).									
	0	6	12	24	36	48	72	96	120	144
Conidial adhesion		+	×							
Germination			+×	×						
Penetration				+×						
Colonization					+	+			×	
Conidiogenesis							+		×	×

+ = B. bassiana, × = M. anisopliae.

DISCUSSION AND CONCLUSION

Entomopathogenic fungi have been reported to invade the host cuticle shortly after germination or after limited hyphal growth (Wraight *et al.* 1990; Leger *et al.* 1991). Fungal infection begins when conidia (asexual spores; the seed of a fungus) attach to insect cuticle, the spores germinate and penetrate the insect skin and enter the host. Once the fungus penetrates the host; it produces toxins that overcome the insect immune system. Thereafter, the hyphae penetrate through the cuticle to the outside and cause sporulation on the insect body.

SEM allowed observing *B. bassiana* and *M. anisopliae* developmental phases on the adult housefly, *M. domestica*. The present study revealed that not all areas of the insect cuticle were equally vulnerable to penetration by propagules of entomopathogenic fungi, the penetration pegs of *B. bassiana* were observed at the base of seta. Conidia were also observed between the ommatidia of the compound eye and at the articulating membrane of legs. *M. anisopliae* conidia were observed in the regions of the host insect as *B. bassiana*, but in smaller concentrations. These observations are consistent with Toledo *et al.* (2010) for development of *B. bassiana* and *M. anisopliae* in the adult plant hopper, *Peregrinus maidis*, Mwamburi *et al.* (2009) for the development of *B. bassiana* in housefly, *M. domestica* and Abd-ElWahed (2011) for the same tested fungi against *Agrotis ipsilon* larvae.

The killing of insect host is attributed to the production of secondary metabolites by the parasitic fungi (Roberts, 1981). These findings may explain the death of *M. domestica* infected with *B. bassiana* or *M. anisopliae*. The present study has shown that, the developmental phases of *B. bassiana* in the adult of *M. domestica* were faster than those of *M. anisopliae*. These results are in agreement with Toledo *et al.* (2010) for the development of *B. bassiana* and *M. anisopliae* in *Peregrinus maidis*.

The present study established the pathogenicity of entomopathogenic fungi, *B. bassiana* and *M. anisopliae* on the housefly, *M. domestica* as biological control agents. Biological control with pathogenic fungi is promising alternative to chemical control against insect pests.

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ARABIC SUMMARY

التأثيرات الممرضة للفطريات بيوفاريا بسيانا ومتارزيم أنزوبلي علي الطور البالغ للذبابة المنزلية مسكا دوميستكا.

أحمد إبراهيم حسب الله، محمد عبد الحي فوده، مصطفي إبراهيم حسن و جمال محمد عمر.
قسم علم الحيوان، كلية العلوم، جامعة الأزهر، مدينة نصر، القاهره..

تم عمل دراسة لتقييم تأثيرات الفطريات الممرضة للحشرات بيوفاريا بسيانا ومتارزيم أنزوبلي علي بعوضة مسكا دوميستكا. تم استخدام الميكروسكوب الالكتروني الماسح لملاحظة الاطوارالمختلفة لنمو الفطريات علي البعوضة.

أظهرت النتائج التي تم الحصول عليها أن المناطق المعرضة للإصابة في جليد الحشرة كانت علي غير ذات القدر من حيث تعرضها للإختراق بواسطة بوغيات الفطريات الممرضة للحشرات. حيث وجدت اثار فطر بيوفاريا بسيانا عند قواعد الشعيرات، كما وجدت غبيرات الفطر بين العيون المركبة للحشرة وعند الغشاء المفصلي للأرجل. اما بالنسبة للفطر الاخر فقد وجدت غبيراته في نفس الاماكن تقريبا ولكن بكميات اقل.

اظهرت لنا هذه الدراسة احتمالية استخدام فطر بيوفاريا بسيانا ومتارزيم أنزوبلي كعوامل مكافحه حيويه فعاله علي بعوضة مسكا دوميستكا. حيث تعد المكافحة الحيوية للافات الحشرية باستخدام الفطريات المسببة للأمراض بديلا واعدا للمكافحة الكيميائية.