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Effect of *Beauveria bassiana* and *Metarhizium anisopliae* on different stages of *Phlebotomus papatasi* (Diptera: Psychodidae)

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Abstract: The sand fly, *Phlebotomus papatasi* (Scopoli, 1786) (Diptera: Psychodidae), is the main vector of *Leishmania major* Yakimoff and Schokhor, 1914, the causative agent of zoonotic cutaneous leishmaniasis North Africa, the Middle East, and North Sinai. The purpose of this study was to determine the effect of fungi on *P. papatasi* larvae, pupae, and adults using light microscopic analysis, scanning electron microscope (SEM), and histopathological studies. Specifically, larvae, pupae, and adult *P. papatasi* were infected with the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. Scanning electron microscope and histopathological methods were used to investigate the destructive impact of the fungi on the external and internal structures of *P. papatasi*. The results revealed propagation of the conidia on the cuticles of all *P. papatasi* life stages, including on the compound eyes, leg setae, thorax, wings, and abdomen of the adults. Histological sections of the control and treated larvae, pupae, and adults showed many alterations and malformations in the body and tissues of all life stages after 72 h. These results demonstrated that *B. bassiana* was more effective than *M. anisopliae* as a biological control of phlebotomine sand flies. Further studies to determine the best methods for delivery and application in the diverse ecological settings of the various leishmaniasis vectors are recommended.

Keywords: Entomopathogenic fungus, sand fly, light microscopic, SEM, histopathology

Introduction

Sand flies (Psychodidae) are vectors for multiple medically important viruses, such as the bacterium *Bartonella baciliformis*, and, most importantly, the protozoan parasites that cause leishmaniasis (WHO 2008). Sand flies are significant biting flies that cause acute dermatitis and delayed-type hypersensitivity reactions in humans (Belkaid *et al*. 2000). *Phlebotomus papatasi* (Scopoli, 1786) (Diptera: Psychodidae) is a species of sand fly that act as a vector for *Leishmania major* Yakimoff and Schokhor, 1914, the causative agent of zoonotic cutaneous leishmaniasis in North Africa, the Middle East, South Asia (WHO 2008), and North Sinai (Fryauff *et al*. 1993). Insecticide resistance in worldwide populations of phlebotomine sand flies is a threat to the success of programs aimed at mitigating the spread of leishmaniasis (Delinger *et al*. 2016).

Difficulties controlling *P. papatasi* using traditional methods have stimulated efforts to develop new control measures with reduced environmental impacts. The biological control of sand flies has enormous potential to provide sustainable and environmentally-friendly alternatives. Various biological control methods have been previously used for sand fly control (e.g. essential oils; Ahmed *et al.* 2018). Entomopathogenic microorganisms are an important alternative pest control method. Specifically, the entomopathogenic fungi (EPF) are possible biological agents for vector control. Some naturally-occurring EPF infect and kill sand flies and other medically important arthropods (Maniania 1998, Scholte *et al*. 2004, Mnyone *et al*. 2009, Amora *et al*. 2009, Kikankie *et al.* 2010, Ansari *et al*. 2011). For example, *Beauveria bassiana* is widely distributed in

nature and has the potential to control a broad range of its approximately 700 insect host species. This fungus is applied as a conidial spray against a large number of field and laboratory insects, such as whiteflies, aphids, and many crop pests. In addition, *B. bassiana* is harmless to a large number of non-target organisms (Irigaray *et al*. 2003). *Metarhizium anisopliae* (Metsch) Sorok (Ascomycota: Clavicipitaceae) is a mitosporic fungus that utilizes asexual reproduction and usually multiplies as blastospores or hyphae structures in their host, similar to yeasticidal activity, then spread into the body cavity via blood fluid movement in the insect. The spores of both of these fungi penetrate insect body via integument contact and result in host death. They produce cylindrical conidia and subcylindrical phialides. The disease caused by *M. anisopliae* is known as green muscardine disease, because of the green color of its spores (Driver *et al*. 2000). Warburg (1991) observed 100% mortality in adult *P. papatasi* infected with the fungus.

Entomopathogenic fungi are generally considered environmentally safe agents with low mammalian toxicity (Siegel & Shadduck 1990, Cox & Wilkin 1996, Moore *et al.* 2000, Batta 2016a). They infect insects by contact. Specifically, fungal conidia attach on the host cuticle, then germinate infectious hyphae that invade the host via direct penetration of the host exoskeleton or cuticle (Clarkson & Chamley 1996, Srivastava *et al.* 2009, Stephou *et al*. 2012, Ortiz-Urquiza & Keyhani 2013). There are many examples of successful, fungus-based insect control programs using *B. bassiana* and *M. anisopliae* (Shah & Pell 2003, Roberts & Leger 2004). One major characteristic of these species is their ability to create resting spores that have facultative or saprophytic properties under unfavorable environmental conditions. Mitosporic fungi, such as *B. bassiana*, *Lecanicillium lecanii* Zare & Gams, 2001, *M. anisopliae*, and *Isaria fumosorosea* (Wize, 1904), are common species worldwide that are capable of infecting Lepidoptera, Hemiptera, Coleoptera, and Diptera species. Anamorphic fungi, like *B. bassiana* and *M. anisopliae*, primarily propagate as blastospores rather than hyphae. The blastospores invade the insect's vital organs by dispersing across the body via hemolymph circulation, and eventually result in insect death due to a clogged circulatory system. After host death, the fungus converts to the facultative feeding phase and initiates hyphal development outwards through the integument, thus building massive amounts of spores on conidiophores that establish new infestations.

Scanning electron microscopy (SEM) is an excellent tool for studying the external morphology (texture), chemical composition, and the crystalline structure and orientation of different materials. Scanning electron microscopes have been used to describe the immature stages (larvae and nymph) of some *Hyalomma* spp. that parasitize camels in Egypt (Abdel-Shafy *et al*. 2011, 2016), and is frequently used to evaluate the infection process of EPF in their insect hosts (Talaeihassanloui *et al*. 2007). The aim of this study was to investigate the external development of *B. bassiana* and *M. anisopliae* after application to the different life cycle stages of *P. papatasi* using light microscopic (LM) analysis and SEM.

Material and methods

Sand fly colony

P. papatasi larvae, pupae and adults were obtained from a laboratory colony maintained at the Department of Zoology, Faculty of Women for Arts, Science & Education, Ain Shams University in Cairo, Egypt. The colonization and rearing procedures from Modi & Tesh (1983) were followed. The sand flies were reared in an environmentally controlled, walk-in insectary regulated to 27°C±2°C, 60–70% relative humidity, and a 12:12 (L:D) photoperiod. Adults were fed

a 30% sucrose solution and hamsters as a blood-source for females. Larval food consisted of dried rabbit pellets and cow blood that had been grinded and autoclaved.

Applied EPF

Strains of *B. bassiana* Balsamo (AUMC5133) and *M. anisopliae* (Metchnikoff) Sorokin (AUMC5130) were provided by the Assiut University Mycological Centre, Assiut, Egypt.

Inoculation of P. papatasi with EPF

Conidial suspensions of *B. bassiana* and *M. anisopliae* were prepared by mixing 1 g spore powder in 10 ml distilled water, then stirring for 30 min before adding 2–3 drops Tween 80 as a dispersant in a glass flask. A dose of 3×10^7 conidia ml^{-1} was used, which was chosen based on previous works by various microbiological control researchers (Wang & Powell 2002, Meikle *et al.* 2007, Halouane 2008, Fan *et al.* 2012, Hamid *et al.* 2013, Khaleil *et al.* 2016).

The sand fly larvae, pupae and adults were directly exposed to *B. bassiana* and *M. anisopliae* fungi. Specifically, larvae and pupae sand flies were topically exposed to the fungal suspension (3×10^7 conidia ml^{-1}) using a micropipette (1 µl) according to the protocol described by Broome *et al.* (1976). The adults were sprayed carefully for 30 seconds with 3×10^7 conidia ml^{-1} that had been put into a small sprayer. The same number of adults were used for the control group that was sprayed with sterile distilled water only.

Larvae, pupae and adult *P. papatasi* were divided into three groups with 30 insects of each development stage used in each group. Group I was the control group of *P. papatasi* with no fungal treatment. Group II included larvae, pupae, and adults exposed to 3×10^7 conidia ml^{-1} of *B. bassiana*. Group III included larvae, pupae, and adults exposed to 3×10^7 conidia ml^{-1} of *M. anisopliae.*

Light microscopy [LM]

The control larvae, pupae and adults, and treated dead larvae, pupae and adults were collected 24, 48 and 72 hours after treatment, then were placed on moist filter paper in Petri-dishes at 27°C±2°C, 60–70% relative humidity, and a 12:12 (L:D) cycle to facilitate fungal sporulation (Farooq & Freed 2016). The dead insects were washed and collected in 10 ml sterile distilled water, then stirred for 30 seconds to extract spores. Phase contrast microscopy (Leica microscope DM2500) was used for the image analysis of the fungus-infected dead insects. Magnification was performed at 10× and 40×.

Scanning electron microscopy [SEM]

All three groups were collected 96 hours after treatment then washed in saline solution, then fixed in 70% alcohol before being mounted on aluminum stubs with double-sided, clear adhesive tape and sputter-coated on an Eduardo S150 Sputter coater with a thin layer of gold. The specimens were examined for image analysis using FEI inspect. The electron scanning microscope with the cathodoluminescence system at the Main Laboratory for Chemical War in Cairo, Egypt was used for SEM analysis.

Histopathological observations

The histopathological observations for all three groups were performed 72 hours after treatment to investigate any changes in the body wall and midgut. Paraffin sections of 6 µm were stained with hematoxylin and eosin, then examined microscopically (400×).

Results

Microscopic investigations

The microscopic analysis of the control, third instar larvae in (Fig. 1A) showed normal body cuticles and body segments. Fig. 1B shows that normal body structures were also observed in the control pupae (Fig. 1B) and the control adults (Fig. 1C), with normal head

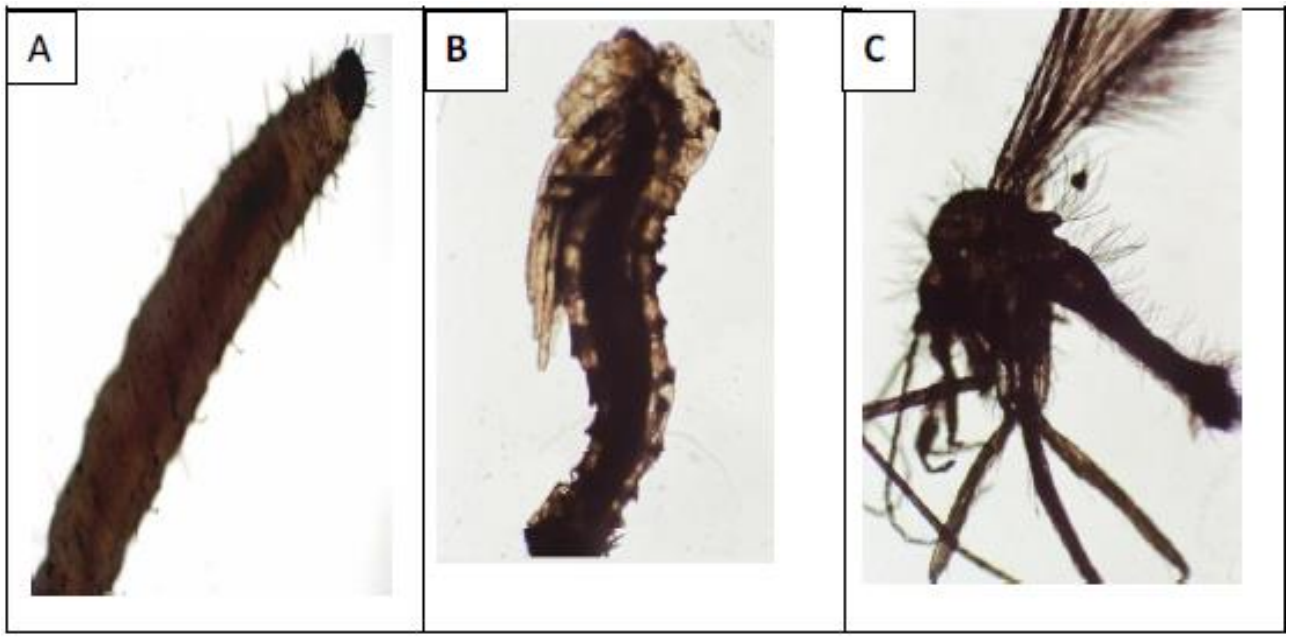

Fig.1. Light microscopic photographs of control larva, pupa and adult of a sand fly *Phlebotomus papatasi*.

structures, obvious compound eyes, and normal wings, legs and abdominal segments.

Fig. 2 and 3 shows the adhesion and penetration of *B. bassiana* and *M. anisopliae* on all three sand fly stages, including conidial adhesion, germination, and penetration through the different stages of sand fly cuticles. Under LM*, B. bassiana* and *M. anisopliae* conidia were observed in three different regions of the insects. The most germinated conidia were observed on the whole body surface of the insects at all three time points (24, 48, and 72 hours post-inoculation). For both fungal species, the germ tubes at 48 hours were short and penetrated directly through the cuticle in regions near the hairs and on the abdomen. After 72 hours, long and errant germ tubes were detected on the cuticular surface. Three different body areas were examined after treatment. Differences between the two species in terms of both the number of conidia and the number of germinating conidia were observed. Examination at all three time points showed that conidia were present in greater numbers on the cuticle after *B. bassiana* treatment for all three examined body regions compared to *M. anisopliae*.

After 24 hours

The *B. bassiana* conidia adhered to the larval integument (Fig. 2A). Although the conidia preferentially adhered to the head region, some conidia could be observed throughout the body of pupae (Fig. 2B) and were starting the germination process and cuticle adherence. Some regions of the adult bodies had groups of conidia adhered to them, while others had conidia that were in the initial phase of germination (Fig. 2C). *M. anisopliae* showed adhesion and penetration structures in the larval stage (Fig. 2D). In addition, the adherence of conidia on the surface of the adult and pupal cuticle (Fig. 2E and 2F) were clearly observed after the direct spray of the conidia.

After 48 hours

A group of conidia remained adhered in many regions of the integument and throughout the larval body 48 hours after exposure to *B. bassiana* (Fig. 3A). Additionally, many conidia were germinating, which was confirmed by the observation of the germ tube, the fungal structure that produces

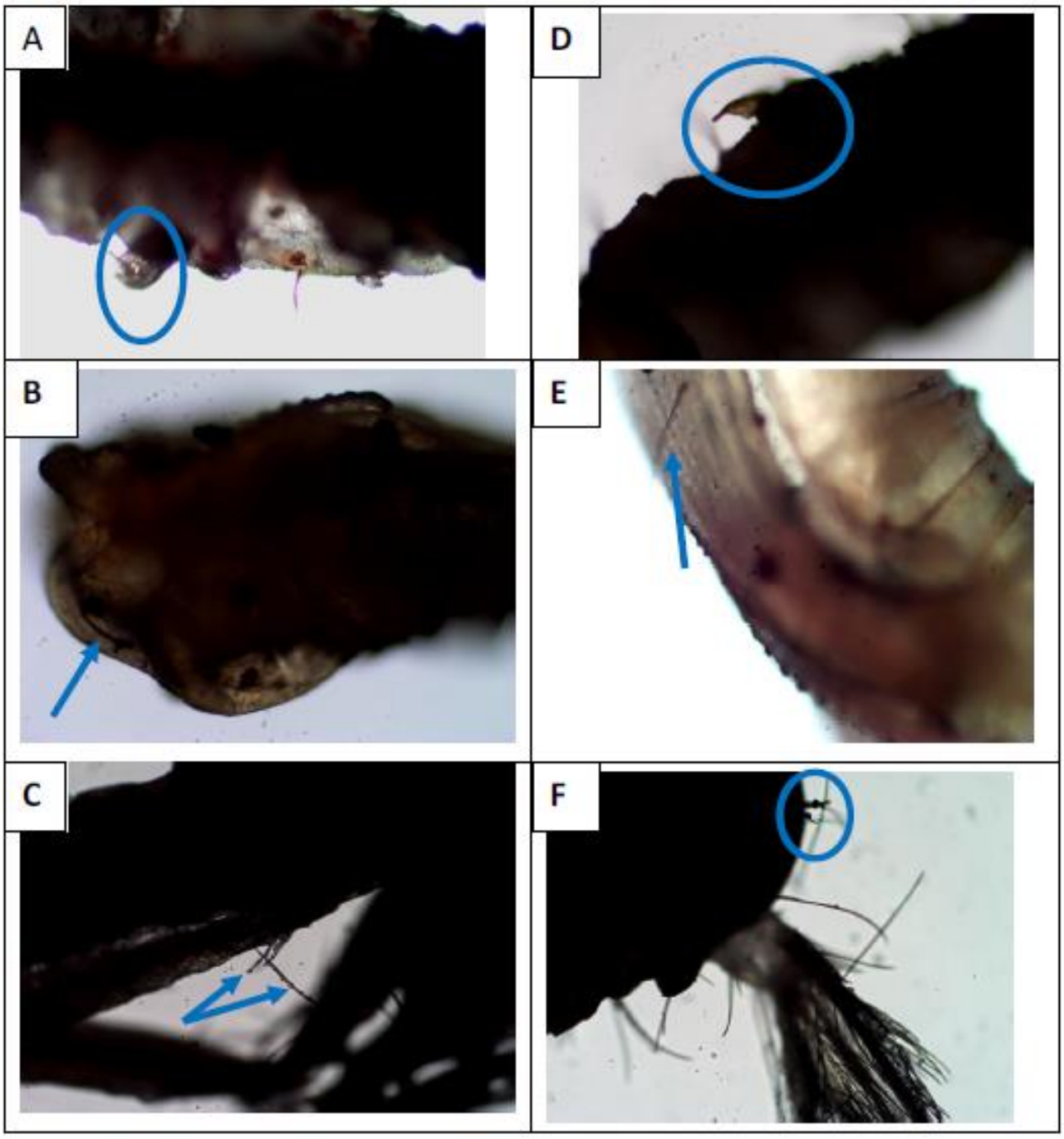

Fig. 2. Light microscopic photographs of treated larva, pupa and adult sand fly *Phlebotomus papatasi,* with light microscope: A, B, C) by *Beauveria bassiana*; D, E, F) by *Metarhizium anisopliae* after 24 h post treatment. Arrows point to fungal bodies.

hyphae (Fig. 3B). The microscopic observations showed that the integument of the pupae was supported by long zigzag filaments, which are transparent hyphae. These are produced on short spikes that give a convex appearance to the conidiogenous cells. In contrast to the larvae, only some conidia had short germ tubes in the adults (Fig. 3C). The germ tubes of *M. anisopliae* at 48 hours were short throughout the body of the larvae (Fig. 3D). Conidial germination and penetration on the pupae occurred between 24 and 48 hours (Fig. 3E). Germ tubes and fungal spores adhering to the cuticle were clearly seen after 48 hours. Post emergence adults also had germ tubes on the dorsal surface of the thorax and the legs, including tarsus region (Fig. 3F).

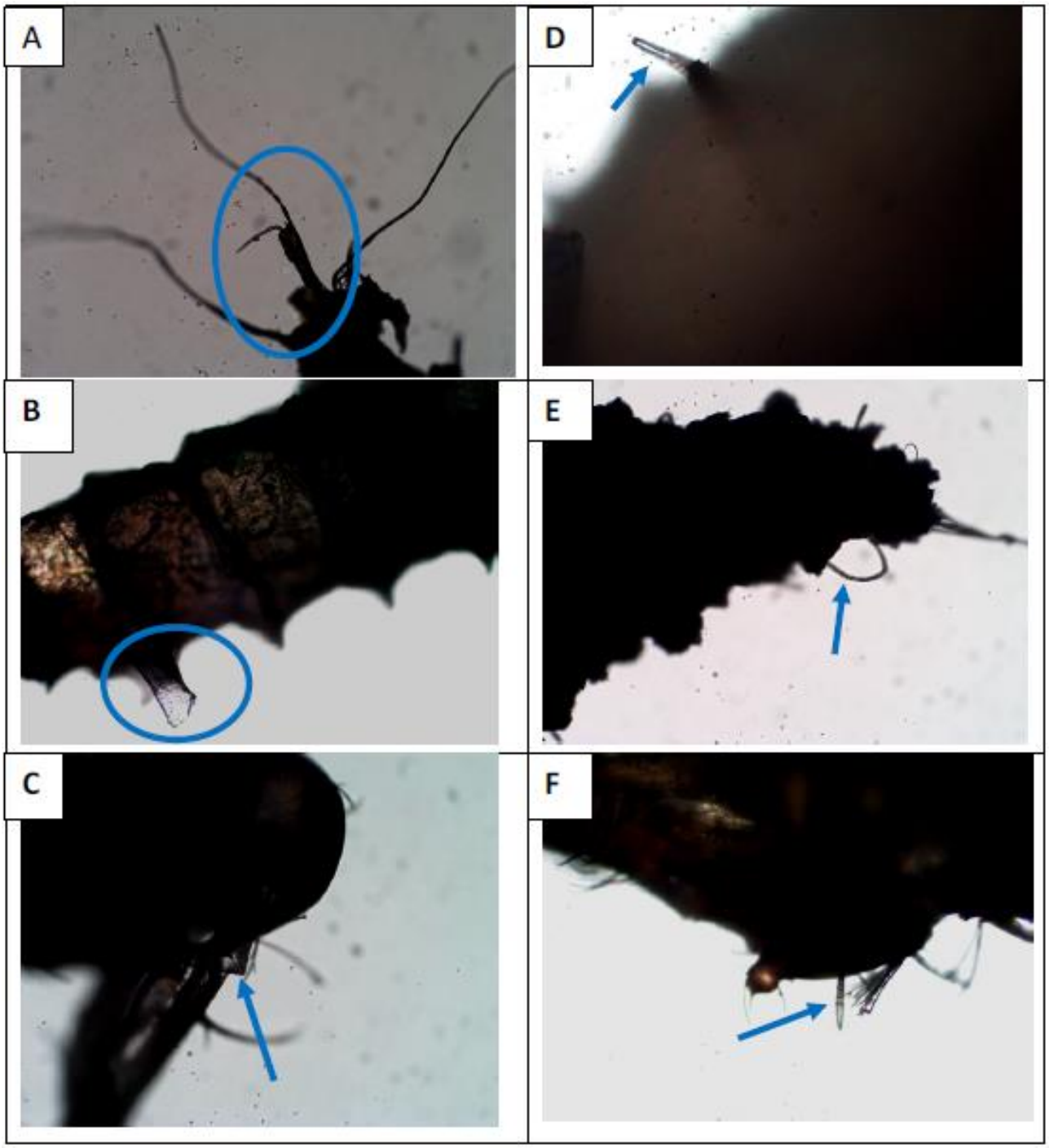

Fig. 3. Light microscopic photographs of treated larva, pupa and adult sand fly *Phlebotomus papatasi*: A, B, C) by *Beauveria bassiana*; D, E, F) by *Metarhizium anisopliae* after 48 h post treatment. Arrows point to fungal bodies.

After 72 hours

The larvae inoculated with *B. bassiana* showed signs of infection after 72 hours, with abundant hyphae observed on the integument of the larvae (Fig. 4A) indicating that *B. bassiana* had started to differentiate penetration structures produced by mycelial hyphae. The microscopic observations revealed that *B. bassiana* hyphae colonized the surface of the pupal integument and overcame the cuticle barrier to reach the interior of the body (Fig. 4B). A dense network was observed in the adults (Fig. 4C). Spores and hyphae of *M. anisopliae* were also observed in the cuticle of the larvae (Fig. 4D). On the pupae, fungal growth and signs of

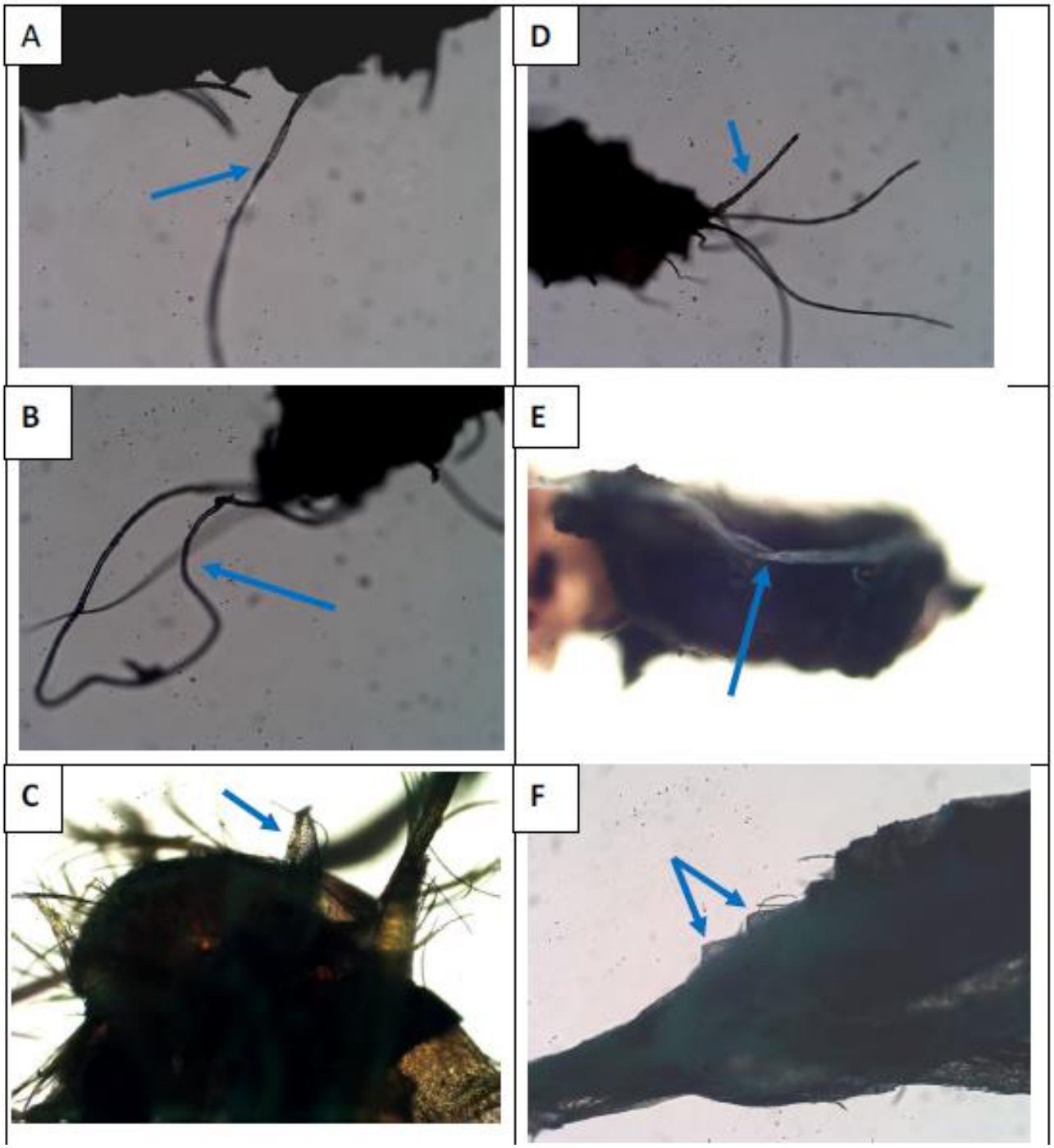

Fig. 4. Light microscope photographs of treated larva, pupa and adult sand fly *Phlebotomus papatasi:* A, B, C) by *Beauveria bassiana*; D, E, F) by *Metarhizium anisopliae* after 72 h post treatment. Arrows point to fungal bodies.

hyphal proliferation and penetration were observed (Fig. 4E). Hyphal bodies of *M. anisopliae* were also observed on the different parts of the adults, as were short, septate hyphae 72 hours post-inoculation (Fig. 4F).

SEM

Control group

The SEM of the control third instars larvae (Fig. 5A) showed normal, intact cuticles that were well formed. Normal pupae with sigmoid, cylindrical shapes were also observed (Fig. 5B). The thoracic, and abdominal segments, and wings of the pupae were clearly visible. The antennal sheath showed sketches of the adult flagellomeres. The abdominal segments progressively

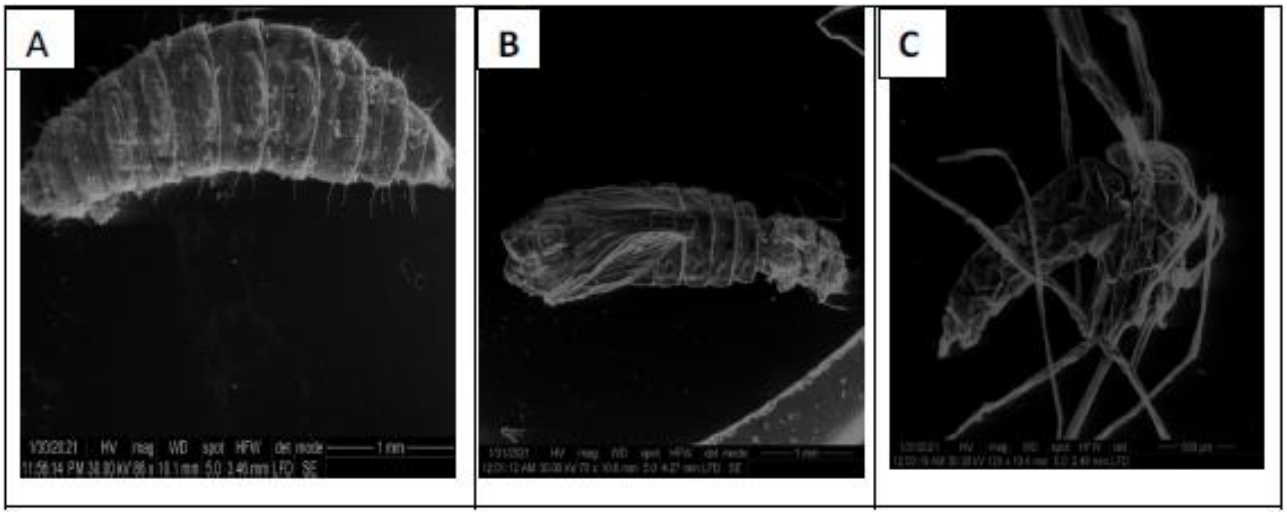

Fig. 5. Scanning electron microscope of sand fly *Phlebotomus papatasi*: A) control larva, bar=1 mm; B) pupa, bar=1 mm; C) adult (bar=500 µm).

decreased in width from the anterior to the posterior extremity, and the 8th and 9th abdominal segments were covered by the exuviae. Normal adults were also observed with normal head structures, obvious compound eyes, normal wings, and normal legs and abdominal segments (Fig. 5C).

B. bassiana*- treated *P. papatasi

The *B. bassiana* infected larvae showed great shrinkage in the abdominal segments and obvious fungal growth in the abdominal region after 96 hours (Fig. 6A). The infected pupae showed clear shrinkage in their body segments, separation of the last abdominal segments, and noticeable hyphal tube growth in the abdominal region (Fig. 6B). The infected adults showed severe destruction of their bodies, and obvious hyphal tube growth in the head region (Fig. 6C).

M. anisopliae*-treated *P.papatasi

Infected larvae showed severe destruction in their abdominal segments and apparent hyphal tube growth in their thoracic segments after 96 hours (Fig. 6D). The infected pupae showed clear shrinkage in their body segments, and obvious hyphal tube growth in their abdominal region (Fig. 6E). In the adults, spores and hyphae were observed in the body wall and clear hyphal tubes were observed in the compound eyes (Fig. 6F).

Histopathological observations

The histological structure of the cuticle in all three control stages was formed of an outer electron-dense layer of epicuticle, followed by a lamellated procuticle consisting of exocuticle, endocuticle, and an inner cellular layer of epidermal cells. The cuticles were followed by fat bodies and muscles layers composed of striated fibers. Each fiber consisted of a number of parallel fibrillates or sacrostyles. The midgut occupied the whole cross section and showed one layer of epithelial cells resting on the basement membrane (Fig. 7A, 8A, 9A). In contrast, the *B. bassiana*- infected larvae showed degenerative lesions that included cuticle lysis and the breakdown of muscles into smaller parts, which are both attributed to the destruction of the sarcolemma and adipose tissue in the body cavity (Fig. 7B). The infected pupae had fungal spores in their cuticular layers and spore penetration in the fat tissues (Fig. 8B). The treated adult had severe gonad destruction and a vacuolization of their fat cells (Fig. 9B). After 72 hours, the larvae, pupae, and adults treated with *M. anisopliae* exhibited muscular lysis and the fungus had clearly aggregated in the body wall. The larvae had cuticle degeneration and fat tissue

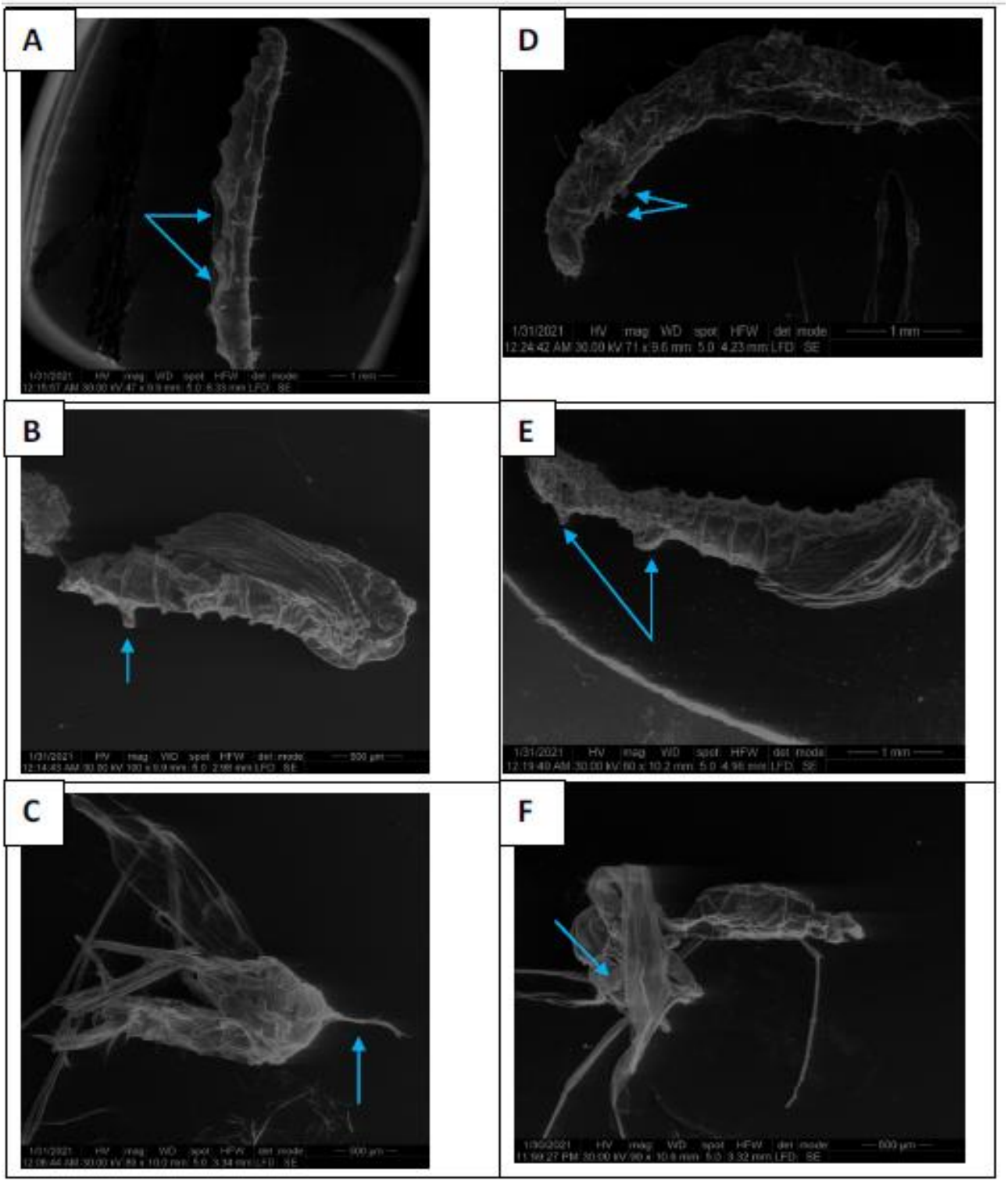

Fig. 6. Scanning electron microscope of treated larva, pupa and adult sand fly *Phlebotomus papatasi*: A) bar=1mm, B) bar=500 µm, C) bar=500 µm, by *Beauveria bassiana;* and D) bar=1 mm, E) bar=1 mm, F) bar=500 µm, by *Metarhizium anisopliae* after 96 h post treatment. Arrows point to fungal bodies.

distortion (Fig. 7C). The infected pupae had cuticles that were no longer visible, and internal organs that had all been completely destroyed (Fig. 8C). The treated adults had mild gonad destruction and fat cell degeneration (Fig. 9C).

Discussion

This study describes the conidia adhesion, germination potential, and the penetration of *B. bassiana* and *M. anisopliae* hyphae in the

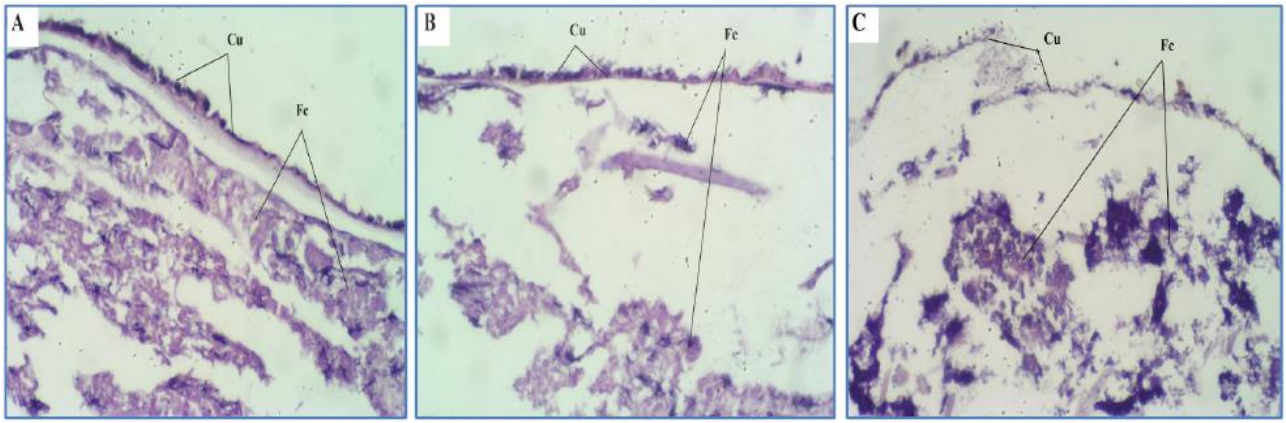

Fig. 7. Transverse H and E-stained sections of larva: A) the cuticle, fat cells of normal larvae of *P. papatasi*, the body wall of normal 3rd instar larvae of the *P. papatasi* has three layers; an outer electron-dense layer of epicuticle, followed by the procuticle, which is composed of the exocuticle and endocuticle, and then the inner layers of epidermal cells; B) the cuticle, fat cells 72 h after being treated with *B. bassiana*; C) the cuticle, fat cells 72 h after being treated with *M. anisopliae*. Cu – cuticle, Fc – fat cells (10x).

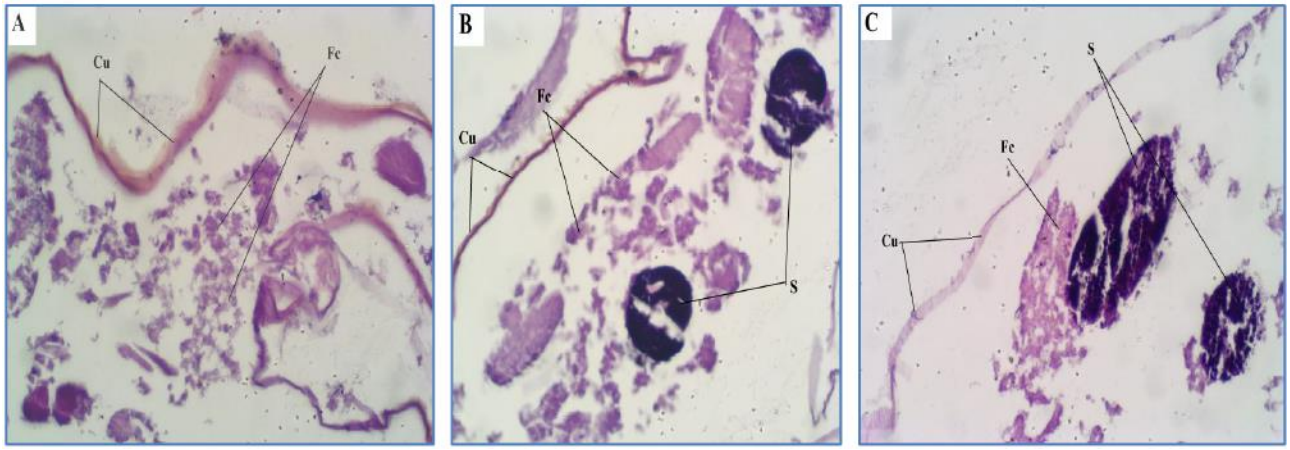

Fig. 8. Transverse H and E-stained sections of pupa: A) the cuticle, muscle of normal pupa of *P. papatasi* the body wall of normal pupa of *P. papatasi* has three layers; an outer electron-dense layer of epicuticle, followed by the procuticle, which is composed of the exocuticle and endocuticle, and then the inner layers of epidermal cells; B) the cuticle, fat cells 72 h after being treated with *B. bassiana*; C) the cuticle, fat cells 72 h after being treated with *M. anisopliae*. Cu – Cuticle, Fc – fat cells, S – spors (10x).

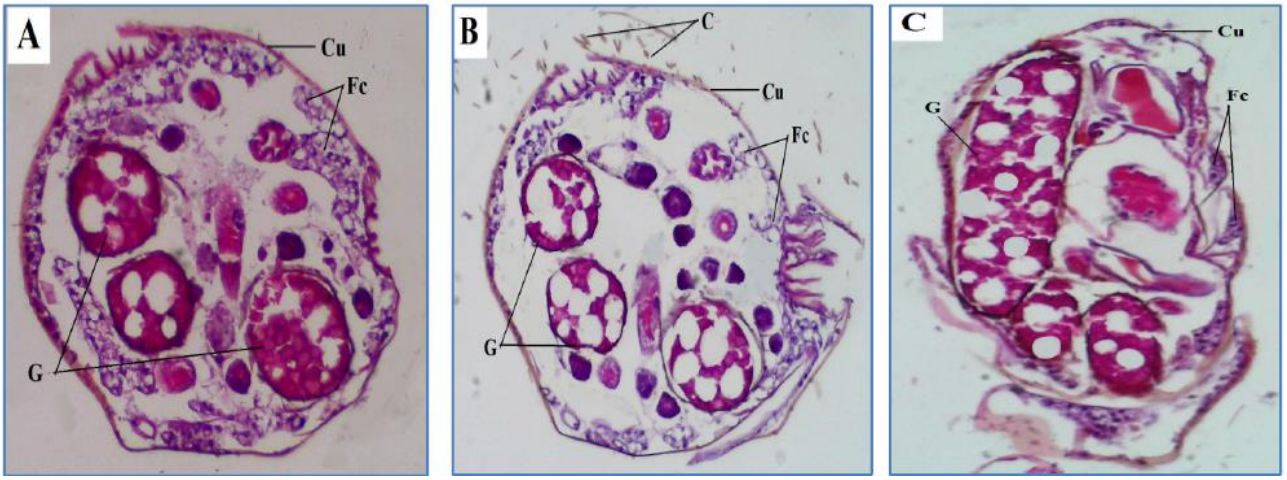

Fig. 9. Transverse and longitudinal H and E-stained sections of adult: A) the cuticle, fat cells and gonads of normal adult of *P. papatasi*; B) the cuticle, fat cells and gonads 72 h after being treated with *B. bassiana*; C) the cuticle, fat cells and gonads 72 h after being treated with *M. anisopliae*. Cu – cuticle, Fc – fat cells, C – conidia, G – gonads (10x).

integument of different sand fly developmental stages, taking into consideration the time needed for these processes to occur in the laboratory. Several studies pertaining to the insecticidal action of *B. bassiana* and *M. anisopliae* on other insects are available, but very few reports describe the histopathological changes for the different stages of sand flies (Travaglini *et al*. 2016, Travaglini 2017), although mortality tests have been reported (Travaglini *et al.* 2016, Travaglini 2017). Diehl-Fleig *et al.* (1988) studied *Atta sexdens* (Linnaeus, 1758) soldiers, and reported that 72 hours of exposure to EPF caused 50% mortality in a controlled environment. Castilho *et al*. (2010) reported that *M. anisopliae* had an 80% mortality rate in *Atta bisphaerica* (Forel, 1908) soldiers 72 hours after inoculation, and also that *B. bassiana* isolates were pathogenic for *A. sexdens* in the same period of time. In this study, the penetration of *B. bassiana* hyphae into body of larvae occurred in less than 48 hours, probably due to the fragile constitution of the exoskeleton during this life cycle stage before the cuticle is fully sclerotized, which is relevant information for this kind of vector control. The presence of sensilla in the adults probably facilitated the adhesion of conidia in these structures, with penetration occurring within 72 hours as expected due to the fully sclerotized cuticle that acts as an efficient physical protection barrier that provides the insect with protection against desiccation, parasitism, and predation (Chapman 1998). It is important to note that the application of the fungi was targeted, and adjuvant may have accelerated the process (Travaglini *et al.* 2016, Arnosti *et al*. 2019); however, reports in the literature on biological control confirm that a single viable conidium that has all the favorable conditions for development is able to cause host death (Arnosti *et al*. 2016). In addition to the integument, the natural openings of the insect body, such as the anus, spiracles, or buccal cavity, have been considered important sites for EPF penetration (Kermarrec *et al*. 1986). These openings have the protection of anatomical structures that prevent the penetration of strange agents into the insect's body (Dunn 1986, Silva 2002). For example, the trachea and tracheoles would act as a barrier for the spiracles.

To better understand these fungal infections, other necessary information needs to be studied, including the method of transmission and dispersion inside the colony after the pathogen has reached the larvae, which are the most susceptible to infection (Travaglini *et al*. 2017). Oi & Williams (2002) studied pathogen transmission and verified that the queen was contaminated after the introduction of *Thelohania solenopsae*-infected larvae and pupae. This study confirmed that conidia adhesion and germination on the cuticle surface of the adults began to occur 24 hours after *B. bassiana* exposure, when it was possible to observe the penetration structure. The process of mass penetration of *B. bassiana* hyphae occurred 72 hours after exposure. After the entomopathogenic fungus spore adheres to the skin of the insect it forms the germination nail and penetrates the epicuticula with the help of oil and protein degrading enzymes and reaches the hypodermis if the climatic conditions are favorable. These hyphae then multiply and spread in the body cavity. Micelles filling the body cavity eventually kill the insect. The fungus then forms chlamydospores that remain alive in the dead insect body. These chlamydospores germinate under appropriate conditions to form conidiophores on the skin of the dead insect. The spores on the conidiophores are also ready to begin new infections (Ferron 1978, Shah & Pell 2003, Goettel *et al*. 2005). Thus, the fungus needs to reach the hypodermis to be successful in transdermal infection. This is very important due to insect molting, especially for insects that molt frequently after only short periods

of time. If the insect molts before the fungal infection reaches the hypodermis, it can be saved from the infection. If the infection reaches the hypodermis first, then the insect cannot save itself from the infection (Öncüer 1984).

The fungal conidia invade the host cuticle shortly after germination, or after limited hyphal growth (Leger *et al*. 1991). Fungal infection begins when the conidia attach to the insect cuticle, the spores germinate and penetrate the insect cuticle, and then circulate in the host. Once the fungus penetrates the host, it produces toxins and secondary metabolites that overcome the insect immune system to grow rapidly (Roberts 1981), which explains the destruction of all *P. papatasi* stages infected by *B. bassiana* and *M. anisopliae* in this study. Specifically, the studied sections showed the lethal effectiveness of both fungi on the internal and external tissues of all three development stages. The formation and multiplication of the fungal hyphal bodies of both species inside and outside the host bodies was marked. Larvae hyphal bodies and spores were seen 24 hours postmortem in the abdominal region, whereas hyphal bodies were seen in the head and wings of the pupae, and in the heads of the adults. Hyphal bodies in the larvae and pupae 48 and 72 hours postmortem were seen in the middle and terminal region of the abdomen, whereas the fungal spores obviously attacked the ommatidia and abdomen of the adults. This finding agrees with Toledo *et al*. (2010), who found that the highest concentration of spores and hyphal bodies was detected in the compound eyes and the terminal region of the abdomen, with lysis of the body fat cells and muscular tissues. The present SEM showed that the conidia *B. bassiana* attached itself to all body regions of the infected flies, particularly in areas with dense hair cuticles. This agrees with Boucias *et al.* (1988), and Boucias *et al.* (1991). In this case, *B. bassiana* conidia were abundant in the hair of infected 4th instar larvae of velvet bean caterpillar (*Anticarsia gemmatalis* Hübner, 1818). *Beauveria* spp. conidia were found between the ommatidia of the compound eye and on the articulating membrane of the adults' legs. This agrees with Hasaballah *et al.* (2017), who recorded *B. bassiana* and *M. anisopliae* conidia on the compound eye, thorax, legs, and abdomen of *Musca domestica* Linnaeus, 1758. Toledo *et al.* (2010) found that conidia of *B. bassiana* isolate biologically controlled *Peregrinus maidi* (Ashmead, 1890) and attacked its compound eyes and legs.

Entomopathogenic fungi have an advantage over other insect pathogens, in that they can infect all developmental stages of their hosts, including the eggs, larvae, pupae and adults. There have been many recent publications of the potential of EPF for the control of disease vectors, such as *Culex*, *Anopheles*, and *Aedes* (Howard *et al.* 2010, Farenhorst *et al.* 2009, Paula *et al.* 2008). LM observation showed that *B. bassiana* conidia were observed in the same regions of the host insect as *M. anisopliae*. The hydrophobic conidia of both fungal species were able to attach to all body regions, with a preference for surfaces containing hairs, as was also reported by Boucias *et al*. (1988). *Beauveria bassiana* conidia were found to be especially trapped by and tightly bound to these hairs, as was also previously reported by Boucias & Pendl (1991). Many germinated conidia were observed at 24, 48, and 72 hours post-inoculation on the whole body surface. For both fungal species, germ tubes were short at 24 and 48 hours, and penetrated directly through the host cuticle. After 72 hours, long and errant germ tubes were detected on the cuticular surface. In all cases, *M. anisopliae* emitted only one germ tube from each conidium, as Schabel (1978) also reported. Neither the bipolar germination nor the appressoria formation observed by Mc Cauley *et al*. (1989) & Vestergaard *et al.* (1999) were detected in this study. The most frequent method of penetration was through the

cuticle (particularly for *B. bassiana*), although *M. anisopliae* germ tubes were observed entering through the hair sockets situated on the forewing venation. McCauley (1968) reported the penetration of *M. anisopliae* through the solid cuticle as the most common method of entering the body cavity of elaterid larvae, after the spiracles and pores of the sense organs. The preferential penetration sites in *Frankliniella occidentalis* (Pergande, 1895) observed by Vestergaard *et al.* (1999) were noted to be on the head, thorax, abdomen, and the thickest part of the wings, where the conidia penetrated directly through the cuticle. In another study, *B. bassiana* conidia were able to penetrate directly through the integument and the respiratory system (Pekrul & Grula, 1979).

Germination on the cuticular surface was observed at 24 hours post-inoculation, but the germination percentages were low (confronted with 95.5% and 100%) *in vitro* for *B. bassiana* and *M. anisopliae*, respectively. In this study, SEM showed that the penetration of *B. bassiana* hyphae in the cuticle body was not fully sclerotized which is relevant information for this kind of control. The penetration of the larvae occurred in less than 24 hours, probably due to the fragile constitution of the exoskeleton in this life cycle. In the adults, the presence of sensilla probably facilitated the adhesion of the conidia on these structures, and the penetration and germination occurred within 48 hours as expected once the integument in this phase is fully sclerotized, providing the insect with protection against desiccation, parasitism, and predation, and acting as an efficient physical protection barrier (Chapman 1998). It is important to note that the application of the fungi was targeted, and that adjuvants may have accelerated the process (Travaglini *et al*. 2016, Arnosti *et al*. 2019). Other studies have investigated the appropriate timing for the conclusion of each step (adhesion, germination, and penetration), which could lead to the successful use of EPF in the control of pest insects (Bot *et al*. 2002, Lacerda *et al.* 2010, Poulsen *et al*. 2002, Vieira *et al.* 2012).

In this study, the control and fungal treated larvae, pupae, and adults were histologically examined after 72 hours by LM. The microscopic examination of the 3rd instar larvae and pupae revealed that the *B. bassiana* and *M. anisopliae* spore suspension brought about massive disintegration and deformation of the fat body and tissues. Severe degeneration was observed in the fat cells, as well as the rupture of the cuticle, which revealed the development and colonization of the fungi inside the insect. The untreated 3rd instar larvae and pupae showed normal body structures with intact cuticles clearly differentiated into epicuticle and endocuticle layers and normal fat cells. The treated individuals had a semi-thin section showing many abnormalities in the insect body. *Spodoptera littoralis* (Boisduval, 1833) larvae treated with five strains of EPF had many malformations in the cuticle (Amer *et al*. 2008). The alterations in the host's cuticle is probably due to physical damage from the mycelium growth, and the beginning of the fungal sporulation process, but may also be due to biochemical degradation (Benserradj & Mihoubi 2014). As mentioned by Haitham & Alleddin (2012), the analysis showed great effects on all parts of the body, including the body wall, intestine and adipose tissues, and especially the degradation of the larval epicuticle into small pieces, in addition to the development of large masses of spores, and the appearance of gaps, which are regions of fungal spore crossings. A large amount of histological changes have been observed in most insects infected with various EPF using light and electron microscopy (Scheneider *et al.* 2013, Gabarty *et al.* 2014, Khaleil *et al*. 2016, Ragavendran *et al*. 2017).

The insect cuticle, with its underlying epidermis, forms the integument. It acts as the exoskeleton and as a barrier between the environment and the animal. It covers the

whole surface of the body, and invaginates in the gastrointestinal tract and the respiratory system. In the present study, the histological changes in the larvae affected the epidermal cells, and karyolysis-type necrosis was detected. The cross section in the gastrointestinal tract showed the effect that the fungus had on the muscular layers, which caused a change in the thickness of these layers compared with those of the controls due to the disappearance of the muscle layer supports. The same effect was observed in the intestinal cells, where degradation was caused by the fungal invasion. In another insect, lepidopteran larvae of *Galleria mellonella* (Linnaeus, 1758) were infected with strains of the fungus *Candida albicans*, then the whole larvae were examined for tissue changes using histological techniques. Various degrees of pathogenicity (strain- or inoculum-related), and the infection time course were described (Perdoni *et al.* 2014). Meanwhile, the examination of histological sections of various portions of the digestive tract showed some changes in *Locusta migratoria* (Linnaeus, 1758) larvae treated with *Pseudomonas fluorescens*, especially at the mesenteron level with the alteration of the peritrophic membrane (Oulebsir-Mohandkaci & Doumandji-Mitiche 2012). The cross sections of the body walls of the different sand fly stages showed the effect that the fungus had on the cuticle and the muscular layers, which caused a change in the thickness of these layers compared with those of the controls as the supports for the muscle layers disappeared. The same effect was observed in the midgut cells and adipose tissue, where degradation was caused by the invasion of the fungi. This was confirmed by the many histological changes seen in most insects infected with different EPF using light and electron microscopy (Scheneider *et al*. 2013, Gabarty *et al*. 2014, Khaleil *et al*. 2016, Ragavendran *et al*. 2017). The alterations in the host cuticle is probably due to physical damages caused by the mycelium growth and the beginning of the fungal sporulation or may be due to biochemical degradation (Benserradj & Mihoubi 2014).

The present study shows *B. bassiana* is a more efficient control agent than *M. anisopliae*. Erler & Ates (2015) noted a similar observation regarding the pathogenicity of *B. bassiana* and *M. anisopliae*, when they found that *B. bassiana* was more effective than both formulations of *M. anisopliae*, causing mortalities up to 79.8% and 71.6%, respectively, against the larvae of *Polyphylla fullo* Linnaeus, 1758 (Coleoptera: Scarabaeidae). In our study, *B. bassiana* had a faster adhesion, germination, and penetration than *M. anisopliae*, which was in contrast with the results obtained by Dimbi *et al*. (2003), who evaluated their effect on adult fruit flies and reported 100% mortality with *M. anisopliae* (ICIPE40/ICIPE41), but only 93.3% with *B. bassiana* (RI/AV/BB) at 4 days post-treatment. Nevertheless, these authors reported *M. anisopliae* as a faster acting pathogen than *B. bassiana.*

This study concluded that the studied fungi have destructive effects on the external and internal structures of the larvae, pupae and adult *P. papatasi*. In addition, *B. bassiana* had a faster adhesion, germination, and penetration than *M. anisopliae*. Thus, fungal spores are recommended as a biological control of the sand fly.

References

Abdel-Shafy S, El Namaky AH, Khalil FHM. 2011. Scanning electron microscopy and morphometrics of nymph and larva of the tick *Hyalomma impressum* (Acari: Ixodidae). *Parasitology Research*, 109: 1509–1518.

Abdel-Shafy S, El Namaky AH, Allam NAT, Hendawy S. 2016. Scanning electron microscopy and morphometrics of nymph and larva of the tick *Hyalomma rufipes* Koch, 1844 (Acari: Ixodidae). *Journal of Parasitic Disease*, 40: 1–10.

Ahmed MM, Kassem HA, Faragallah, AM, Fahmy AR, Georgi JM, El Sawaf BM. 2018. Larvicidal activity of *Eucalyptus globulus* (Myrtaceae) and *Origanum majorana* (Lamiaceae) Essential oils against the Sand fly *Phlebotomus papatasi* (Diptera: Psychodidae). *Egyptian Academic Journal of Biological Sciences*, 10: 31–38.

Amer MM, El-Sayed TI, Bakheit HK, Moustafa SA, El-Sayed YA. 2008. Pathogenicity and genetic variability of five entomopathogenic fungi against *Spodoptera littoralis*. *Research Journal of Agriculture and Biological Sciences*, 4: 354–367.

Amora SSA, Bevilaqua CML, Feijo FMC, Silva MA, Pereira RHMA, Silva SD, Alves ND, Freire FAM, Oliveira DM. 2009. Evaluation of the fungus *Beauveria bassiana* (Deuteromycotina: Hyphomycetes), a potential biological control agent of *Lutzomyia longipalpis* (Diptera, Psychodidae). *Journal of Biological Control*, 50: 329–335.

Ansari MA, Pope EC, Carpenter S, Scholte E, Butt TM. 2011. Entomopathogenic Fungus as a Biological Control for an Important Vector of Livestock Disease The Culicoides Biting Midge. *PLoS ONE*, 6: e16108.

Arnosti A, Delalibera I, Conceschi MR, Travaglini RV, Camargo-Mathias MI. 2016. Morphological mapping of the integument of adult females of *Diaphorina citri* Kuwayama, targeting the development of control strategies. *International Journal of Advance Agricultural Research*, 4: 57–64.

Arnosti A, Delalibera I, Conceschi MR, D'alessandro CP, Travaglini RV, Camargomathias MI. 2019. Interactions of adjuvants on adhesion and germination of Isariafumosorosea on adults of *Diaphorinacitri*. *Scientia Agricola*, 76: 487–493.
DOI: 10.1590/1678-992x-2017-0240

Batta YA. 2016a. Recent advances in formulation and application of entomopathogenic fungi for biocontrol of stored grain insects. *Biocontrol Science and Technology*, 26: 1171–1183.

Belkaid Y. Mendez S, Lira R. 2000. A natural model of *Leishmania major* infection reveals a prolonged "silent" phase of parasite amplification in the skin before the onset of lesion formation and immunity. *Journal of Immunology*, 165: 969–977.

Benserradj O, Mihoubi I. 2014. Larvicidal activity of entomopathogenic fungi *Metarhizium anisopliae* against mosquito larvae in Algeria. *International Journal of Current Microbiology and Applied Sciences*, 3: 54–62.

Bot ANM, Ortius-Lechner D, Finster K, Maile R, Boomsma JJ. 2002. Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. *Insectes Sociaux*, 49: 363–370.
DOI: 10.1007/ PL00012660

Boucias DG, Pendland JC, Latge JP. 1988. Nonspecific factors involved in attachment of entomopathogenic deuteromycetes to host insect cuticle. *Applied and Environmental Microbiology*, 54: 1795–1805.

Boucias DG, Pendland JC, Latge JP. 1991. Attachment of mycopathogens to cuticle: The initial event of mycoses in arthropod hosts. In: Cole GT, Hoch HC. (Eds.) *The Fungal Spore and Disease Initiation in Plants and Animals*, pp. 101–127.

Broome JR, Sikorowski PP, Norment BR. 1976. A mechanism of pathogenicity of *Beauveria bassiana* on larvae of the imported fire ant, *Solenopsis richteri*. *Journal of Invertebrate Pathology*, 28: 87–91.
DOI: 10.1016/0022-2011(76)90074-4

Castilho AMC, FRAGA ME, Aguiar-Meneze EL, Rosa CAR. 2010. Seleção de isolados de *Metarhizium anisopliae* e *Beauveria bassiana* patogênicos a soldados de Atta bisphaericae *Atta sexdens rubropilosa* em condições de laboratório. *Ciência Rural*, 40: 1243–1249.

DOI: 10.1590/S0103- 84782010005000100

Chapman R. 1998. *The Insects: Structure and Function (4th ed.)*. Cambridge, Cambridge University Press, 770 pp.
DOI: 10.1017/CBO9780511818202

Clarkson JM, Chamley AK. 1996. New insights into the mechanisms of fungal pathogenesis in insects. *Trends in Microbiology*, 4: 197–203.

Cox PD, Wilkin DR. 1996. The potential use of biological control of pests of stored grain. Home-Grown cereals authority research review (36). HGCA, London, UK, p. 53.

Denlinger DS., Creswell JA, Anderson JL, Reese CK, Bernhardt SA. 2016. Diagnostic doses and times for *Phlebotomus papatasi* and *Lutzomyia longipalpis* and flies (Diptera: Psychodidae: Phlebotominae) using the CDC bottle bioassay to assess insecticide resistance. *Parasites and Vectors*, 15: 9–212.
DOI: 10.1186/s13071-016-1496-3

Diehl-fleig E, Silva ME, Pacheco MRM. 1988. Testes de patogenicidade dos fungos entomopatogênicos *Beauveria bassianae Metarhizium anisopliae* em *Atta sexdens piriventris* (Santschi, 1919) em diferentes temperaturas. *Ciência e Cultura*, 40: 1103–1105.

Dimbi S, Maniania NK, Lux SA, Ekesi S, Mueke JM. 2003. Pathogenicity of *Metarhizium anisopliae* (Metsch.) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin to three adult fruit fly species: *Ceratitis capitata* (Weidemann), *C. rosa var. fasciventris* Karsch and *C.cosyra* (Walker) (Diptera: Tephritidae). *Mycopathologia*, 156: 375–382.

Driver F, Milner RJ, Trueman WHA. 2000. A taxonomic revision of *Metarhizium* based on sequence analysis of ribosomal DNA. *Mycological Research*, 104: 135–151.

Dunn PE. 1986. Biochemical aspects of insect immunology. *Annual Review of Entomology*, 31: 321–339.
DOI: 10.1146/annurev.en.31.010186.001541

Erler F, Ates AO. 2015. Potential of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. *Journal of Insect Science*, 15: 44.
DOI: 10.1093/jisesa/iev029

Fan Y, Borovsky D, Hawkings C, Ortiz-Urquiza A, Keyhani NO. 2012. Exploiting host molecules to augment myco-insecticide virulence. *Nature Biotechnology*, 30: 35–37.

Farenhorst M, Mouatchob JC, Kikankieb CK, Brookeb BD, Hunt RH, Thomas MB, Koekemoerb LL, Knols BGJ, Coetzee M. 2009. Fungal infection counters insecticide resistance in African malaria mosquitoes. *PNAS*, 106: 17443–17447.

Farooq M, Freed S. 2016. Infectivity of housefly, *Musca domestica* (Diptera: Muscidae) to different entomopathogenic fungi. *The Brazilian Journal of Microbiology*, 47: 4807–4816.

Ferron P. 1978. Biological control of insect pests by entomogenous fungi, *Annual Review of Entomology*, 23: 409–442.

Fryauff DJ, Modi GB, Mansour NS, Kreutzer RD, Soliman S, Youssef FG. 1993. Epidemiology of cutaneous leishmaniasis at a focus monitored by the multinational force and observers in the northeastern Sinai desert of Egypt. *American Journal of Tropical Medicine and Hygiene*, 49: 598–607.

Gabarty A, Salem HM, Fouda MA, Abas AA, Ibrahim AA. 2014. Pathogencity induced by the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in *Agrotis ipsilon* (Hufn.). *Journal of Radiation Research and Applied Sciences*, 7: 95–100.

Goettel MS, Eilenberg J, Glare T. 2005. *Entomopathogenic fungi and their role in regulation of insect populations*. In: Gilbert LI, Iatrou K, Gill SS. (Eds.). Comprehensive Molecular Insect Science. Amsterdam, pp. 361–405.

Halouane F. 2008. Research on the entomopathogenic *Beauveria bassia*

als.) Vuill. (Sordariomycetes- Clavicipi-taceae): Bioécologie, production and application on *Schistocerca gregaria* (Forskàl, 1775) and *Locusta migratoria* (Linné, 1758) (Orthoptera, Acrididae), Doctoral memory on agronomic Science, Agronomic National Institute of EL-Harrach.

Haitham MAH, Alleddin AH. 2012. Histological study of *Culex pipiens* larvae and adults infected with *Beauveria bassiana. Baghdad Science Journal*, 9: 187–193.

Hamid S, Halouane F, Bissaad FZ, Benzina F. 2013. Study about the effect of *Beauveria bassiana* (Vuillemin, 1912) on the aquatic stages of *Culex pipiens* (Linné, 1758). *International Journal of Bio Technology and Research*, 3: 31–42.

Hasaballah AI, Fouda MA, Hassan MI, Omar GM. 2017. Pathogenicity of *Beauveria bassiana* & *Metarhizium anisopliae* on the adult house fly L. *Egyptian Academic Journal of Biological Sciences*, 10: 79–86.

Howard AFV, N'Guessan R, Koenraadt CJM, Asidi A, Farenhorst M, Akogbéto M, Thomas MB, Knols BGJ, Takken W. 2010. The entomopathogenic fungus *Beauveria bassiana* reduces instantaneous blood feeding in wild multi-insecticide-resistant *Culex quinquefasciatus* mosquitoes in Benin, West Africa. *Parasites & Vectors*, 3: 2–11.

Irigaray FJSDC, Marco-Mancebón V, Pérez-Moreno I. 2003. The entomopathogenic fungus *Beauveria bassiana* and its compatibility with triflumuron: effects on the two spotted spider mite *Tetranychus urticae*. *Biological Control*, 26: 168–173.

Kermarrec A, Febuay G, Decharme, M. 1986. *Protection of leaf-cutting ants from biohazards: is there a future for microbiological control?* In: Lofgren S, Vander Meer RK. (Ed.). Fire ants and leaf-cutting ants: biology and management. Westview Studies in Insect Biology, London, pp. 339–356.

Khaleil M, El-Mougith A, Hashem H, Lokma N. 2016. Biocontrol Potential of Entomopathogenic Fungus, *Trichoderma Hamatum* against the Cotton Aphid, *Aphis Gossypii*. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 10: 11–20.

Kikankie CK, Brooke BD, Knols BGJ, Koekemoer LL, Farenhorst M, Hunt RH, Thomas MB, Coetzee M. 2010. The infectivity of the entomopathogenic fungus *Beauveria bassiana* to insecticide-resistant and susceptible *Anopheles arabiensis* mosquitoes at two different temperatures; *Malaria Journal*, 9: 71.
DOI:10.1186/1475-2875-9-71

Lacerda FG, Della lucia TMC, Serrão JE, Cecon PR, Souza LM, Souza DJ. 2010. Morphometry of the metapleural gland of workers engaged in different behavioral tasks in the ant *Atta sexdens rubropilosa*. *Animal Biology*, 60: 229–236.
DOI: 10.1163/157075610X496315

Leger SRJ. 1991. *Integumental as a barrier tomicrobial infections*. In: Retnakaran A, Binnington K. (Eds.). *Physiology of Insect Epidermis*. Commonwealth Scientific and Industrial Research Organization, Melbourne, pp. 286–308.

Maniania NK. 1998. A device for infecting adult tsetse flies, Glossina spp., with an entomopathogenic fungus in the field. *Journal of Biological Control*, 11: 248–254.

Mc Cauley VJE, Zacharuk RY, Tiline RD. 1989. Histopathology of green muscardine in larvae of four species of Elateridae (Coleoptera). The Journal of Invertebrate Pathology, 12: 444–459.

Meikle W, Mercadier G, Girod V. 2007. The micro-fungus, new hope in biological fighting against Varroa destructor. *Bees & Co.*, 118: 3.

Mnyone LL, Tanya LRTL, Issa N, Lyimo IS, Lwetoijera DW, Matthew JKMJ, Christian L C. 2009. First report of *Metarhizium anisopliae* IP 46 pathogenicity in adult *Anopheles gambiae*s. and *An. arabiensis* (Diptera: Culicidae) *Parasites & Vectors*, 2: 59.
DOI: 10.1186/1756-3305-2-59

Modi GB, Tesh RB. 1983. A simple technique for mass rearing *Lutzomyia longipalpis* and *Phlebotomus papatasi* (Diptera: Psychodidae) in the laboratory. *The Journal of Medical Entomology*, 20: 568–569.

Moore D, Lord JC, Smith SM. 2000. *Pathogens*. In: Subramanyam BH, Hagstrum DW. (Eds.) Alternatives to pesticides in stored product IPM. Kluwer Academic Publishers, Dordrecht, pp. 193–227.

Oi DH, Williams DF. 2002. Impact of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) on Polygyne colonies of red imported fire ants (Hymenoptera: Formicidae). *Journal of Economic Entomology*, 95: 558–562.
DOI: 10.1603/0022- 0493-95.3.558

Öncüer C. 1984. Entomopathogenic fungi in biological control against harmful insects (Zararlı böceklere karşı biyolojik savaşta entomopatojen funguslar). *Türkiye Bitki Koruma Dergisi*, 8: 177–189.

Ortiz-Urquiza A, Keyhani NO. 2013. Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects*, 4: 357–374.

Oulebsir-Mohandkaci H, Doumandji-Mitiche B. 2012. Study of the biological impact of *Pseudomonas fluorescens.* on haemolyphatic metabolites and digestive tract histology of L5 larvae of *Locusta migratoria* (Linnaeus, 1758). *Lebanese Science Journal*, 13: 99–115.

Paula AR, Brito ES, Pereira CR, Carrera MP, Samuels RI*.* 2008. Susceptibility of *adult Aedes aegypti* (Diptera: Culicidae)to infection by *Metarhizium anisopliae* and *Beauveria bassiana*: prospects for Dengue vector control. *Biocontrol Science and Technology*, 18: 1017–1025.

Perdoni F, Falleni M, Tosi D, Cirasola D, Romagnoli S, Braidotti P, Clementi E, Bulfamante G, Borghi E. 2014. A histological procedureto study fungal infection in the wax moth *Galleria mellonella. The European Journal of Histochemistry,* 58: 24–28.

Pekrul S, Grula EA. 1979. Mode of infection of the corn earworm (*Heliothiszea*) by *Beauveria bassiana* as revealed by scanning electron microscopy. *The Journal of Invertebrate Pathology*, 34: 238–247

Poulsen M, Bot ABM, Nielsen MG, Boomsma JJ. 2002. Experimental evidence for the costs and hygienic significance of the antibiotic metapleural gland secretion in leaf-cutting ants. *Behavioral Ecology and Sociobiology*, 52: 151–157.
DOI: 10.1007/s00265-002-0489-8

Ragavendran C, Mariappan T, Nataraja D. 2017. Larvicidal, Histopathological Efficacy of *Penicillium daleae* against Larvae of *Culex quinquefasciatus* and *Aedes aegypti* Plus Biotoxicity on *Artemia nauplii* a Non-target Aquatic Organism. *Front Pharmacology*, 8: 1–14.

Roberts DW. 1981. Toxins of entomopathogenic fungi. In: Burges HD. (Ed.). *Microbial Control of Pests and Plant Diseases*. Academic Press, NY, pp. 441–464.

Roberts DW, Leger RJ. 2004. *Metarhizium* spp. cosmopolitan insect pathogenic fungi: mycological aspects. *Advances in Applied Microbiology*, 54: 1–70.

Shah PA, Pell JK. 2003. Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology*, 61: 413–423.

Schabel HG. 1978. Percutaneus infection of Hylobius pales by *Metarhizium anisopliae*. *The Journal of Invertebrate Pathology*, 31: 180–187.

Schneider LCL, Silva VC, Pamphile JA, Cone H. 2013. Infection, colonization and extrusion of *Metarhizium anisopliae* (Metsch) Sorokin (Deuteromycotina: Hyphomycetes) in pupae of *Diatraea saccharalis* F. (Lepidoptera: Crambidae), *The Journal of Entomology and Nematology*, 5: 1–9.

Scholte EJ, Knols BGJ, Samson RA, Takken W. 2004. Entomopathogenic fungi for mosquito control: A review. *Journal of Insect Science*, 24: 4–19.

Siegel JP, Shadduck JA. 1990. *Safety of*

microbial insecticides to vertebrates-humans. In: Laird M, Lacey LA, Davidson EW. (Eds.). Safety of microbial insecticides. CRC Press, Boca Raton, pp. 101–113.

Silva CCA. 2002. Aspectos do sistemaimunológico dos insetos. *Biotecnologia, Ciência e Desenvolvimento*, 24: 68–72.

Srivastava CN, Maurya P, Sharma P, Mohan L. 2009. Prospective role of insecticides of fungal origin: review. *Entomological Research*, 39: 341–355 .

Stephou VK, Tjamos SE, Paplomatas EJ, Athanassiou CG. 2012. Transformation and attachment of *Beauveria bassiana* conidia on the cuticle of *Tribolium confusum* and *Sitophilus oryzae* in conjunction with diatomaceous earth. *Journal of Pest Science*, 85: 387–394.

Toledo AV, Lenicov AMM, Lastra C. 2010. Histopathology caused by the entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, in the adult planthopper, *Peregrinus maidis*,a maize virus vector, *Journal of Insect Science*, 10: 1–6.

Travaglini RV, Forti LC, Arnosti A, Camargo RS, Silva LC, Camargo-Mathias MI. 2016. Mapping the adhesion of different fungi to the external integument of *Atta sexdens rubropilosa* (Forel, 1908). *International Journal of Agriculture Innovations and Research*, 5: 118–125.

Travaglini RV. 2017. *Bases para o controle microbiano de formigas cortadeiras*. Tese (Doutorado em Proteção de Plantas) – Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, pp. 21–89.

Vestergaard S, Butt TM, Bresciani J, Gillespie AT, Eilenberg J. 1999. Light and electron microscopy studies of the infection of the Western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) by the entomopathogenic fungus *Metarhizium anisopliae*. *Journal of Invertebrate Pathology*, 73: 25–33.

Vieira AS, Morgan ED, Drijfhout FP, Camargomathias MI. 2012. Chemical composition of metapleuralgland secretions of fungus-growing and non-fungus-growing ants. *Journal of Chemical Ecology*, 38: 1289–1297.
DOI: 10.1007/ s10886-012-0185-8

Wang C, Powell JE. 2002. Isolation and Evaluation of *Beauveria bassiana* for Control of *Coptotermes formosanus* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Sociobiology*, 41: 1–13.

Warburg A. 1991. Entomopathogens of Phlebotomine sandflies: Laboratory experiments and natural infections. *Journal of Invertebrate Pathology*, 58: 189–202.

WHO 2008. Leishmaniasis. Accessed online: http://www.who.int/leishmaniasis/en/).

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