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The Pathogenic Effects of Some Bioinsecticides on *Culex pipiens L.* (Diptera: Culicidae)

A thesis

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By

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ABSTRACT

The present study examined the pathological effectiveness of two entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) against 3rd instar *Culex pipiens* larvae in the laboratory. As judged by a comparison of LC_{50} values resulted from the bioassay test, *Metarhizium anisopliae* was more effective with a recorded LC_{50} value of $0.36X10^{8}$ spore/ml than *Beauveria bassiana* with an obtained LC_{50} value of $0.72X10^{11}$ spore/ml. The biochemical effects of the two entomopathogenic fungi were studied. Results revealed significant reduction in the total proteins of the treated *Cx. pipiens* larvae compared to the control larvae. Protein analysis using Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) revealed that there was a reduction in the number of protein bands due to the treatment with either of the two fungi.

Light microscopy and transmission electron microscopy were used to examine different tissues of the control and treated larvae including cuticle, muscles, and midgut. Examination of several tissues of the treated larvae revealed that the epicuticle layer is completely deformed and detached. Muscles were gradually disorganized over the treatment time in treated larvae with appearance of rupture and tearing in the muscles. In addition, the mid gut epithelial cells showed loss of microvilli at the midgut cell apex and loss of adhesion between adjacent cells. Moreover, deeply altered intracellular organelles gave rise to vacuoles in the cytoplasm of the epithelial cells.

These interpretations suggested that toxins secreted by germinating spores caused damage to the larval tissues, leading to their necrosis, and finally death.

Key words: *Culex pipiens*; Entomopathogenic fungi; Bioassay; Total proteins; SDS-PAGE; Ultrastructural studies.

I- Introduction

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Mosquitoes (Diptera: Culicidae) species are vectors responsible for the transmission of infectious diseases of medical and veterinary importance including filariasis, malaria, and arboviruses (Goddard 2008; Mullen & Durden 2009; Medlock *et al.* 2012). Risk for human infection considerably enlarged during the last decades due to climatic changes and increasing global trade (Reiter 2001; Medlock *et al.* 2012; Boukraa *et al.* 2013). Integrated pest management is now encouraged due to harmful side effects of the chemical insecticides classically used for mosquito control and insect resistance development (Nauen 2007; Rattner 2009; Rivero *et al.* 2010).

Development of an effective control method against the *Culex pipiens* is urgently needed since it does transmit serious diseases to people, especially children, in Egypt. There is a serious interest in the use of safe microbial insecticides for biological control, as alternatives to chemical control, since they neither leave toxic chemical residues in the environment nor do they induce resistance in their insect hosts (Evans, 1999). And hence, the public awareness and concern for environmental

quality has led to more focused attention on research aiming at developing biological agents (Hidalgo *et al.*, 1998). A promising strategy with good potential to control and, at the same time, to minimize the adverse effects of chemical insecticides is the use of entomopathogenic fungi.

There are 90 genera and more than 700 species of entomopathogenic fungi that are considered as insect infecting fungi including Orthoptera, Lepidoptera, Diptera and Homoptera that represent about all the major classes of known fungi (Hajek and St. Leger, 1994).

Beauveria bassiana (Balsamo) (Ascomycota: Cordycipitaceae) and *Metarhizium anisopliae* (Metsch) (Ascomycota: Clavicipitaceae) are well known entomofungi with a broad host ranged and regarded as safe biopesticides (Bidochka and Khachatourians, 1987; McCoy, 1990; Gisbert *et al*., 2007). Fungal biocontrol agents reduce the use of chemical insecticides, their subsequent residues, and side effects in agriculture. However, there are many factors influencing the host range and specificity of fungal pathogens. These include the fungal strain, the host's physiological state, nutrition, defense mechanisms, cuticle and epicuticular microorganisms (Wan, 2003).

Entomopathogenic fungi are progressively studied in a biological control background regarding their ability to infect and kill insect hosts with more or less selectivity (Shah & Pell 2003; Becker *et al.* 2010). Fungal pathogens including *Beauveria bassiana* and *Metarhizium anisopliae* have been widely studied due to their simple life-cycle and thereby easy production of stable aerial spores which are the infectious propagules (Scholte *et al.* 2004; Kanzok & Jacobs-Lorena 2006; Seye *et al.* 2013).

The infection process of insect starts by contact of the spores to the host cuticle. Sometimes, conidium attaches to the cuticle or secretes mucus for adhesion during its germination and swelling (Hajek and St. 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 through the cuticle and insect epidermis, the fungus multiplies into the body cavity of insect and also more adapted to aquatic environments or simply ingested by larvae. Some ingested spores may mechanically block the mouth parts while others can attach inside the digestive tract (Federici, 1981; Butt *et al.* 2013).