An - Najah National University
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Bio - Control of Tobacco Whitefly *Bemisia tabaci* Using the Entomopathogenic Fungus *Metarhizium anisopliae*

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Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environment, Faculty of Graduate Studies, at An - Najah National University, Nablus, Palestine.

بسم الله الرحمن الرحيم

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صدق الله العظيم

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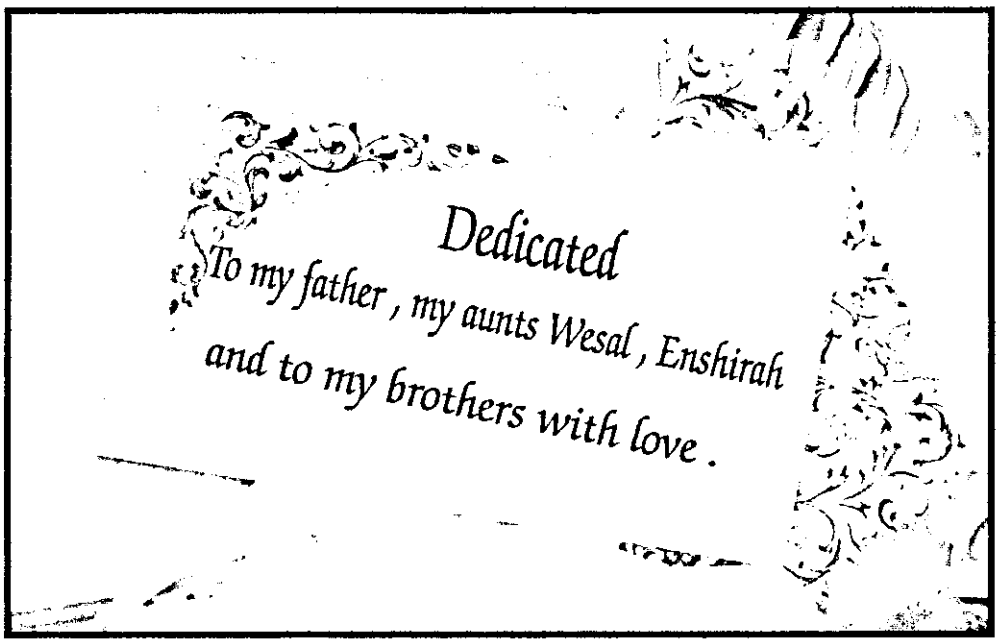

Acknowledgments

All praise to Allah for this accomplishment.

Thanks to Dr. Yacoub Batta for his guidance, encouragement and supervision during the study and dissertation preparation.

I would like to record my special thanks to my father, my aunts for efforts in all steps of my life and combine harvesting.

Thanks to my brothers especially Fadi for encouragement, upholding, facilitating means of exploration, statistical analysis and printing.

At the end, my thanks to the many other people who helped in this work.

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Abstract

This research aimed at evaluation of efficacy of the entomopathogenic fungus *Metarhizium anisopliae* against the immature stages (larvae and pupae) of the sweetpotato whitefly *Bemisia tabaci*. The fungus was mainly applied in form of invert emulsion (water - in - oil formulation) after being introduced into the emulsion in form of conidia. Two types of experiments were carried out to evaluate the efficacy, the first type was under laboratory conditions (20 ± 1°C) and a humidity of using formulated and non - formulated forms of the fungus, in addition to the control treatment; the second type was carried out on pots of eggplant seedlings kept under outdoor conditions during fall season using the above forms of the fungus, in addition to the control treatments. Results obtained from the two types of experiments indicated that affected larvae and pupae of *B. tabaci* due to the attack of the fungus turned into black - greenish in color (natural color of larvae is whitish, but it is yellow to dull - yellow for pupae) whereas, the values of mortality percent in the immature stages of *B. tabaci* under laboratory conditions were 100 %, 83.75 % and 0 % for the formulated form of *M. anisopliae*, non - formulated form and the control, respectively. Comparatively, the mortality percent in *B. tabaci* immature stages under the outdoor conditions were 92.269 %, 27.994 % and 1.000 % for the treatments mentioned above, respectively. Significant differences

($P > 0.05$) were obtained between the mean mortalities for the treatments with the formulated form of the fungus, the non - formulated form and the control. Moreover, it is recommended to confirm the efficacy of the fungus against *B. tabaci* especially in the formulated form under the open field conditions before the commercial use of the fungus against the whitefly.

CHAPTER ONE
Introduction

Chapter One
Introduction

Sweetpotato whitefly [*Bemisia tabaci* Genn.] is an important insect pest on many crops . It sucks the plant sap and transmits viral diseases to host plants (Cohen & Berlinger, 1986). The only means available for the control of this pest is the use of chemical pesticides. However, in recent decades, elevated awareness of the impacts of chemical pesticide use on the environment and human health have resulted in efforts to reduce reliance on chemical controls using insecticides. Many countries have instituted more stringent regulation of insecticides manufacture, registration and use, thereby increasing the cost and decreasing the availability of these tools. In many cases the pests themselves have indicated the need for change, in addition to that research efforts are increasingly being directed towards developing biocontrol strategies against this insect pest. These biocontrol strategies could be used as a part of an overall integrated pest management (IPM) program to reduce environmental and public - health hazards resulting from using chemicals. In addition, these strategies may be a more economical alternative to some insecticides (David et al., 1997). One of the biocontrol strategies of whitefly is based on using fungi that act as microbial insecticides . These bio - insecticides could be applied by using conventional spray equipments. To the best of our knowledge, no attempts

were made so far to apply the entomopathogenic fungus *Metarhizium anisopliae* to control *B. tabaci* although it has been applied by some investigators to control other insect pests (Samson et al., 1999; Driver et al., 1998; Driver et al., 2001; Milner and Rowland, 1998; Zimmermann, 1993). Therefore, the objective of this study was to test the efficacy of the fungus against the immature stages of *B. tabaci*. For this purpose, formulated and non - formulated forms of the fungus using invert emulsion (water - in - oil formulation) were tested under laboratory and outdoor conditions.

CHAPTER TWO
Literature Review

Chapter Two
Literature Review

1. Sweetpotato whitefly [*Bemisia tabaci* Gennadius]

This insect pest belongs to the family: Aleyrodidae, order: Homoptera, class: Insecta or Hexapoda.

1.1. Distribution

There are more than 1200 species of whiteflies in the world. The sweetpotato whitefly *B. tabaci* is one of the most pestiferous of the group. This pest was first described as *Aleyrodes tabaci* from tobacco in Greece in 1889 (Cock, 1986). It has been reported as a serious pest of cultivated crops in tropical and subtropical areas including Africa, Asia, Central America, South America, and the West Indies where it is also known as the tobacco whitefly and cotton whitefly (Cock, 1986).

1.2. Host Plants

The sweetpotato whitefly is widely polyphagous; it attacks more than 500 species of plants representing 74 plant families (Greathead, 1986). It has been a particular problem on members of the family Cucurbitaceae: squash, melon, cucumbers, pumpkins; Solanaceae family: tomato, eggplant, potato; Malvaceae family: cotton, okra, hibiscus; Fabaceae family: beans, soybean, peanuts; other families containing: salvia, poinsettia,

diseases associated with sweetpotato whitefly include: Lettuce necrotic yellows, irregular ripening of tomato, silver leaf of squash, cotton leaf curl, tomato leaf curl, tobacco leaf curl, and cassava mosaic (Cohen and Berlinger, 1986; and Cohen, 1999).

1.4. Description

It is important to be able to properly identify the sweetpotato whitefly because its susceptibility to control measures is quite different from that of other whiteflies. An understanding of the life cycle is also important for the proper timing of control measures . The description includes the following life stages:

1.4.1. Adults: The sweetpotato whitefly adult has a small size with 0.8 mm body length. At the resting state, it holds its white wings as a roof over its pale yellow body. It inhabits the lower surface of the host plant leaves and feeds by sucking the plant sap with its piercing - sucking mouthparts. The insect's snow - white color is attributed to the secretion of waxy powder on its body and wings (Hoelmer et al, 1991).

1.4.2. Eggs: The females deposit their eggs on the undersides of leaves, where they are usually clustered in groups. The number of eggs deposition per female ranges from 50 - 400 eggs [average = 160 eggs] (Butler et al, 1983). The eggs are very small of about 0.2 mm long, and 0.1 mm in width. Each egg is attached by a stalk or pedicel to the leaf; it is somewhat

elliptical in shape, tapering towards the unattached end. Newly laid eggs are smooth and whitish - yellow in color but turn brown when approach hatching five to seven days after laying (Butler et al., 1983).

1.4.3. Larval Stages: This insect goes through four larval instars ranging in an approximate size from 0.3 mm as first instar (crawlers) to 0.6 mm as fourth instar. The first instar or crawlers is flattened, oval in shape that attaches itself to the underside of the leaf near the empty egg shell. It remains there through three more molts . Late third & fourth instars begin to develop distinctive eye spots and are often referred to as red - eyed instars . These immature stages are thin and flat , elliptical in shape , and greenish - yellow

in color (Butler et al., 1983) .

1.4.4. Pupal Stage: At the end of the larval stages, the whitefly enters into what is commonly referred to as the pupal stage. It is yellow in color & about 0.7 mm long . The pupa has very prominent eye spots and is oval in shape and flat with rounded external margin (Butler et al., 1983). When the adult whitefly emerges from the pupa, it leaves a distinctive T - shaped dorsal split in the pupal case.

1.5. Biology and Life Cycle (Fig. 1)

The life cycle of sweetpotato whitefly, from egg to adult, requires 2 - 3 weeks in warm weather, but may take as long as 2 months under cool

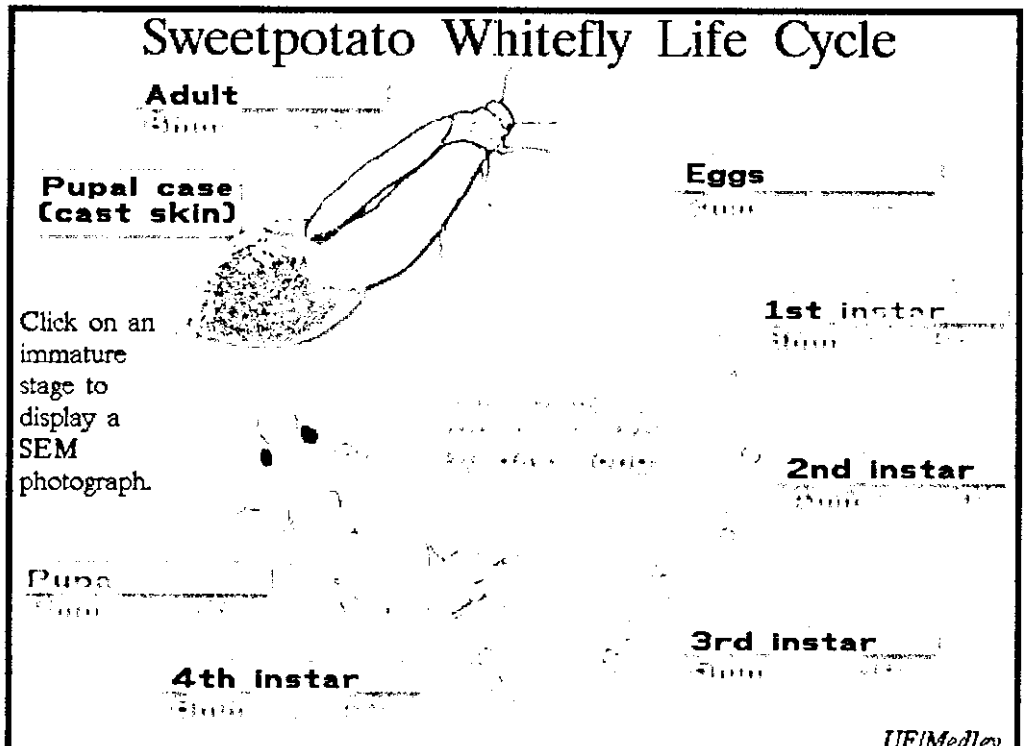

Fig. 1: Life cycle of sweetpotato whitefly *Bemisia tabaci* (Butler et al., 1983, http://whiteflies.ifas.ufl.edu/wfly0036.htm).

conditions (Butler et al., 1983). The number of eggs produced per female is also greater in warm weather than in cool weather. The rate of reproduction of sweetpotato whitefly varies with the host plant (Arnal et al., 1993). Oviposition occurs from 1 - 8 days after mating (pre - oviposition period). Adult life span ranges from 6 - 55 days depending on temperature. Reproduction may occur with or without copulation. Unmated females can reproduce by parthenogenesis in which the female produce only male progeny (Butler et al., 1983). As the life cycle progresses from one stage to another, molting occurs and the cast skins (particularly from the pupal) remain on the leaves. These structures are empty, silver in color and resemble small fish scales on the leaves.

1.6. Control of *B. tabaci*

1.6.1. Chemical Control

Conventional chemical control of the sweetpotato whitefly is difficult to achieve because of the distribution of the immature stages primarily on the underside of the leaves .The older larvae and pupae, are located lower in the plant canopy. The diversity of the attacked cultivated and weed host plants contributes to the source of infestation . A number of insecticides have effectively controlled this pest in the past but resistance has developed rapidly . Several new materials, including systemic insecticides, insect growth regulators and new pyrethroid insecticides, appear promising.

Current reliance on chemical control must be considered to be a temporary measure until a satisfactory IPM program can be developed . Acephate, carbaryl, diazinon, endosulfan, kinoprene, malathion, methomyl, oxamyl, phosdrin and telstar are the most important chemicals, which are currently used against *B. tabaci* (Omer et al., 1993). Organic and inorganic materials with completely different mode of action have been identified as effective against the sweetpotato whitefly. In addition, oils used as insecticides, botanical extracts such as Margosan - O (Azidirachtin) and glandular secretions from species of *Nicotiana* (tobacco) are highly toxic to larvae of *B. tabaci.* An additional difficulty for chemical control is caused by the behavior of this insect since adult feeding, mating and oviposition and larval development occur on the lower surfaces of leaves (Coudriet et al., 1985).

1.6.2. Cultural Control

Barriers such as row covers and repellent mulches that affect phototactic responses of whiteflies may be used as cultural control methods. Tomatoes mulched with yellow polyethylene sheets delayed the spread of tomato yellow leaf curl virus for 20 days (Cohen and Melamed - Madjar, 1978; Cohen and Berlinger, 1986). In addition, intercropping as a cultural control method can be used as an alternative method for the reduction of the insect pests in certain situations such as intercropping of tomatoes with cucumber

have been shown to be beneficial in reducing whitefly population on tomato by functioning as preferable host (Cohen and Berlinger, 1986).

1.6.3. Host - Plant Resistance

Integrated pest management (IPM) should include certain elements in its components for suppression of whitefly populations (Berlinger, 1986). Examples of these elements are the use of smooth - leaf (glabrous) cotton rather than hairy - leaf cotton to reduce the impact of whiteflies on yield, in addition to using resistant commercial cultivars although it takes time to develop resistant varieties for many crops (Berlinger and Dahan, 1987).

1.6.4. Biological Control Using Parasitoids

Several types of parasites may attack the sweetpotato whitefly. Large efforts are being underway to locate the appropriate biological control agents especially parasitoids (parasitic insects) (Polaszek et al., 1992), where five parasitoid genera of wasps are known to parasitize the tobacco whitefly: *Amitus* (Fam.: Platygasteridae), *Encarsia* (Fam.: Aphelinidae), *Eretmocerus* (Fam.: Aphelinidae), *Signiphora* (Fam.: Signiphoridae), and *Metaphycus* (Fam.: Encyrtidae). Among these parasitoids, *Encarsia* and *Eretmocerus* are the two main parasitoids attacking the tobacco whitefly.

1.6.4.1. *Encarsia*: This genus comprises over 200 species. The majority of these species are parasitoids on whiteflies . Twenty - two of these species were described and at least 3 species are undescribed, but they have been

reared from *Bemisia tabaci* (Gerling and Foltyn, 1987). Males of many species of *Encarsia* are a delphoparasites (i.e. males develop on the females of their own or different species). Members of this genus have 6 funicle segments [5 in some males], the tarsal formula is usually 5 - 5 - 5 [5 segments on each of the 3 tarsi], but several members of *Encarsia* that attack *B. tabaci* are 5 - 4 - 5.

1.6.4.2. *Eretmocerus*: There are 36 described species of this genus, in addition to many undescribed species (Powell and Bellows, 1992). Both male and female of the described species are known as primary parasitoids. Members of this genus are easily recognized by the two short funicle segments (absent in the male), long antennal club and the 4 - 4 - 4 tarsal formula (Hayat, 1972).

1.6.4.3. *Signiphora*: There are 18 described species of this genus, in addition to many undescribed species (Gerling, 1990). *Signiphora aleyrodis* Ashmead is the common species reared from *B. tabaci*. Members of this genus are easily recognized by their bare forewing with long marginal fringes , 3 - segmented funicle and long antennal club and 5 - 5 - 5 tarsal formula . *S. aleyrodis* is yellow with dark, transverse bands across the mesosoma (abdomen). The forewing also has a dark brown band across the middle region (Gerling, 1990).

1.6.4.4. *Amitus*: There are 17 described species of this genus; all of them are parasitoids of whiteflies. *Amitus bennetti* Viggiani and Evans is the

America and Asia, particularly Japan (Hoddle et al., 1998). These are areas where increased use of *E. formosa* would be possible. *Encarsia formosa* females are small (0.6 mm in length), have a black head and thorax and yellow abdomen. Males are rare and dark in color.

Habitat: Principal greenhouse crops in which *E. formosa* is used include tomato (*Lycopersicon esculentum*) and cucumber (*Cucumis sativus*). The parasitoid is also used, or is being tested, on much smaller areas of eggplant (*Solanum melongena*) and gerbera (*Gerbera jamesonii*), poinsettia (*Euphorbia pulcherrima*), marigolds (*Tagetes erecta*), and strawberry (*Fragaria* x *ananassa*). Virtually nothing is known about the ecology of *E. formosa* in outdoor agricultural systems (Hoddle et al., 1998). It parasitizes at least fifteen species of whiteflies belonging to eight genera. Most work has looked at the ability of this parasitoid to control sweetpotato whitefly infesting plants grown in greenhouses (Hoddle et al., 1998).

Life Cycle: To successfully reproduce in greenhouses, *E. formosa* must locate its potential hosts, assess host quality, and use nymphs appropriately for host feeding or parasitism. Following release into the host's habitat (i.e., greenhouses), the parasitoid employs visual and olfactory cues to find infested host plants (Guerrieri, 1997). When searching new leaves, the parasitoid does not distinguish between upper and lower surfaces and shows no preference for middles or edges of leaves. The rate at which hosts are encountered is dependent on the parasitoids walking speed, whitefly

size, and number of hosts on a leaf. Walking speed is reduced by leaf venation; high trichome densities, excessive honey dew, encounters with nymphs suitable for host feeding and parasitism, decreasing temperature, low barometric pressure, and smaller egg loads (Hoddle et al., 1998). *Encarsia formosa* is a solitary endoparasitoid that produces 8 -10 eggs per day. Daily egg maturation and oviposition rates decline as wasp ages. Adults obtain energy and nutrients by consuming honey dew and hemolymph of hosts that are pierced with the ovipositor, but in which no egg is deposited. Killing hosts for nutritional purposes is termed host feeding. *E. formosa* will oviposit in all immature stages of *B. tabaci* older than the settled first instar nymph. It prefers to oviposit in third, fourth, and prepupal nymphs of *B. tabaci.* The rate of successful emergence of the parasitoid is highest from these preferred stages, but it does not oviposit in up to 50% of suitable hosts in the preferred stages even when these are not parasitized or damaged from host feeding. Such hosts may be parasitized at a later encounter. Failure to oviposit in such hosts may result from defensive host movements (Hoddle et al. 1998). Exposure of females to antibiotics or high temperatures (31°C) for two or more generations suppresses microbial activity, allowing females to successfully produce male offspring. Fecundity is reduced once symbionts are eliminated. Males develop as primary endoparasitoids of whiteflies. The mating behavior of *E. formosa* has been described but males are unable to successfully

inseminate females (Zchori-Fein et al., 1992). Four distinct methods of releasing *E. formosa* into greenhouses for whitefly control have been suggested.

This includes; the pest in first, the dribble, the banker plants, and the repeated release method. The "pest in first" method begins with the deliberate introduction of adult whiteflies into greenhouses at a fixed rate (e.g., two whitefly adults per plant). *E. formosa* is later introduced one to three times at a standard rate (e.g., eight parasitized nymphs per plant) at regular intervals, which coincide with availability of host stages suitable for parasitism. This method has not been widely adopted due to concern over releasing pests onto the crop. With the "dribble method", parasitoid introductions begin at planting in anticipation of natural development of a whitefly population. Regular parasitoid releases at a low rate (e.g., one parasitized nymph per plant) continue until parasitized nymphs are found in the crop. The "banker plant" system utilizes established breeding colonies of whiteflies and parasitoids on earlier grown plants from which wasp and whitefly disperse into the crop. Banker plants are introduced at a fixed rate (e.g., one banker plant per 352 crop plants). Mesh screens can be used to cage banker plants to contain whiteflies while allowing the smaller adults of *E. formosa* to migrate into crop production areas. Inundative programs require regular releases of high numbers *E. Formosa*; establishment and reproduction of the parasitoid in the crop are not expected. This method is

applied most frequently to ornamental crops (Hoddle et al., 1998). In floral crops, the presence of whiteflies at even very low densities (e.g., 0.02 to 0.03 nymphs per cm^2 in poinsettias [unpublished]) is considered damaging and market standards require greater levels of whitefly suppression than are used for vegetable crops (e.g. 7.0 nymphs per cm^2). Consequently, use of *E.Formosa* has been more extensive in vegetables than in floral crops.

The fourth approach, in which repeated parasitoid releases are made throughout the cropping season, is used when a reproducing population of parasitoids is not expected to develop, either because the cropping season is too short or the whitefly or host plant are unfavorable. Whitefly mortality results from host feeding or superparasitism.

1.6.5. Biological Control Using Predators

There are many types of predators that may attack whiteflies. This includes the following types: true bugs (Hemiptera: especially Anthocoridae and predatory Miridae), beetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae, Hemerobiidae, Coniopterygidae), flies (Diptera: Dolichopodidae, Syrphidae, Anthomyoodae), ants (Hymenoptera: Formicidae), spiders (Araneida), mites (Acarina, Phytoseiidae, Stigmaeidae) (Breene et al., 1992; Cohen and Byrne, 1992; Hoelmer et al., 1993; and Hoelmer et al 1994). Some of these are opportunistic predators of adult whitefly, others are general feeders of leaf - feeding Homoptera like whiteflies, and still others are specific predators of

whiteflies. It is important to note that very little information is available on the biology and impact of the above predators on sweetpotato whitefly, especially in field crops (Rendiz Ruiz, 1993).

1.6.6. Biological Control Using Fungi

Although many fungi have been found in association with *Bemisia tabaci*, only few of them have been demonstrated to be pathogenic on it such as: *Verticillium lecanii, Paecilomyces fumosoroseus, Paecilomyces farinosus Aschersonia aleyrodis* and *Beauveria bassiana* (Fransen, 1992; Osborne and Landa, 1992; Brownbridge et al., 1993; Meade and Byrne, 1991). The extent of control by the majority of these fungal pathogens is not clearly known, except for *B. bassiana.*

Beauveria bassiana: (Deuteromycotina: Hyphomycetes) is a common soil borne fungus that occurs world wide. It attacks a wide range of both immature and adult insects. They have also occasionally been found in the lungs of wild rodents and nasal passages of horses, man, and giant tortoises. It has one of the largest host lists among the imperfect fungi and occurs in soil as a ubiquitous saprophyte (McCoy et al., 1988; Tanada and Kaya, 1993). This fungus is not a natural regulator of whitefly populations, it is only occasionally found infesting whiteflies in agro - ecosystems. In comparison to other fungal pathogens, little work has been done to evaluate the efficacy of this natural enemy against whiteflies. Commercially

available strains of *B. bassiana* exhibit high levels of pathogenicity in laboratory studies and *B. tabaci* nymphs and adults are susceptible to infection. Temperature and humidity levels affect *B. bassiana* pathogenicity and maximum mortality is inflicted at moderate temperatures (< 20°C) and high relative humidity (> 96%) when these conditions persist for at least 24 hours. Whitefly nymphs infected with *B. bassiana* display a pronounced red pigmentation. *B. bassiana* produces spores that are resistant to environmental extremes and act as the infective stage of the fungal life cycle. The spores, which are called conidia, infect directly through attacking the external surface of the insect's skin. Under favorable temperature and moisture conditions, a conidium adhering to the host cuticle will germinate, then fungal hypha growing from the spore secretes enzymes, which attack and dissolve the cuticle, allowing it to penetrate the skin and grow into the insect body. Once inside the insect, it produces a toxin called Beauvericin that weakens the host's immune system (Roberts, 1981). After the insect dies, an antibiotic (oosporein) are produced that enables the fungus to outcompete intestinal bacteria. Eventually, the entire body cavity is filled with fungal mass, and when conditions are favorable, the fungus will grow through the softer parts of the insect's body, producing the characteristic "white bloom" appearance. Relative humidity must be 92% or more for *B. bassiana* to grow outside the insect. These external hyphae produce conidia that ripen and are released into the

environment, completing the cycle. *B. bassiana* is available commercially as a microbial insecticide since the fungus can be produced massively by fermentation process and be formulated to enable the fungus to withstand ultraviolet light, and temperature and humidity extremes which are commonly encountered in the field. There are several products that contain *B. bassiana,* including Naturalis® and Mycotrol®; and other products will be registered by the EPA. However, it takes 3 - 7 days to kill an insect with *B. bassiana* so it will take some time to suppress the pest population when using these products. In addition, thorough spray coverage is essential when applying these products because fungal spores must be in contact with the insect for infection to occur.

Our literature search concerning biological activity of *Metarhizium anisopliae* indicated that there were no trials on using this entomopathogenic fungus against *Bemisia tabaci.*

2. *Metarhizium anisopliae*

This deuteromycetous fungus was formerly known as *Entomophthora anisopliae*, it is a widely distributed soil inhabiting fungus. The first use of *M. anisopliae* as a microbial control agent against insects was in 1879, when Elie Metchnikoff used it in experimental tests to control the wheat grain beetle: *Anisoplia austriaca* with cultures of *M. anisopliae* produce

destruxins A, B, C, D and E and des-methyl destruxin B. These substances are toxic to insects (Metchnikoff, 1879; Suzuki et al., 1966, 1970 and 1971). The rapid production of destruxins in the attacked larvae causes its death. In addition, it also produces toxic proteolytic enzymes (Kucera, 1980).

2.1. Description

M. anisopliae is categorized as green muscardine fungus due to green color of the sporulating colonies; it forms a white mat that upon formation of conidia turn green. The conidiophores are hyaline , branched , forming a sporulating layer . Phialides are single or in pairs or in whorls . Conidia (phialospores) are produced in basipetal chains, compacted into columns. They are elongate to ovoid or cylindrical one - celled , hyaline or slightly pigmented , olive - green in mass . The fungus is parasitic on insects or saprophytic in soil (Speare, 1920; Barnett and Hunter, 1998; and Tanada and Kaya, 1993).

2.2. Host Range

M. anisopliae is reported to be capable of infecting more than 100 different insect species belonging to a variety of insect orders (McCoy et al., 1988).

2.3. Pathogenicity

Infection with *M. anisopliae* generally takes place through the insect

integument. However , the exact site of infection is dependent on the stage of the insect and environmental conditions . In addition, the insect cuticle is penetrated with the aid of enzymes secreted by the apex of the penetrating hyphae and these hyphae give rise to hyphal bodies before death of the host which become distributed throughout the body cavity and give rise to secondary hyphae (McCoy et al., 1988) .In moist, warm weather conditions, the fungal hyphae emerge a few days after the insect's death. This emergence usually occurs through weaker parts of the integument, and the conidia are produced on conidiophores by the millions. This fungus also produces several toxic compounds that may kill the host (McCoy et al., 1988). Briefly, the infection cycle of hyphomycetous fungi is consisting of conidial attachment, germination, germ tube penetration, vegetative growth, and finally the conidia formation or reproduction (McCoy et al., 1988).

2.4. Role in Controlling Insects

Due to the environmental concerns and health risks associated with the application of synthetic insecticides, efforts have been developed to apply biological control programs including the entomopathogenic fungi as alternatives or supplements to these chemicals since these asexual fungi are easily cultured on culture media and be able to produce large quantities of conidia and have been economically produced for commercial applications

(Soper and Ward, 1981; Curran et al., 1994).

2.5. Biological Activity and Mode of Action

M. anisopliae generally enters insects through its respiratory pores or spiracles. Once gets inside the insect , the fungus produces a lateral extension of hyphae which eventually proliferate and consume the internal contents of the insect . Hyphal growth continues until the insect is filled with the mycelia and, thus the internal contents have been consumed then, the fungus breaks through the cuticle and sporulates making the insect appears " fuzzy " (Quarles, 1995 b). The fungus can also release spores or conidia under low humidity conditions (< 50 %), in addition to its ability for obtaining nutrition from the lipids entering in the composition of cuticle. It can also produce secondary metabolites (Thomas et al., 1995). However, it is reported that the fungus is sensitive to temperature extremes since the spore viability decreases as storage temperatures increase and virulence decreases at low temperatures (Latch, 1965; Daoust and Roberts, 1983). Moreover, at later stages of infection, the insects often turn into a characteristic pink color and then become dark green as the fungus sporulates (Hunter and Spurgin, 1999).

2.6. Formulations of the Fungus

Several attempts were made to formulate the fungus and until present, the following types are attempted:

2.6.1. Bioblast

It is commercially available and could be used to control termites (*Reticulitermes* sp.) . The formulated fungus may be applied on wood containing active termite galleries . This formulation, which is in form of wettable powder, could be sprayed directly on the termites in their galleries (Quarles, 1995 a).

2.6.2. Anti - Pasture Pest's Formulation

The successful development of dry mycelial preparations of *M. anisopliae*, which was reviewed by Zimmermann, 1993, has increased the use of this fungus throughout the world. Moreover, Rath, 1992 and other investigators have developed *M. anisopliae* as a commercially prepared control agent for cockchafer larvae in Tasmania, New Zealand. On the other hand, *M. anisopliae* was re-evaluated as a biological control agent for New Zealand pasture pests where the isolate F16 was originally isolated from *Costelytra zealandica* larvae collected by Nelson in 1990. Also, the isolate F36 was isolated from grass grub larvae in 1991. The black field cricket (*Teleogryllus commodus*) is a serious pest of pastures in the winter rainfall zone of western Victoria, New Zealand (Farrow et al., 1993). For its control, they use a myco - insecticide with conidia of *M. anisopliae* as the active ingredient (isolate F11037 of the fungus was applied at 1.5×10^{13} conidia / ha as effective and standard treatment), (Milner et al., 1996).

2.6.3. Destruxin

This formulation is specifically manufactured to enhance the insecticidal activity and biological stability of fungus spores and its secondary metabolites. It is in form of wettable powder containing conidia, mycelia and destruxins of *M. anisopliae*, in addition to fermentation solids and stabilizers (Laverlam, 1999). The rate required for *Perkinisiella* sp. and *Aenolamia* sp. control is 25 - 50 g per acre, but for the control of larval stages of *Ancognatha* sp. and *Phyllophaga* sp. is 50 g per acre, whereas to control 1st and 2nd instar larvae of *Spodoptera frugiperda* and *Sogatodes oryzicolus* in rice, 25 g per acre is recommended (Laverlam, 1999).

2.6.4. Biostop & Meta - Gaurd

It is a contact wettable powder termiticide (50 % wettable powder formulation). Since the spores of *M. anisopliae* must come into direct contact with the termites, it has no effect by ingestion or vapor, but can actually be spread from exposed termites to unexposed termites. This could allow the fungus to be spread through the colony killing additional termites (AJAY Bio - Tech (India), 1999).

2.6.5. Anti - Locust Formulation

Since 1990, multi - organizational efforts to harness the capabilities of the

naturally occuring fungus *M. anisopliae* var. acridium for control of locusts and grasshoppers recently culminated with commercial registration of the fungus - based mycoinsecticide in South Africa. The product is labeled " Green Muscle " and it is available in the market since 1999, it is produced by CABI Bioscience (UK). This mycoinsecticide became the " first environmentally friendly non - chemical insecticide for locust control " . It is a virulent strain of the fungus *M. anisopliae*, but it is kept in check by the absence of moisture - needed for germination in the arid zones frequented by locusts. The formulated fungus takes 6 - 21 days to kill locust depending on local conditions and it may be applied at ultra low volume spray (ULV) (CABI Bioscience (UK), 1999; Prior and Greathead, 1989; Lomer et al, 1997; Auld, 1992; Bateman, 1997; Cherry, et al, 1999). The biological control of locusts and grasshoppers (Acrididae) has led to the development of biopesticides based on naturally occuring pathogen *M. anisopliae* var acridum flavoviride is being developed for use as a microbial control product by the LUBILOSA program. The aim of this program has been directed to determine how host - pathogen interaction influence the transmission of the disease (i.e. secondary cycling) between insects. Abiotic conditions, notably temperature and infecting dose are important to determine insect mortality rates; these conditions have little impact on the outcome of infection. However, following host death, abiotic conditions are important to the sporulation of the pathogen. Efforts to

predict secondary cycling should focus on the local level (Thomas, 1999; Thomas et al., 1995).

2.6.6. Granular Formulation

The effect of soil sprays with *M. anisopliae* against coffee berry borer. (*Hypothenemus hampei*) was demonstrated by suspending the fungal conidia in water then applied on the trees at a dosage of $1.0x10^9$ conidia / tree (Fargues & Robert 1985, Gaugler et al .1989, Li & Holddom 1993). However, the sprayed conidia can persist for a short periods of time in the soil. Therefore, the efficacy of *M. anisopliae* to control *H. hampei* could be improved by the use of more appropriate formulations such as granular formulation since this kind of formulation could avoid the loss of conidia through rainfall, maintain high conidia viability, and prevent movement from the upper layers of the soil where contact with the borers takes place.

2.6.7. Biopath and Bengal

Are two new cockroach bait stations that use the fungus *Metarhizium anisopliae* encased in a plastic disk. The two products are the same, except that Biopath is black and it is used by the licensed pest control operators only, but Bengal is green and it can be purchased by the public. The station contains an agar plate that keeps humidity at the right level for fungus infection, and the water in the formulation attracts roaches (the station contains no food attractant). Infected roaches carry the fungus back to their

hideouts and infect other roaches. Upon using this formulation, German cockroaches were reduced by 50 % within three weeks and by 80 % after four weeks in laboratory tests (close to optimal conditions). The recommended use rate is about 10 to 20 stations for every 100 square feet of space. Bait stations sealed in packaging have a shelf - life of two years or more, and once opened should remain active for at least three months (Quarles, 1995 b).

Finally, some investigators have carried out trials using *Metarhizium anisopliae* conidia as a repellent where the conidia are mixed with soil to repel the termites and to prevent damage on susceptible timber. Applied conidia gave protection of up to 3 years in cool , dry conditions , but only for 6 months in tropical conditions . In addition, experiments are being conducted to test the efficacy of the product for control of cockroaches with encouraging results (AJAY BIO - Tech (India), 1999).

CHAPTER THREE
Materials and Methods

Chapter Three
Materials and Methods

1. Materials

1.1. Plant Materials

Lantana camara L. as a host plant for rearing tobacco whitefly under insectary conditions. In addition to eggplant (*Solanum melongena*) variety: " Red thin "to be infested with whiteflies in order to be used in carrying out biological efficacy tests.

1.2. Fungal Materials

The entomopathogenic fungus *Metarhizium anisopliae* (strain: Meta 1) was used in the biological efficacy tests. This strain was obtained from " Galilee Regional Research and Development Center " as pure culture of the fungus grown on Sabouraud medium.

1.3. Insect Materials

The tobacco whitefly (*Bemisia tabaci* Genn.). Adults and pupae of this insect, which were used in starting the insect's culture, were obtained from infested eggplants grown under field conditions at Tulkarm area.

1.4. Chemical Materials

Water - soluble wax (Dehymuls K), Glycerin, Commercial plant - origin

oils (coconut and soybean oils), Oil - soluble emulsifier (Tween 20), and oat meal agar (OMA) culture medium.

2. Methods

2.1. Technique of culturing the fungus

The fungus was subcultured on oat meal agar (OMA) medium under aseptic conditions then, incubated at 20 ± 1 °C and 16 hours of illumination (growth chamber conditions) for 10 - 14 days in order to obtain enough quantities of fungal conidia (Fig. 2 and 3).

2.2. Rearing the insect and infestation technique

Immature stages [Larvae & pupae] of tobacco whitefly were collected from infested leaves of eggplants grown under field conditions during summer season and then placed on previously grown ornamental plants of *Lantana camara* L. under the insectary conditions in order to have a culture of the insect. These plants were confined with cages made of transpirant plastic sides and cheez - cloth top to prevent whiteflies escape. This culture was involved in conducting the artificial infestation of eggplant seedlings by introducing them into the culture for 48 hours to have enough whitefly eggs deposited on the eggplant leaves.

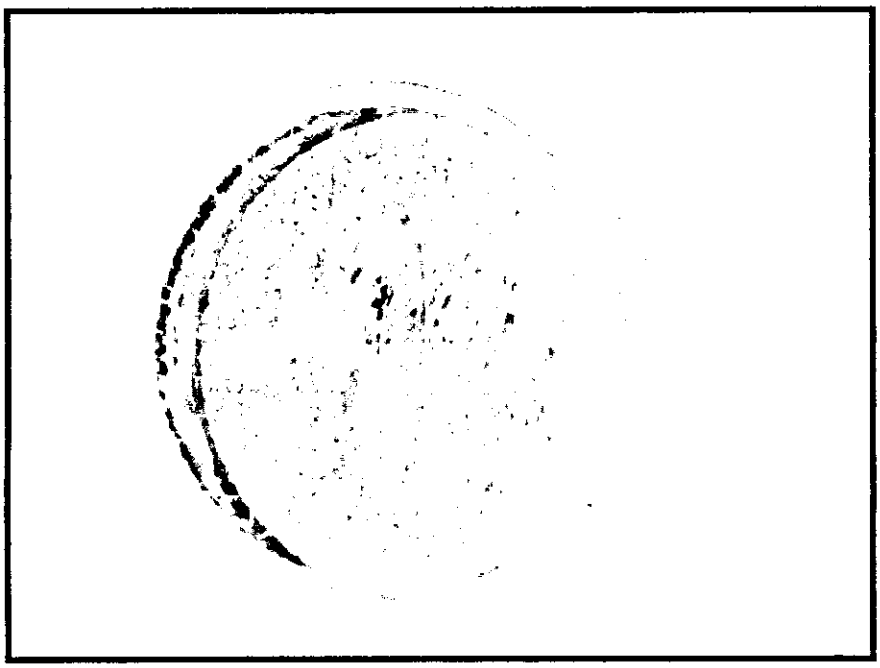

Fig. 2: Typical culture of *Metarhizium anisopliae* on oat meal agar medium (14 days - old culture).

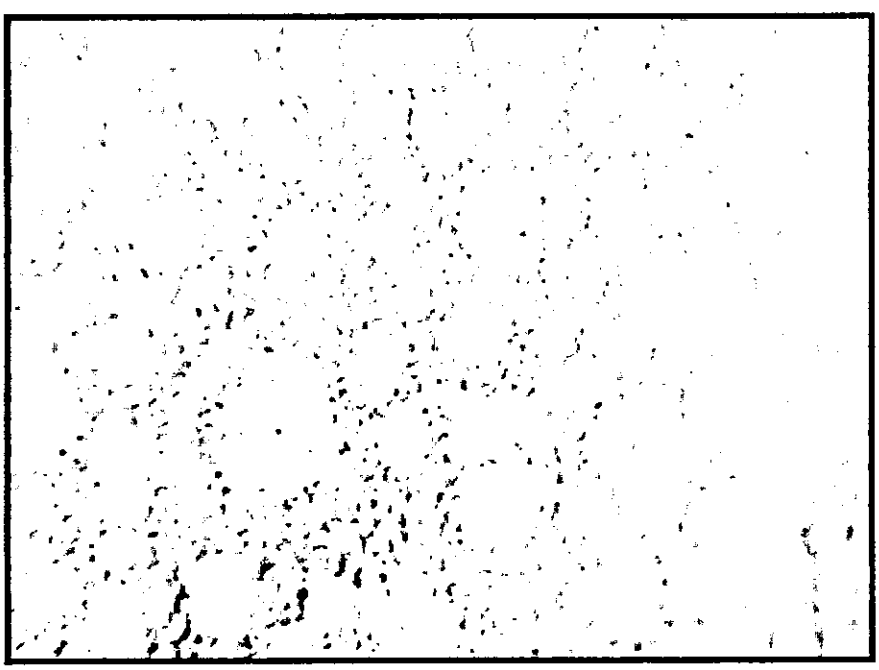

Fig. 3: Magnification of a part of *M. anisopliae* culture showing layers of conidia formed on the culture medium surface (40 x).

2.3. Technique of invert emulsion preparation

One hundred eighty one grams of sterilized distilled water and 3 grams of water - soluble wax (Dehymuls K) were heated at 75 °C using water bath for 10 - 15 minutes in order to dissolve the wax in water (becoming milky texture when properly dissolved). The contents were then mixed with 16 grams of glycerin in order to obtain 200 grams of aqueous phase of the emulsion. The contents of this phase were added to the ingredients of the oil phase to produce water - in - oil emulsion. The latter phase was consisting of a mixture of 75 grams of coconut oil, 115 grams of soybean oil and 10 grams of Tween 20. This mixture constitutes the oil phase of the emulsion. Agitation with a spoon for one minute then, final mixing of all ingredients through a homogenizer[1] at the speed no. 5 (18000 rpm) for 1.5 minute was carried out. Resulted emulsion has a white milky texture with low viscosity (28 cps) (measured by a viscometer[2]) and high stability (91 % emulsion layer). It was stored after preparation in an incubator at (20 ± 1 °C) using screw - capped bottles (Fig. 4). The above formulation of invert emulsion was selected as a result of long screening program followed by Basalat, 2002 during preparation of his M.Sc. thesis. The selected formulations have being tested as a carrier of antagonistic microorganisms to insects, plant pathogens or weeds in order to develop bio - pesticides

[1]Homogenizer Diax 900, Heidolph Com., Germany.
[2]Viscometer VT – O3, Rion Com., Japan.

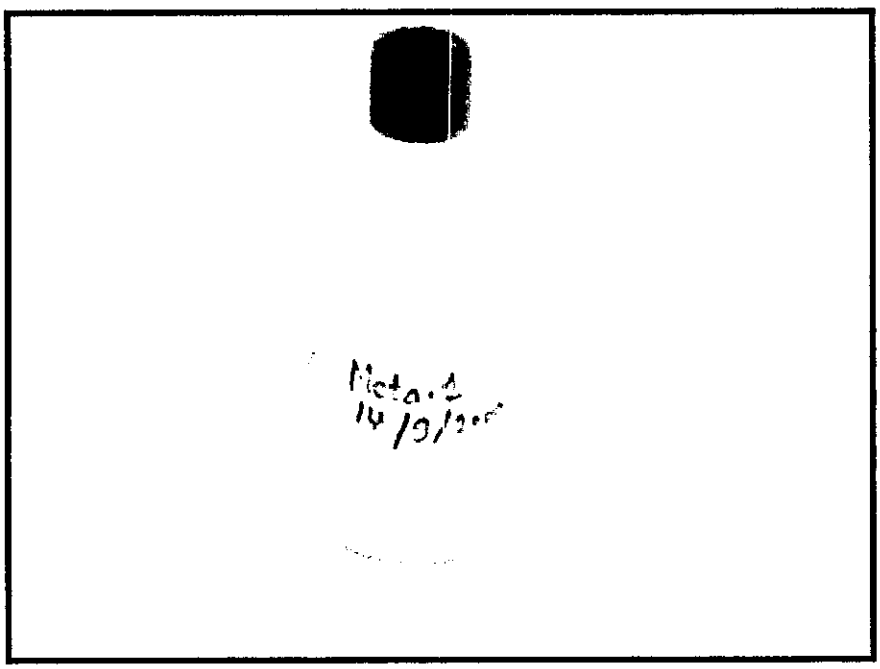

Fig. 4: Prepared invert emulsion containing *M. anisopliae* stored in screw - capped bottle.

based on invert emulsion formulation.

2.4. Technique of fungus introduction into the invert emulsion

Metarhizium anisopliae (strain: Meta 1) was introduced into the invert emulsion as conidial suspension. This introduction was carried out during the emulsion preparation by substituting 90 grams out of 181 grams of sterilized distilled water entering in the aqueous phase of the emulsion with titrated conidial suspension (for example 5.0×10^6 conidia / ml in 90 grams of the suspension). Titration of the conidia obtained from 10 days old culture of the fungus was done by using the hemocytometer . The final concentration of the conidia in the resulted invert emulsion was calculated according to the volume of the emulsion (1.125×10^6 conidia / ml in 400 ml).

2.5. Technique of viability measurement

Viability test was carried out to study the period that the fungal strain remains viable in the invert emulsion. It was done by spreading 100 µL of the invert emulsion containing the fungus on the surface of plates containing oat meal agar (OMA) medium. These plates were then incubated at (20 ± 1 °C) for 1 week to observe if the fungus grows on the OMA or not. This test was repeated each 2 weeks to indicate how many days that conidia are remaining viable in the invert emulsion.

2.6. Biological efficacy of the fungus

The efficacy of the fungus *M. anisopliae* post introduction into the invert emulsion was studied as follows:

2.6.1. Efficacy under laboratory conditions

Two methods were used in testing this efficacy:

- Glass - slide inside petri - dishes with moistened filter paper.
- Leaf - discs (20 mm Ø) inside petri - dishes with moistened filter paper.

All petri - dishes in both cases were incubated at 20 ± 1 °C. Three types of treatments were used during efficacy testing in both cases:

- Sterilized distilled water (SDW) used as a control treatment, where either picked immature stages from infested eggplant leaves or entire leaf - discs taken from infested sites of eggplant leaves were placed directly on the glass - slide.
- Non - formulated form of the fungus in form of conidial suspension (concentration of conidia = 1.75×10^6 conidia / ml) applied in two procedures as in the first treatment (glass – slide, leaf – discs).
- Formulated form of the fungus (concentration of conidia in the formulation of invert emulsion = 3.9×10^5 conidia / ml) applied in two procedures as in the above treatments (glass – slide, leaf – discs).

2.6.2. Efficacy on potted outdoor plants

For testing the efficacy of *M. anisopliae*, formulated and non - formulated forms of the fungus were sprayed directly on eggplants infested with the

immature stages of whitefly (64 days - old). Three treatments were used:

- Five pots of whitefly - infested eggplant seedlings were treated with formulated form of the fungus (concentration of conidia in the formulation = 5.1×10^6 conidia / ml).
- Five pots of whitefly - infested eggplant seedlings were treated with non - formulated form of the fungus (concentration of conidia in the conidial suspension = 7.2×10^6 conidia / ml).
- Five pots of whitefly - infested eggplant seedlings were used as control (sprayed only with sterilized distilled water).

Each treated seedling was sprayed with approximately 10 ml of the above treatments using a small hand sprayer (1 L volume), and then kept under outdoor conditions during the fall season (average daily temp. 25 °C). (Fig. 5).

2.7. Evaluation of the efficacy tests

Observations on petri - dishes or seedlings of eggplant treated with formulated and non - formulated forms of the fungus were made 3 and 5 days after treatment. These observations were mainly constituted of the mortality percent in the immature stages of *B. tabaci* and color changing due to infection with the fungus.

Fig. 5: Pots of eggplant seedlings treated with formulated (right) and non – formulated (middle) forms of *M. anisopliae*, in addition to control (left) treatment.

CHAPTER FOUR
Results

Chapter Four

Results

1. Laboratory Experiments

1.1. Immature stages of *B. tabaci* treated with formulated form of *M. anisopliae*

1.1.1. Glass - slide procedure (Fig. 6)

Results indicated that when the immature stages of *B. tabaci* were treated with formulated form of *M. anisopliae* (strain: Meta1), the attacked larvae and pupae were becoming black - greenish in color after three days of treatment and with dark - brown mycelial growth after five days of the treatment.

1.1.2. Leaf - disc procedure (Fig. 7)

Similar results were obtained with this procedure but with more dense mycelial growth (dark - brown) observed on the surface at the site of infestation with *B. tabaci* after five days of treatments.

1.2. Immature stages of *B. tabaci* treated with non - formulated form of *M. anisopliae*

1.2.1. Glass - slide procedure (Fig. 8)

Immature stages of *B. tabaci* treated with non - formulated form of the

fungus (conidial suspension only) indicated that attacked larvae and pupae placed on glass - slide became also black - greenish in color but with less density of superficial mycelial fungus growth and little sporulation compared with the formulated form.

1.2.2. Leaf - disc procedure

Similar results to glass - slide procedure were obtained here but with less density of superficial mycelial fungus growth.

1.3. Non - treated immature stages of *B. tabaci* with *M. anisopliae*

1.3.1. Glass - slide procedure

Immature stages of *B. tabaci* treated with sterilized distilled water showed that the color of larvae (whitish to yellowish in color) and pupae (yellow or dull yellow in color) were not changed and the emergence of adults from pupae was normal but the development of larvae on glass - slide was slow due to the lack of feeding.

1.3.2. Leaf - disc procedure (Fig. 9 & Fig. 10)

Similar results were obtained as in the glass - slide procedure (1.3.1), but with normal development of larvae on the surface of leaf discs.

1.4. Viability of the fungus after introduction into the formulation

Results indicated that the viability of fungal conidia introduced into the formulation of invert emulsion was affected during 3 months of its

introduction (Table 1). The fungal conidia were still viable and able to germinate within this period of time.

1.5. Effect of treatment with *M. anisopliae* on the mortality of the immature stages of *B. tabaci* in petri - dishes

Results indicated that the highest mortality percent (100 %) in the immature stages of *B. tabaci* placed on glass - slides was obtained after treatment with formulated form of the fungus compared with mortality percent in case of treatment with the non - formulated form of the fungus or the control (80 and 0 % respectively) (Table 2). Similar results were obtained in case of treatment of whitefly immature stages on leaf discs (Table 2).

2. Outdoor Experiments

As a result of the laboratory experiments, another type of experiments were conducted under the outdoor conditions on potted eggplant seedlings. Results obtained were as follows:

2.1. Observations on treated potted eggplant seedlings

The color of larvae and pupae of whitefly infesting plants was changed during three days after treatment where attacked larvae and pupae were

becoming at first, brownish to yellowish in color then during two additional days, its color became black - greenish compared to dull yellow color in non - attacked pupae by the fungus and whitish to yellowish color in non - attacked larvae. On the other hand , evaluation of the adult emergence from pupae in treated and non - treated immature stages revealed that this emergence was normal or regular in the non - treated pupae , whereas it did not occur in treated immature stages with both formulated and non - formulated form of the fungus due to attack of the fungus on these immature stages . The attacked pupae were characterized by black - greenish color as a result of its death . However, little injury in form of phytotoxicity was observed on the margins of some lower leaves of the treated plants with formulated form of the fungus (Fig. 11).

2.2. Effect of treatment with *M. anisopliae* on the mortality of immature stages of *B. tabaci* infesting potted eggplants

Treatment of whitefly immature stages infesting potted eggplant seedlings placed under outdoor conditions showed that significant differences were obtained in the mortality of whitefly immature stages after the treatment with formulated and non - formulated forms of the fungus (Table 3). The mortality percent was significantly higher in case of treatment with formulated form of the fungus compared with non - formulated form

(conidial suspension of the fungus only) or non - treated using sterilized distilled water only. This proves that formulation of the fungus in invert emulsion increased its efficacy in comparison with its efficacy in non – formulated form (increase from 27.994 to 92.269 %) (Table 3).

Table 1

Viability of *Metarhizium anisopliae* (Meta 1) in the prepared formulation of invert emulsion.

Weeks after introduction of the fungus into the formulation during preparation	Viability test of the fungus on oat meal agar medium plates
0	+ + +
2	+ + +
4	+ + +
6	+ + +
8	+ + +
10	+ +
12	+
14	+
16	- - -
18	- - -
20	- - -
22	- - -
24	- - -
26	- - -

+ + + → positive on all petri – dishes (3 plates).

+ + → positive on 2 petri – dishes.

+ → positive on 1 petri – dish.

- - - → negative (no growth) on 3 petri – dishes.

Table 2

Effect of formulated and non - formulated forms of *M. anisopliae* on the immature stages of whitefly *B. tabaci* under laboratory conditions (20 ± 1 °C).

Treatment of immature stages	Mortality percent in the immature stages		
	Formulated form	Non-formulated form	Control (non - treated)
Larvae & Pupae on slides	100 (15 / 15)	80 (12 / 15)	0 (0 / 15)
Larvae & Pupae on leaf discs	100 (9 / 9)	87.5 (7 / 8)	0 (0 / 8)

Formulated = Invert emulsion.

Non – formulated = Conidial suspension.

Control = Sterilized distilled water.

Percentage of mortality = dead immature stages / no. of larvae & pupae counted x 100.

Table 3

Effect of formulated and non - formulated forms of *M. anisopliae* using invert emulsion on the mortality of immature stages of *B. tabaci* on eggplant seedlings kept under outdoor conditions.

Plant number	Mortality percent in the immature stages of *B. tabaci*					
	Formulated form		Non - formulated		Control	
	Leaf 1	Leaf 2	Leaf 1	Leaf 2	Leaf 1	Leaf 2
1	100	100	35.29	42.86	0	0
2	100	90	11.11	50	0	0
3	80	100	12.5	40	0	10
4	100	83.3	10	18.18	0	0
5	88.89	80	50	10	0	0
Mean mortality percent	93.778	90.66	23.78	32.208	0	2
Grand mean mortality	92.269 % c		27.994 % b		1.000 % a	

- Randomly taken leaves from plants of the different treatments were done in order to count the dead and alive larvae & pupae of whitefly to calculate the mortality percent five days after treatment.

- Mean mortalities followed by the same letter were not significantly different at 5 % probability level according to ANOVA table and Duncan's Multiple Range Test for the CRD.

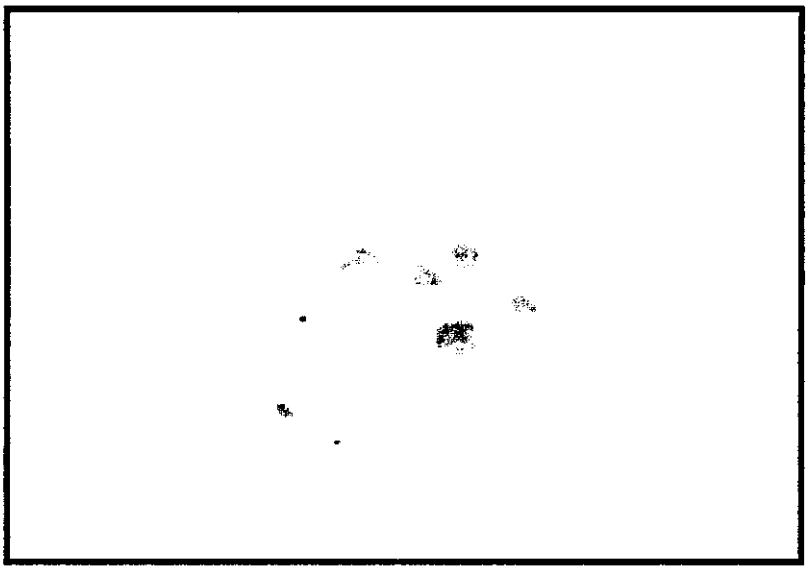

Fig. 6: Effect of formulated form of *M. anisopliae* on larvae & pupae of *B. tabaci* placed on glass - slide inside petri - dish (black - greenish color observed on attacked larvae & pupae with dark - brown mycelial growth). (40 x)

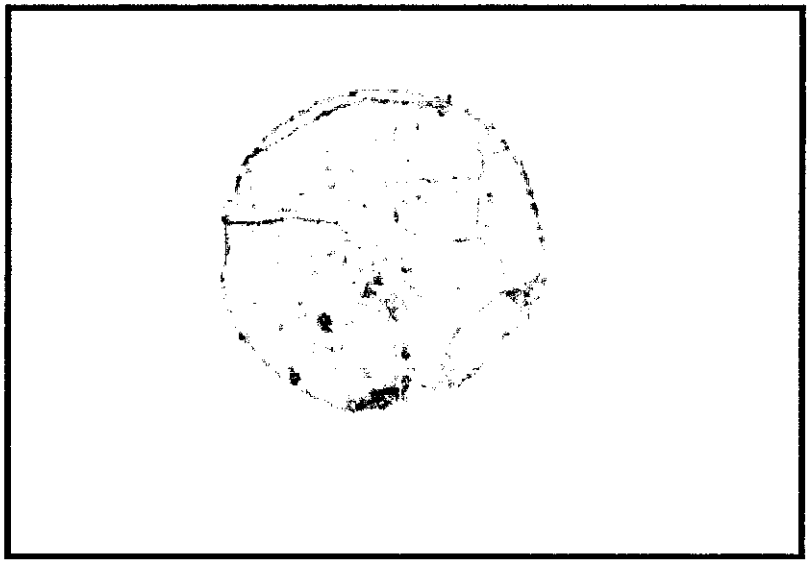

Fig. 7: Immature stages of *B. tabaci* treated with formulated form of *M. anisopliae* on leaf - discs inside petri - dish (attacked larvae & pupae appeared with black - greenish in color).

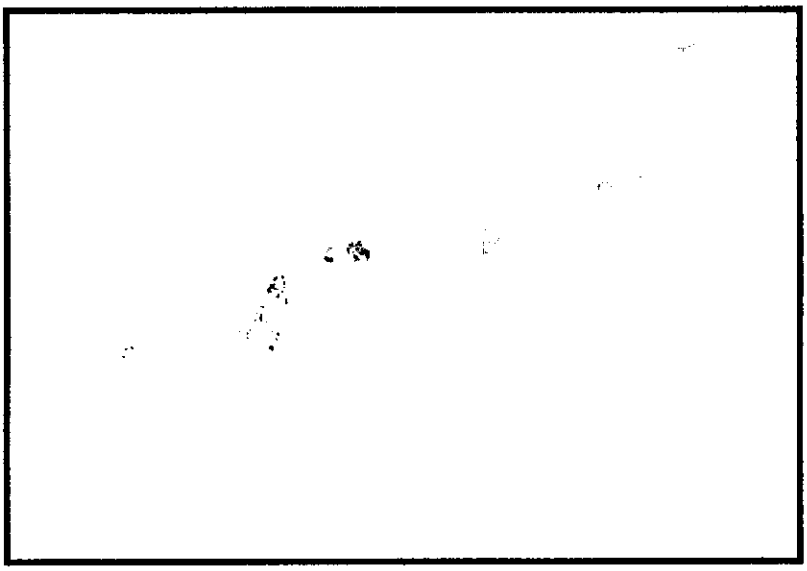

Fig. 8: Effect of treatment with conidial suspension of *M. anisopliae* on larvae & pupae of *B. tabaci* (attacked larvae & pupae became black - greenish in color, but with less superficial mycelial growth and sporulation compared with the formulated form) (40 x).

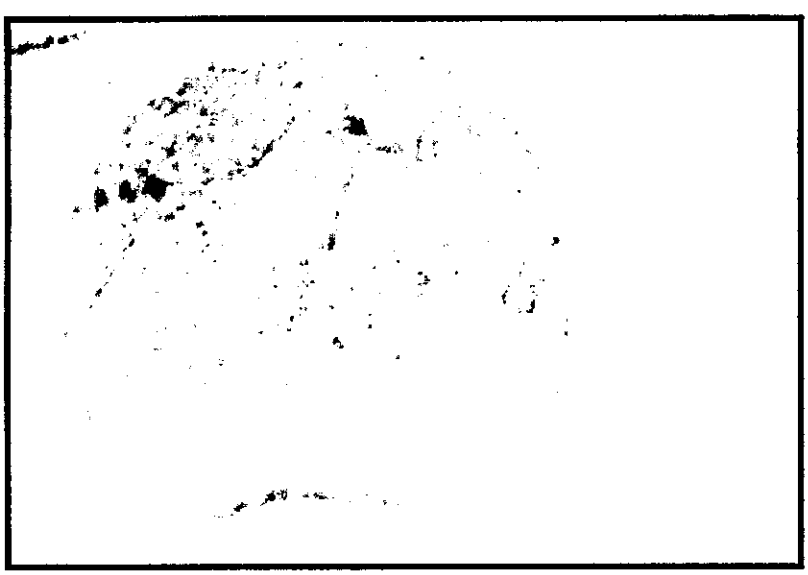

Fig. 9: Whitefly pupae (yellow or dull yellow color), on leaf disc of eggplant.

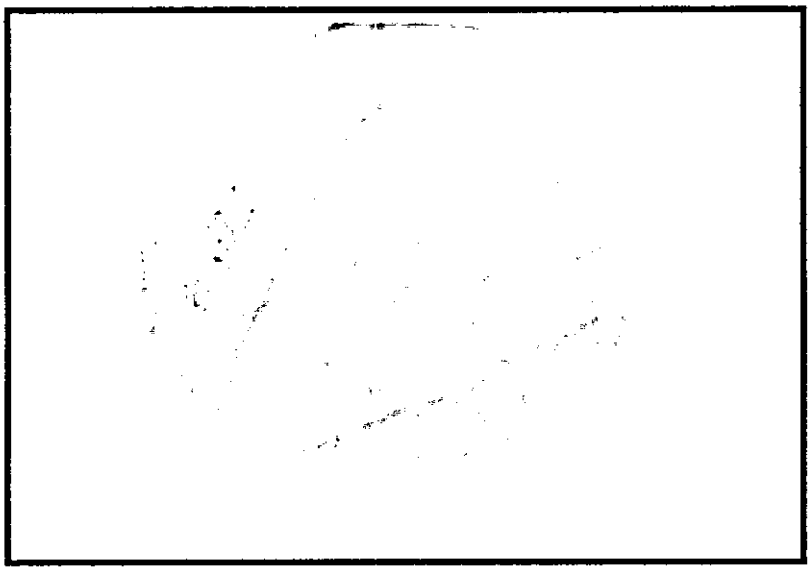

Fig. 10: Whitefly larvae (whitish to yellowish color), on leaf disc of eggplant.

Fig. 11: Effect of formulation of the fungus on the leaves of eggplant seedlings kept outdoor (slight phytotoxic effect on the margins of leaves especially lower ones).

CHAPTER FIVE
Discussion

Chapter Five

Discussion

The control of sweetpotato whitefly *B. tabaci* is extremely difficult since this insect pest has a rapid life cycle as it can develop from egg to adult in as little as three weeks under summer conditions (Butler et al., 1983). This means that, in case of using chemical pesticides to kill them (knowing that adult stage which is usually affected), spraying must be done regularly each 2 weeks to control the severe outbreak of the insect pest. An additional difficulty for applying chemical control is caused by behavior of this insect pest since adult feeding, mating and oviposition, in addition to larval development occur on the lower surfaces of infested leaves (Coudriet et al., 1985). However, egg mortality is usually minimal in case of using chemicals, but weather and predation may cause high mortality rates during the crawler and first nymphal stage development (Omer et al., 1993). Large efforts are now underway to locate the appropriate biological control agents as alternative or supplement to rapidly killing chemicals. This includes the predators (Hoelmer et al., 1993; Van Lenteren and Woets, 1988), parasites (Polaszek et al., 1992; Butler and Henneberry, 1993; Gerling and Foltyn, 1987), and entomopathogenic fungi (Brownbridge et al., 1993; Fransen, 1992; Meade and Byrne, 1991; Osborne and Landa, 1992). Moreover, entomopathogenic fungi have recently also assumed

importance in agriculture and household insects, but the extent of *B. tabaci* control by the majority of chemical alternatives is not clearly known except for the parasitoid *Encarsia formosa* (Hoddle et al., 1998) and the fungus *Beauveria bassiana* (Roberts, 1981; McCoy et al., 1988).

The attack of *Metarhizium anisopliae* on *B. tabaci* was not cited in literature up to our best knowledge, but the fungal attack on other insect pests was cited since, for example *M. anisopliae* was registered by the EPA in 1993 for the control of cockroachs and in 1995 for the control of termites (Auld, 1992; Bateman, 1997; Butt et al., 1995; Kleespies and Zimmermann, 1998; Stenzel et al., 1992; Verkleij et al., 1992; Milner et al., 1998). The efficacy of this bio-agent for combating insect pests is generally based on the antagonistic ability of this agent which leads to insect pest destruction. In this study, we used *M. anisopliae* as bio-agent against the immature stages (larvae & pupae) of *B. tabaci* under laboratory and outdoor conditions. The laboratory experiment indicated that when using formulated form of *M. anisopliae* in invert emulsion, the mortality of *B. tabaci* was very high (100 %) (Table 2) and the color of the controlled larvae and pupae became black - greenish with dark - brown mycelial growth, that means infection with fungus has occurred and fungus has penetrated the insect cuticle, then mycelium has largely expanded on the surface of affected larvae and pupae in comparison with less superficial mycelial growth in case of using non - formulated form of *M. anisopliae*

(conidial suspension only) (Fig. 8). This result was in concordance with the results obtained by other investigators (Quarles, 1995a; Milner et al., 1996; Auld, 1992; Butt et al., 1995), which indicated the growth of *M. anisopliae* on the surface of attacked termites, black field crickets, locusts, and cockroachs after infection.

As a result of the success in getting good results on biological effect of *M. anisopliae* against the immature stages of *B. tabaci* under laboratory conditions (Table 2, Fig. 6, 7, 8), the biological effect of the fungus was also tested against *B. tabaci* infesting eggplant seedlings grown under the outdoor conditions. This test showed that attacked larvae and pupae became also black - greenish in color with 92.269 % mortality in case of using formulated form of the fungus compared to the non - formulated form and the control (27.994 % and 1.000 % respectively) (Table 3). Also, significant differences ($P > 0.05$) in the mean mortalities obtained in the three types of treatment were shown. These results are confirmed by the effect of *M. anisopliae* on Japanese beetles, mosquito larvae, and coconut leaf beetles (Villani et al., 1994; Daoust et al., 1982; Fry et al., 1997; Liu et al., 1989).

It is noteworthy to mention that some old leaves of treated eggplants with formulated form of *M. anisopliae* in the outdoor experiment showed little injury in form of slight phytotoxicity on their margins (Fig. 11). This may be attributed to some ingredients entering in the formulation of *M.*

anisopliae (probably the oil) which may affect plant cells upon increasing temperature due to the direct sunlight during the early fall season. Further experiments to verify the real reason of this phytotoxicity should be conducted in the future.

The overall results obtained in this research demonstrated the efficacy of *M. anisopliae* against *B. tabaci* where high mortality percent was obtained in larvae and pupae of *B. tabaci* especially in case of applying the formulated form of *M. anisopliae* in invert emulsions. However, it is well - known that the whitefly life cycle is consisting of four life stages: egg, larvae, pupae and adult, and the duration of these stages is largely affected by the seasonal temperature (Butler et al., 1983; Arnal et al., 1993; Byrne and Bellows, 1991). Therefore, selection of the intermediate stages of the life cycle, namely larvae and pupae for testing efficacy of the fungus aimed also at avoiding the probable resistance of insect adult to the fungus which may be developed rapidly in nature especially in case of using insecticides. This selection may constitute an environment - friendly tool in controlling the insect pest (Leger et al., 1996).

Many trials to use this fungus and groundwork formulation were attempted, results obtained in this respect until present, concentrated at obtaining high efficacy rate without damaging the environment surrounding the treated insects. Examples on these formulations and insects treated are: termite control (Bioblast), (Quarles, 1995a); black field cricket control (Anti -

pasture pests), (Milner et al., 1996); locust and grasshopper control (Green Muscle), (Cherry et al., 1999; Science Magazine in Africa, 2001; Lomer et al., 1997); coffee berry borer control (Granular formulation), (Fargues and Robert, 1985; Li and Holddon, 1993; Gaugler et al., 1989); and cockroach control (Biopath and Bengal), (Quarles, 1995a). Therefore, formulation of *M. anisopliae* in invert emulsion was not previously attempted up to our best knowledge.

Finally, as a complementary work to that accomplished in this research, the efficacy of *M. anisopliae* against *B. tabaci* is recommended to be confirmed on eggplants grown under open field conditions or in the greenhouse where natural infections with *B. tabaci* may take place.

In conclusion, since this study constitutes the first trial to use the antagonistic fungus *M. anisopliae* (especially in formulated form using invert emulsion) against *B. tabaci*, it may be considered as the first step towards using *M. anisopliae* in biocontrol of *B. tabaci* commercially. However, further experiments are recommended to be conducted before carrying out this commercial use such as confirmation of the fungus efficacy against *B. tabaci* under fully opened natural conditions; the side - effects (if any) of the formulation when applied under natural conditions.

ARABIC ABSTRACT

الملخص

المكافحة الحيوية لذبابة التبغ البيضاء باستعمال فطريات مضادة

يهدف هذا البحث إلى تقييم فعالية الفطر المضاد (*الميتاريزيوم انيسوبليا*) ضد الأطوار غير الناضجة (اليرقة ، الشرنقة) لذبابة البطاطا الحلوة أو ذبابة التبغ البيضاء(*البيميزيا تباسي*) . لقد تم استعمال الفطر بشكل رئيسي كمستحلب منعكس (ماء في زيت) بعد إدخاله إلى المستحلب المنعكس بشكل كونيديا وتم تطبيق نوعين من التجارب لتقييم الفعالية : التجربة الأولى تمت في ظروف المختبر باستعمال الفطر بشكل مستحلب منعكس ، وبشكل محلول مائي يحتوي على كونيديا الفطر بالإضافة إلى المعاملة الشاهد ،وأما التجربة الثانية فقد طبقت على قوارير تحتوي أشتال باذنجان موضوعة في البيئة الخارجية خلال فصل الخريف و باستعمال نفس المعاملات المذكورة سابقا . لقد دلت النتائج خلال التجربتين السابقتين أن اليرقة أو الشرنقة بعد تأثرها بالفطر تتحول إلى اللون الأخضر الغامق ، علما بأن اللون الطبيعي لليرقة هو الأبيض ، ولون الشرنقة الطبيعي هو الأصفر– الأصفر الفاتح ، ونسبة الموت في الأطوار غير الناضجة للحشرة في ظروف المختبر كانت ١٠٠ % ، ٨٣,٧٥ % ، ٠ % للمستحلب المنعكس المحتوي على *الميتاريزيوم انيسوبليا* ، والمحلول المائي المحتوي على الفطر بشكل كونيديا ، والشاهد على التوالي مقارنة بنسبة الموت في الحشرة في البيئة الخارجية للأطوار غير الناضجة من الذبابة البيضاء حيث كانت هذه النسبة ٩٢,٢٦٩ % ، ٢٧,٩٩٤ % ، ١,٠٠٠ % باستعمال نفس المعاملات السابقة على التوالي . لقد وجد أن هناك فروقات معنوية (الاحتمالية ٠,٠٥) عند مقارنة متوسط الموت للحشرة في المعاملات بالمستحلب المنعكس المحتوي على الفطر والمحلول المائي المحتوي على كونيديا الفطر والشاهد ومع ذلك فانة ينصح بإجراء مزيد من

التجارب لتأكيد فعالية الفطر ضد الذبابة البيضاء خاصة استعمال المستحلب المنعكس المحتوي على الفطر في ظروف الحقل المكشوف قبل استعمال الفطر والمحلول المنعكس بشكل تجاري ضد الذبابة البيضاء .

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