Symptomatology of Tobacco Whitefly and Red Spidermite Infection with the Entomopathogenic Fungus *Metarhizium anisopliae* (Metsch.) Sorokin

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**ABSTRACT**

The present research aimed at describing the macroscopic and microscopic symptoms of infection with the entomopathogenic fungus *Metarhizium anisopliae* on tobacco whitefly (*Bemisia tabaci*) and red spidermite (*Tetranychus cinnabarinus*). For this, conidial suspensions of the fungal strain Meta 1 were applied against larvae and pupae of *B. tabaci*, and adults and nymphs of *T. cinnabarinus* infesting eggplants under laboratory and simulated field conditions. Results obtained have indicated that infected larvae and pupae of *B. tabaci* turned into black-greenish color due to the fungus-attack 3 days after treatment and incubation at 20±1°C and 100% RH using two types of incubation techniques. Infected nymphs and adults of *T. cinnabarinus* also turned into dark-brown color 3-4 days after treatment and incubation under similar conditions to *B. tabaci*. The same color change in both pests due to the fungus-infection under simulated field conditions (19-30°C and >90% RH) was also obtained. Dark-brown fungal outgrowth and sporulation was observed on the cadavers of treated individuals with the fungus in both pests 5 days after treatment and incubation under the above-mentioned conditions. Microscopic examination of infected individuals of *B. tabaci* or *T. cinnabarinus* have indicated that typical mycelia and conidia of *M. anisopliae* were observed on their surface. Moreover, dense mycelial growth of the fungus was also observed under higher microscopic magnifications in the hemocoel of attacked individuals of *B. tabaci* or *T. cinnabarinus*. The latter observation was realized after a careful superficial sterilization of the infected individuals of both pests, then mounting and clearing them in lactophenol before examination. Overall results refer to the possibility of using the fungus in biocontrol of both pests.

**KEYWORDS:** Tobacco whitefly, Red spidermite, *Metarhizium anisopliae*, Macroscopic and Microscopic symptoms.

1. **INTRODUCTION**

*Metarhizium anisopliae* (Metsch.) Sorokin is a hyphomycetous fungus which belongs to class Deuteromycetes (Barnett and Hunter, 1998). It is entomopathogenic and has been reported to infect one hundred species of insects which belong to a variety of insect orders (McCoy et al., 1988; Zimmermann, 1993). Practically, few insect species were reported to be biologically controlled by this fungal pathogen such as wheat grain beetles (*Anisoplia austriaca*) (Ferron, 1981), termites (*Reticulitermes* sp.) (Quarles, 1995), black field cricket (*Teleogryllus commodus*) (Milner et al., 1996), the wax moth (*Galleria melonella*) (Kucera, 1980), Western flower thrips (*Frankliniella occidentalis*) (Ludwig and Oetting, 1998), Cockroaches (Shripat et al., 1999), Locusts and Grasshoppers (Prior and Greathead, 1989; Lomer et al., 1997), and Pasture scarab (*Adoryphorus coulon*) (Rath, 1992; Farrow et al., 1993).

Pathogenicity of *M. anisopliae* on some of the above mentioned insect pests was indicated by excessive growth of the fungal mycelium in the body cavity or hemocoel of attacked-insects. This fungal growth induces death of attacked-insects (McCoy et al., 1988). In addition, the fungus produces toxins and secondary metabolites such as destruxins and proteolytic enzymes during the infection process (Kucera, 1980; Thomas et al., 1995; Hunter and Spurgin, 1999).
Tobacco whitefly (*Bemisia tabaci* Genn.) and red spidermite (*Tetranychus cinnabarinus* Boisd.) are serious pests on eggplants growing under our local conditions especially under field conditions during summer and fall seasons. Mixed infestations with the two pests are frequent during the hot seasons causing serious damage to infested eggplants. Such infestations are usually controlled by applying huge quantities of insecticides and acaricides. Effective, non-chemical control measures such as biocontrol measures using entomopathogens are currently searched.

To the best of our knowledge, no attempts were made to demonstrate pathogenicity of *M. anisopliae* on *B. tabaci* or *T. cinnabarinus* or apply the fungus in biocontrol of both pests. The objective of the present research was to test the fungus-infectivity on both pests by describing the macroscopic and microscopic symptoms of infection with the fungus on these pests. If this infectivity is proved, this will constitute the first step towards applying the fungus in biocontrol of the two pests.

2. Materials and Methods

2.1. Insect and Mite Strains

Strain Btl of tobacco whitefly (*Bemisia tabaci*) and strain Tr4 of red spidermite (*Tetranychus cinnabarinus*) were used in this study. They were obtained from infested eggplants grown under open field conditions in Tulkarm district - Palestine, then reared on caged eggplant seedlings kept in the insectary at 25±2°C. Adults and immature stages of both pests obtained after rearing were used in the experiments of infection with the fungus.

2.2. Fungus Strain

Strain Meta 1 of *Metarhizium anisopliae* was used in this study. It was first isolated from infected larvae of the ground beetles (*Harpalus caliginosus*; Carabidae, Coleoptera) then, subcultured on oat meal agar medium for 10 days to obtain the characteristic sporulating-layer of the fungus formed on culture-surface (Fig. 1-G). Harvested conidia from 10-day old fungus-culture (Fig. 1-H and I) were used in preparing conidial suspensions with a concentration 5.0×10⁶ conidia/ml to be used in tests of fungal infection.

2.3. Plant Material

Eggplant seedlings (variety: Red Thin) were used as a host-plant for the tobacco whitefly and red spidermite. Also, artificial infections with the fungus were carried out on whitefly and red spidermite adults and immature stages reared on eggplant seedlings or infesting its leaves. Proper irrigation and fertilization were practiced as usual and according to the plant requirements grown in pots.

2.4. Technique of Fungus-inoculation on *B. tabaci* and *T. cinnabarinus*

2.4.1. Under Laboratory Conditions

A 25-μl droplet (containing 125,000 conidia) of fungal conidial suspension was directly deposited onto larvae and pupae of *B. tabaci* or nymphs and adults of *T. cinnabarinus*. Concentration of the fungal conidia in the conidial suspensions used in this study was 5.0×10⁶ conidia/ml. Two techniques were used for incubating treated life-stages of the two pests either in Petri-dishes with moistened filter paper (100% RH) on leaf-disc (20mm diameter) cut from previously infested leaves or laid directly on glass-slides placed in Petri-dishes with moistened filter paper (100% RH) (Fig. 1-A and E; Fig. 2-A, B and D). The treated life-stages were then kept at 20±1°C and 16:8 light to dark for 5 days before being evaluated. Fifteen individuals (larvae and pupae) of *B. tabaci* or 15 individuals (nymphs and adults) of *T. cinnabarinus* were examined per incubation technique. For the treated individuals of *B. tabaci* or *T. cinnabarinus* which were laid directly on the glass-slides, no food was provided during the five days of treatment depending on the food reserve in their bodies. Also, during the treatment, the active individuals of *T. cinnabarinus* were prevented from escape by encircling them with a small ring of vaseline before depositing the droplet of fungal conidial suspension. Percent of fungus infection on the examined life-stages of the two pests was calculated.

2.4.2. Under Outdoor Conditions

Healthy potted eggplant seedlings (6-week old; variety: red thin) were artificially infested with eggs of whitefly or red spidermite by introducing them into caged cultures of *B. tabaci* or *T. cinnabarinus* for 48 hours. Adult whiteflies or red spidermites remained on the seedlings after infestation period were removed then, the seedlings were treated 7 days later. The time period extended to 7 days after egg hatching is sufficient to obtain an infestation with larvae and pupae of *B. tabaci* or nymphs and adults of *T. cinnabarinus* on the seedlings. Conidial suspensions with a concentration 5.0×10⁶ conidia/ml were used in tests of fungal infection.
conidia/ml and sterile distilled water as control treatment were sprayed onto the seedlings infested with both pests. Ten ml of each one of the above treatments were sprayed per potted seedling using small hand sprayer (1.5L capacity). The treated seedlings were properly irrigated 3h before the treatment then directly covered with plastic bags after the treatment for 72h to maintain >90% RH since this value of RH was necessary to fungus infection. The seedlings were then kept under outdoor conditions (19-30°C, and >90% RH) during the experiment. Randomly chosen leaves were examined 5 days after the treatment, to evaluate the infection with the fungus in treated pests population. Five potted eggplants which represent 5 replicates per treatment were used in the study. The Percentage of fungus infection in each pest was calculated.

2.5. Technique of Studying Macroscopic Symptoms of Fungus-Infection on *B. tabaci* and *T. cinnabarinus*

The visual or macroscopic symptoms of fungus-infection on larvae and pupae of *B. tabaci*, and adults and nymphs of *T. cinnabarinus* were studied within 5 days after treatment (Fig. 1-B, and C; Fig. 2-A, B, C and D). To study these symptoms, samples of fungal-treated individuals (10 randomly chosen samples per life stage per pest) were taken, then examined for the following criteria: color change of infected individuals after the fungus treatment and fungal outgrowth and sporulation on dead infected individuals or cadavers. The time periods needed for appearance or observation of the above-mentioned criteria were also defined.

2.6. Technique of Studying Microscopic Symptoms of Fungus-Infection on *B. tabaci* and *T. cinnabarinus*

The microscopic symptoms of fungus-infection on larvae and pupae of *B. tabaci*, and adults and nymphs of *T. cinnabarinus* were also studied within 5 days after treatment (Fig. 3-A to H). To realize this purpose, 10 samples which represent 10 individuals or specimens of each pest life-stage were taken. These samples were randomly taken then, examined microscopically after mounting and clearing them in lactophenol for 48 hours. The microscopic examination aimed at observing the typical fungal mycelia and conidia formed on the surface of treated individuals of both pests, in addition to observing the internal mycelial growth of the fungus in infected whitefly-pupae or red spidermite-adults. To clearly observe the internal mycelium under the microscope, a superficial sterilization of infected whitefly or red spidermite individuals or specimens was done using 70% ethyl alcohol then, rinsing with sterile distilled water (3 times successively) before mounting and clearing the specimens in lactophenol for 48 hours. Examination was, then, carried out at higher magnification (400X).

3. Results

3.1. Macroscopic Symptoms of *M. anisopliae* Infection on *B. tabaci* and *T. cinnabarinus*

3.1.1. Color Change of Infected Individuals After the Fungus Treatment

Treated individuals of larvae and pupae of *B. tabaci* with the fungal conidial suspension appeared to be black-greenish in color when infected with the fungus (Fig. 1-A to C). Such color change was observed in both types of incubation techniques 3 days after treatment and incubation at 20±1°C compared to no color change of the individuals treated with sterile distilled water only (Fig. 1-D and F). Mean percent of fungus-treated individuals with black-greenish color was 78.8.

Similar results were obtained on treated individuals of nymphs and adults of *T. cinnabarinus* with the fungal conidial suspension, but the color of the treated individuals when infected with the fungus was dark-brown. Such color change was observed 3-4 days after treatment and incubation at the same conditions and incubation techniques of *B. tabaci* (Fig. 2-A to E). Mean percent of fungus-treated individuals with dark-brown color was 68.6.

The color change of treated individuals of both pests into black-greenish or dark-brown also occurred in case of applying the above-mentioned treatments on entire infested potted eggplant seedlings kept under outdoor conditions (10-30°C). Mean percent of fungus-infected individuals due to color change was 71.2 for *B. tabaci* and 62.9 for *T. cinnabarinus*.

3.1.2. Fungal Outgrowth and Sporulation on Infected Individuals

The fungal outgrowth and sporulation on treated larvae and pupae of *B. tabaci*, and nymphs and adults of *T. cinnabarinus* were observed 5 days after treatment and incubation at 20±1°C using the fungal conidial suspension. If compared to treatments with sterile
distilled water, no fungal outgrowth or sporulation will be observed on the treated individuals of B. tabaci or T. cinnabarinus in the two types of incubation techniques. The color of the fungal outgrowth and sporulation which was observed on the surface of pests’ cadavers (dead infected individuals) was dark-brown (Fig. 1-B and C; Fig. 2-C and D).

3.2. Microscopic Symptoms of M. anisopliae-infection on B. tabaci and T. cinnabarinus

When specimens of fungus-attacked pupae and larvae of B. tabaci or adults and nymphs of T. cinnabarinus were examined under the microscope, mycelia and conidia typical of M. anisopliae were observed on the surface of whitefly and red spidermite cadavers 5 days after the treatment (Fig. 3-A to C and F). This microscopic observation was assured when whitefly-pupae or spidermite-adult cadavers were examined under higher magnifications (200 and 400X) (Fig. 3-D, F and H). In carefully surface-sterilized specimens of fungal-attacked whitefly-pupae or spidermite-adults, typical mycelium of M. anisopliae was also observed under the microscope (400X) growing densely in the hemocoel of infected specimens (Fig. 3-G). The latter observation was realized when the specimens were mounted and cleared in lactophenol following surface-sterilization.

4. Discussion

Results obtained on M. anisopliae infection on treated B. tabaci or T. cinnabarinus life stages indicate the presence of a prominent pathogenic effect of the fungus on both pests. This shows clearly the possibility of using the fungus in biocontrol of the two pests. To the best of our knowledge, infectivity of M. anisopliae on B. tabaci and T. cinnabarinus is something new and has not been reported by other investigators. This is because until present, biocontrol of B. tabaci has been carried out either by using certain parasitoids such as Amitus (family platygastridae), Encarsia (family · Aphilinidae), Eretmocerus (family Aphilinidae), Signiphora (family Signiphoridae), and Metaphycus (family Encyridae) (Polaszek et al., 1992; Gerling and Foltyn, 1987; Gerling, 1990; Powell and Bellows, 1992; Viggiani and Evans, 2001; and Butler and Henneberry, 1993), or by using certain entomopathogenic fungi such as Verticillium lecanii, Paecilomyces fumosoroseus and P. farinosus, Aschersonia aleyrodensis and Beauveria bassiana (Brownbridge et al., 1993; Fransen, 1992; Meade and Byrne, 1991; Osborne and Landa, 1992; Lacey et al., 1996 and 1999). Also, biocontrol of T. cinnabarinus has been carried out either by using some acaropathogenic fungi such as Hirsutella thomsonii, H. kirchneri, H. necatrix, Neozygites floridana, Cephalosporium diversiphialidum, and Paecilomyces terricola (Sztejnberg et al., 1997; Cehrnin et al., 1997; Gardner et al., 1982; Smith and Furr, 1975; Balazy, 1973; Kenneth et al., 1971; and Van Der Geest et al., 2000), or by using certain acaropathogenic bacteria such as Bacillus thuringiensis and Wolbachia (Royalty et al., 1990; Guo et al., 1993; Breeuwer and Jacobs, 1996; and Tsagkarakou et al., 1996).

The microscopic observations realized in the present research showed the presence of dense mycelial growth of M. anisopliae in the hemocoel of attacked B. tabaci and T. cinnabarinus life stages, in addition to the presence of fungal outgrowth and sporulation of M. anisopliae on both pests' cadavers. These observations confirm the above-mentioned evidence on M. anisopliae-infectivity on the two pests. These results also coincide with the results obtained by other investigators on the mode of fungus infection on other insect hosts (McCoy et al., 1988; Kucera, 1980; Thomas et al., 1995; Hunter and Spurgin, 1999). Moreover, the special technique of infection with the fungus followed in the present study enabled us to follow the external fungal growth and its sporulation on the attacked whitefly and red spidermite hosts due to incubation of these hosts under humid conditions using Petri-dishes with moistened filter papers.

In conclusion, M. anisopliae has a great potential to infect whiteflies and red spidermites. This potential indicates the possibility of using the fungus in biocontrol of both pests, but the application of the fungus against both pests requires >90% RH to be succeeded. The availability of high RH is more frequent in greenhouses and protected cultures than in open fields where the success of fungal application is expected to be more. The lack of high RH in open fields could be effectively overcome when the fungus is applied in formulated form. In this regard, using proper formulation such as the invert emulsion (water-in-oil type) can compensate the lack of high RH and may increase the success of fungal application. The following step in our research program will be the fungus formulating, then testing the prepared formulation against the two pests especially under field conditions.
Figure 1: Effect of treatment with *M. anisopliae* (strain: Meta 1) on *B. tabaci*. A- treatment of larvae and pupae of *B. tabaci* with conidial suspension of *M. anisopliae* after being laid on glass-slide in Petri-dish under humid conditions. attacked immaturity stages (arrows) appeared black-greenish in color (magnification:5X). B and C- enlarged group of larvae and pupae of *B. tabaci* treated in the same way as in A and appeared black-greenish in color with dark-brown fungal mycelial growth on the surface (magnification:10X and 20X, respectively). D- non-attacked larvae (whitish in color) and pupae (yellow in color) of *B. tabaci* (magnification:15X). E- infected eggplant leaf-discs (20mm²) with larvae and pupae of *B. tabaci* placed on glass-slide in Petri-dish under humid conditions for being treated with conidial suspension of *M. anisopliae*. F- indicates the whitish colored larva and yellow colored pupae of *B. tabaci* (arrows) on eggplant. G- typical pure culture of *M. anisopliae* (strain: Meta 1) grown on oat meal agar medium to be used in treatments with fungal conidial suspension. H- enlarged rectangular area in G showing the typical layer of *M. anisopliae* conidia (arrows) formed on the surface of culture medium (magnification:20X), I- typical conidia of *M. anisopliae* (arrows) obtained from the typical culture of the fungus in G (magnification:400X).
Figure 2: Effect of treatment with M. anisopliae (strain: Meta 1) on T. cinnabarinus. A and B- treatment of adults and nymphs of T. cinnabarinus infesting leaf-discs of eggplant (30 mm²) with conidial suspension of M. anisopliae, attacked nymphs and adults (arrows) appeared dark-brown in color (magnification: 10X and 20X, respectively). C and D- treatment of adults and nymphs of T. cinnabarinus with conidial suspension of M. anisopliae after being laid on glass-slide in petri-dish under humid conditions, attacked nymphs and adults (arrows) appeared dark-brown in color (magnification: 10X and 20X, respectively), in addition to appearance of dark-brown fungal mycelial growth on the surface of attacked T. cinnabarinus. E- non-attacked adult of T. cinnabarinus (magnification: 20X). F- numerous non-attacked nymphs and adults of T. cinnabarinus (arrows) infesting eggplant leaf taken from infected eggplant seedlings (magnification: 5X).
Figure 3: Microscopic examination of *Bemisia tabaci* and *Phytoseiulus persimilis* life stages after infection with the antioptic fungus *Metarhizium anisopliae* strain I-21. A: a group of infected larvae and pupae of *B. tabaci* after treatment with the fungus (magnification: 400X). B: enlarged larva infected with the fungus (arrows indicate the mycelium and fungal apposition on the larval surface; magnification: 200X). C: enlarged *B. tabaci* pupa infected with the fungus (arrows indicate the mycelium and fungal apposition on the pupal surface; magnification: 200X). D: enlarged, rectangular area in C shows the mycelium and fungal growth of the fungus (arrows); this growth may be on the surface or inside the pupal body (magnification: 400X). E: infected adult of *T. cinnabarinus* (arrow indicates the adult mouthparts; magnification: 400X). F: infected adult of *T. cinnabarinus* with *M. anisopliae* (arrow indicates superficial mycelial growth (arrows; and black circular area) which grows density in the hemocoel of *B. tabaci* pupae and *T. cinnabarinus* adult (magnification: 400X). G: typical young and mature conidia of *M. anisopliae* (arrows) obtained from the aggregated conidia on the surface of infected larve and pupae of *B. tabaci* (magnification: 400X).
REFERENCES


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سلسلة الأعراض المتعلقة باصابة كل من نباتة التبغ البيضاء والعجلات الأحمر بفطر
(Metarhizium anisopliae)

ملخص

يهدف هذا البحث إلى وصف سلسلة أعراض الأصابة الظاهرة والميروسكونبية على كل من نباتة التبغ البيضاء والعجلات الأحمر بعد معاملتها بالفطر (M. anisopliae). وتحقق ذلك فقد تم اعمال مادة التفاعل باستخدام نوعين من أنواع الفطر بشكل متساو على الأطوار غير المعتادة في الباعة البيضاء والآلوية للعجلات الأحمر تحت ظروف مختارة وما يشبه ظروف الطقس. أوضحت النتائج التي تم الحصول عليها أن الأطوار غير المعتادة في الباعة البيضاء قد تحولت إلى لون أصفر مختلط بسبب الأصابة بالفطر بعد مرور 3 أيام على المعاملة وقد بين مختلفين على درجة حرارة 25\degree C ودرجات حرارة 20\degree C. أما الأطوار المعتادة في الباعة والعجلات الأحمر فقد تحول لونها إلى اللون أصفر الغامق بعد مرور 3-4 أيام على المعاملة عند درجة حرارة 25\degree C ودرجات حرارة بين 20\degree C و15\degree C. وكان هناك أيضاً نمو عالياً بون بني غامق على السطوح الخارجية للأطوار المصابة والتي تم المعاملتها بالفطر كلا النوعين من الأطوار بعد مرور 5 أيام على المعاملة. لقد أظهر الفحص الميروسكونبي وجود نمو نباتي طفيف في الفطر خلال الأطوار المصابة لكل من الباعة البيضاء والعجلات الأحمر، كما لوحة نحو مينا نباتي في الفطر بشكل كفي بنما وعاء نباتي في السطح الخارجي للأطوار المصابة. وقد ثم الفحص الأخير بعد إجراء تعقيم وصفي جيد للأطوار المصابة ثم جرى تأثريها على سلافيس لاتكوبولز قبل فحصها. ان سجل النتائج التي تم الحصول عليها يشير إلى إمكانية استعمال الفطر في الكفاح الحيوية للكلا النوعين من الأطوار.

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