2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

STUDIES ON FUNGAL PATHOGENS FOR CONTROLLING THE TWO-SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* KOCH

Mahmoud E. El-Naggar and Rania H.M. Hassan
Plant Protection Research Institute
ARC
M.A. Zaher and M.F. Hassan
Zoology and Nematology Department
Fac. of Agric.
Cairo University

Abstract

Recently, fungal pathogens have shown considerable effect in controlling some phytophagous mites. Therefore , this work included survey on fungi associated with the two-spotted spider mite *T. urticae* infesting ,broad bean and maize in the three governorates, Menoufia, Kafr El-Sheikh and Giza. Also, laboratory bioassay and field study on the effect of some fungal pathogens on *T. urticae* and the phytoseiid predator *E. scutalis* was carried out. Results revealed the following:

1. Fungi *Neozygites sp*., *Cephalosporium sp*., *Verticillium lecanii*, *Alternaria sp*. and *Aspergillus sp*. Were collected in association with *T urticae*.
2. Fungi *Beauveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae* were used under laboratory conditions.
3. Direct and indirect method for testing effect of *B. bassiana*, *V. lecanii* and *M anisopliae* on the predator *E. scutalis* was undertaken. In a field experiment, *B. bassiana* decreased *T. urticae* population from 10% to 45 % and from 10% to 25 % for *V. lecanii* and *M anisopliae* when concentrations increased from 106 to 100 x 106 respectively.

Introduction

Scientists all over the world have a growing interest in reducing dependence on chemical pesticides as means of controlling pests. Natural antagonists are considered most promising biological mean of pest control. Consequently, invertebrate pathology received much attention in the second half of the twentieth century of which insect pests had the main interest. However, in recent years studies on mite pathogens mainly concerning with fungi infecting eriophyidae and tetranychoid mites have been growing on. Fungi pathogen to mites are known to include fungi belonging to Zygomycetes [Entomophthoralean species *(Neozygites* sp.)] (Fisher, 1951), Deuteromycetes *(Hirsute/la thompsonii)* (McCoy, 1975), *Vertici//ium lecanii* (Sewify and Mabrouk. 1991), *Paecilomyces* sp. (Pena *et al,* 1996) and *Beauveria bassiana* (Pellagatti, 1989 and Pena *el al,* 1996). The recorded epizootic, effectively, on mite populations were recorded in several countries by Zygomycetes and Deuteromycetes fungi. The efficacy of fungal pathogens as microbial control agents against mite and insect pests, is largely influenced by weather factors of which temperature and humidity are the main one. Under tropical and subtropical conditions, these two factors play an important role with regard to infection, incubation period and its propagation. Consequently, for successful development as microbial control agents, pathogenic fungi have to be adapted to the environmental conditions, temperature in particular, in the area in which they are to be applied. Therefore, it is important to select fungal isolates as a bio-control agent that grow rapid and infect at temperatures prevailing after spore application. In Egypt, climatic conditions are considered more suitable for fungal pathogens as bio-control agents (Sewify, 1999). Usually, biological control agents, have been selected for their efficacy towards a specific pest, and therefore, have a limited spectrum of targets. This target specificity is a disadvantageous with regard to practical use of these bio-control agents, since have to be compatible with other pathogens required to control other pests and diseases affecting the same crop at the same time. To avoid these shortcomings strategies, incorporating several biological control agents having complementary on synergistic modes of action against these pests have to be developed.

The objectives of this study were to survey and isolate of fungal pathogens associated with the two-spotted spider mite, *T. urticae* on the three Governorates; Giza, Menoufia and Kafr El-Sheikh; and their pathogencity to its host mite, as well as non-target mites. Susceptibility of *T urticae* and its predatory phytoseiid mites *Euseius scutalis* (A.-H.) to some fungal pathogens, was examined. Also, the investigation included control application against the spider mite by using fungal pathogen *B. bassiana* under field conditions.

Materials and Methods

Survey of Entomopatbogenic Fungi Associated with Spider Mite *Tetranychus urticae*

A survey was conducted in two governorates; Giza, Kafr El-Sheikh , and Menoufia in the period from June 2000 to January 2002 (on broad bean and maize plants). Leaves with mummies of dead mites showing any symptoms of fungi infection were collected in plastic bags and transferred to the laboratory. The collected dead mummies were kept in Petri dishes with mois-

tened filter paper and examined daily. If any mycosis, symptoms were manifested the dead mites were mounted with lacto phenol and examined microscopically (X l00).

Isolation

The method of isolation was as follows: The whole mite mummies showing mycosis were maintained directly in potato dextrose agar (PDA) (Sewify, 1989), and incubated at 25^{0}C.

Identification

Postulates and steps advised by the German Bacteriologist Robert Koch (1843-1910), provided general outlines when conducting infective tests. If these steps are carried out with positive results, it may be considered conclusive evidence that the microorganism in question is the cause of fungal infectivity experiments in the present study. These steps were as follows:

1. The whole mummies or part of mycelium mounted on slide, stained with lacto phenol, and examined with light microscope, other part was cultured in PDA media.
2. The fungus isolated in PDA, was inoculated at 25^{0}C for 15 days. A small part of mycelium was mounted on slide, stained with lacto phenol and examined with light microscope to insure that it was the same fungus.
3. Infection of the original host with fungus culture was manifestation of the mycosis symptoms.
4. Mycosis was examined with light microscope to prove that the fungus was the same as that isolated in "1" as well as it is pathogenic (George *et al.,* 1984).

Identification of isolated fungus species was carried out using keys mentioned by Burges (1981) and Sewify (1989).

Culturing of Fungi

Isolated fungi were grown using autoclaved Potato Dextrose Agar media "PDA" (250 g potato, 20 g dextrose, *25* g agar and *5* g streptomycin in 1000 ml distilled water). This method was applied in the cultures of *Verticillium lecanii, Beauveria bassiana* and *Metarhizium anisopliae* and incubated at 25^{0}C for 10 days (Sewify, 1989).

Mite Maintenance

The mite material necessary for the present study was obtained from cultures reared under laboratory conditions, under 27-30^{0}C, in the Center of Biological Control, Faculty of Agriculture, Cairo University.

The Susceptibility of the Two-Spotted Spider Mite *T. urticae* Stages to the Pathogenic Fungi

The susceptibility of the two-spotted spider mite *T urticae* stages was carried out to the fungi *B. bassiana, V lecanii* and *M anisopliae*.

Bioassay Procedures

The fungi were cultured on autoclaved PDA media. The inoculated PDA with fungal spores was incubated for 2 weeks at 25^{0}C. Spores were harvested by rinsing with sterilized distilled water, then filtered through cheese cloth to reduce mycelium clumping. The spores were counted in the suspension using a haemocytometer. Five concentrations : 106, s x 106, 10 x10^6, 50 x 106 and 100 x 106 spore/ml of each isolate were prepared.

a) The susceptibility of egg stage: The uninfected leaves of plant were transferred to the laboratory and cleaned by sterilized tissue paper. Petri dishes (30 mm in diameter) with its bottom smeared with a layer 3 mm of 1.5 % water agar for maintaining a relative humidity at 100 %. Leaf discs, each 2.5 cm in diameter and with 25-30 mite eggs was painted with 2 ml spore suspensions of different concentrations, allowed to dry for 20 mm. Each disc surrounded with Tungel-foot was kept in a Petri dish. A rubber band was used to fasten the two parts of the Petri dish together. The control was tested with distilled water. Percentage of hatching eggs was assessed in both treated and control experiments.
b) The susceptibility of larvae and adult stages: The aforementioned experiments for eggs was used in testing larvae and adults except using 10 individuals of each for every disc. After the dish was closed, percentage mortality was assessed for 5 days daily after inoculation.
c) The effect of pathogenic fungi *B. bassiana, V. lecanii* and *M. anisopliae* on the predator *Euseius scutalis* (A.-H.)

1- Direct Effect

Five concentrations 106, *5* x 106, 10 x 106 and 50 x 106 and 100 x 106 spores/ml of ***B. bassiana, V*** *lecanii* and *M anisopliae* were tested separately against the adult stage under laboratory conditions. Adults were contaminated by dipping method for 5 sec.(EI- Hady, 1995) in conidial spore suspension and in sterilized distilled water as a control. Each individual of the predator was transferred to a clean fresh castor leaf disc, one square inch in diameter then treated with the suspension of fungi. After treatment, the leaf discs holding the mites were placed on wet cotton pads in 10 cm diameter Petri-dishes put inside a box maintaining *95* % R.H. *T. urticae* was introduced daily as a diet for the predator (10 mites/one predator) in each Petri dish, and kept at 25^{0}C for *5* days. The rate of consumption was recorded for five days after inoculation.

2- Indirect Effect

Three treatments of infected adult of the two- spotted spider mite *T urticae* were carried out using the three isolates as previously mentioned at five concentrations 106,5 x 106, 10 x 106, 50 x 106 and 100 x10^6 spore/ml. These infected adults were used as diet source for the predator adults (10 infected mites/predator). Four replicates were used and separated in Petri-dishes. The experiment was kept at 25^{0}C and the total consumed *T urticae* was recorded daily for *5* days.

Field Trials

Production of Infective Spores: B. bassiana. Fungal conidia of *B. bassiana* were used in field experiments. Conidia were produced on barley substrate (Aregger, 1992), which contained 50 gm barley, 35 ml distilled water and 2 ml sunflower seed oil. The barley mixed with water and oil was autoclaved in Erlenmeyer Flasks vigorously. The flasks were cooled at room temperature, inoculated with 1 ml of conidia suspension 106 spores/ml, and incubated for 2-3 weeks in the dark at 25±1 ^{0}C (Photo 1). The conidia were harvested by suspending them in 50 ml of distilled water and 0.01 % Tween-80. The suspension was filtered through a double layers of muslin cloth and the desired concentrations for field application was adjusted by adding distilled water. Total spores were counted before application using haemo-cytometer.

Field Application

Application was carried out against the two- spotted spider mite 7'. *urticae* on soybean plants and its effect on the predator *E. scutalis.* One field experiment was conducted at the Agricultural Experiment and Research Station, Faculty of Agriculture, Cairo University, Giza, Egypt. During 2001 season, variety "Crawford" of soybean was planted. A split-split plot design with four replications was used. Soybean was assigned to sub-plot. Each sub-plot contained 10 ridges, 3.5 m long and 60 cm wide. Soybean was planted on May 10 and 23 in 2001. Seeds were sown in hills, 10 cm apart within ridges, plants were thinned 21 days after emergence, leaving 2 plants/hill. All other agricultural practices were done as recommended for ordinary soybean production in the area. The area was divided longitudinally into 2 parts, each contains 3 parts. These three parts were separated by two rows of maize plants, the 2 parts were sprayed by *B. bassiana* with concentrations of 100 x10^6 spores/mi and mixed with water, each liter contained 0.4 % sunflower seed-oil + 0.01 Tween-80. The control was sprayed with similar formulation without fungal spores. Plants were sprayed to "run-off' on the ventral leaf surfaces with *5* liters of suspension by using hand sprayer compressor machine. The application was repeated four times at intervals 3, *5,* 7 and 14 days after the first application. Adult mites of 7'. *urticae* and the predator *Euseius scutalis.* were counted on 20 plants in each treatment (3 leaves/plant) with the aid of a binocular microscope. The efficiency of each treatment was based on the reduction in mite population in relation to control according to the formula of **Henderson and Tilton *(1952):***

Reduction percentage [1 – (Cl x T2 / C2 x Ti)] 100 where:

Cl = Population in control before application.
C2 = Population in control after application.
Ti = Population in treatment before application.
T2 = Population in treatment after application.

Statistical Analysis

The statistical analysis of the obtained data were based on the analysis of variance and linear regression analysis (Snedecor, 1956). The probit analysis was also employed (Bliss, 1952), in bioassay testes.

Results

Survey of Pathogenic Fungi Associated with the Two-Spotted Spider Mite

1-Tetranychus urticae Koch. A survey on pathogenic fungi associated with the two-spotted spider mite *T. urticae* Koch was conducted out on maize, and broad bean plants in Menoufia, Giza and Kafr E1-Sheikh Governorates from June 2000 to February 2001. The survey revealed the presence of 5 identified fungi species associated with the two-spotted spider mite, *T. urticae,* (Table 1).

2-Neozygites sp. The fungus *Neozygites* sp. was found in the population of the two-spotted spider mite T. *urticae* on broad bean, *Vicia faba* during January 2001 in Menoufia Governorate. Infected cadavers remained attached to leaves and were covered with a whitish dust. The microscopic examination showed that the hyphal bodies are spherical. Primary conidia are subglobose, with relating flattened basal papilla 14-25 gm x 14-16 gm (19 x 15 µm). Secondary conidia capilliconidia are almond-shaped, 20-30 lam x 10-14 gm (25 x 12 gm), supported by a capillary conidiophore's (25 gm). No resting spores were observed.

3-Verticillium lecanii (Zimm.). The fungus was isolated from *T. urticae* cadavers on maize, *Zea maize* plants sampled in Giza region during September 2001. The fungus was recognized by appearance of the white mycelium on infected mite. Conidiogenous cells (phialides) in whorls (verticils) or solitary on hyphae, conidia hyaline, born in slime droplets or dry.

4- Cephalosporium roseo-griseum. This fungus was isolated from mummies of *T. urticae* collected from maize plants, *Zea maize,* in Kafr EI-Sheikh Governorate during September 2000-2001.

5- Alternaria sp. and Aspergillus sp. These species were isolated from the two-spotted spider mite *T. urticae* sampled from *Zea maize* and *Vicia faba* plants in Kafr EI-Sheikh Governorate during 2000-2002. The two fungi species were known as common fungal contaminants of insect cadavers.

Susceptibility of *T. urticae* Koch Adult, Larval and Egg Stage to Pathogenic Fungi *Metarhizium anisopliae, Beauveria bassiana* and *Verticillium lecanii*

Adult Stage. *Tetranychus urticae* adult stage was treated with different concentrations of the fungal conidia of *B. bassiana, V. lecanii* and *M. anisopliae.* The calculated LC_{50} values were shown in Table (2). Fungus E *lecanii* was the most pathogenic to T. *urticae* (LC_{50} = 4.4 x 106 spores/ml), followed by M. *anisopliae* (LC_{50} = 9.5 x 10^6 spores/ml), and finally the least pathogenic fungus was *B. bassiana* (LC_{50} = 19 x 10^6 spores/ml). The rapid mortality was recorded in the case of adult mite treated with each of *V. lecanii* and M. *anisopliae* (LT_{50} = 3.5 and 3.6 days, respectively), while B. *bassiana* induced slow mortality (LT_{50} = 5.4 days).

Larval Stage. Data in Table (4) showed the susceptibility of *T. urticae* larval stage exposed to the different concentrations of fungi *V. lecanii, M. anisopliae* and *B. bassiana.* Here again as in adults, fungus V. *lecanii* was the most pathogenic to T. *urticae* larvae (LC_{50}= 1.28 x 10^6 spores/ml) followed by M. *anisopliae* (LC_{50} = 3.35 x 106 spores/ml), while the least pathogenic fungus was *B. bassiana* (LC_{50} =3.76 x 10^6 spores/ml). In case of larvae treated with previous fungi, a rapid mortality was recorded by *M. anisopliae* (LT_{50} = 4.03 days), and *V. lecanii* (LT_{50} = 4.40 days), respectively. *B. bassiana* induced slower mortality (LT_{50} = 5.75 days) than the other two fungi species.

4.2.3- Egg Stage. The effect of fungal infection on egg hatching was carded out. Three day-old eggs were treated with different concentrations of conidiophores of fungi *V. lecanii, M. anisopliae,* and *B. bassiana.* The egg hatching decreased as a result of fungal infection (Table 4). Increasing the fungi concentration decreased the egg hatching. On the contrary, fungus *B. bassiana* proved to be highly effective at all tested concentrations compared with fungi V. *lecanii and M. anisopliae.* The percentage of egg hatching was 0, 2.5 and 11.25 %, when 3-day old eggs were treated with *B. bassiana, M. anisopliae* and *V. lecanii* at 100 x 10^6 spores/ml, and 5 days after treatment, respectively. These results suggested differences in pathogencity between the three fungi and the T. *urticae* stages exposed. The results indicated that, T. *urticae* larvae were more susceptible to all fungi in a descending order V. *lecanii, M. anisopliae* and *B. bassiana.* Data also showed that fungus *V. lecanii* caused highest mortality to adult and larval stages.

From the previous results, it could be concluded that *V. lecanii* was the most effective fungal pathogen for T. *urticae* adult and larvae, while being the least for egg, while *B. bassiana* was the best to control egg hatching.

Effect of Fungal Pathogens *V. lecanii B. bassiana* and *M. attisopliae* on the Phytoseiid Predator *Euseius scutaIis* (A.-H.)

1- Direct Effect. The susceptibility of adults of the predatory mite *E. scutalis* against the three fungi pathogens *V. lecanii M. anisopliae* and B. *bassiana* was carried out. Obtained results in Table (5) showed that treated E. *scutalis* adults were moderately affected by infection of fungus M. *anisopliae,* whereas the percentage of adult mortality was 25 % at concentration of 100 x 106. However, *V. lecanii* gave the highest effect with mortality percent 45 % at 100 x 10^6 spores/ml, while *B. bassiana* proved to be the least effective as it gave only 10 % mortality at the same concentration.

2- Indirect Effect. The obtained results in Table (6) showed that, the feeding capacity of the predator *E. scutalis* adult stage was slightly affected when fed on T. *urticae* immature treated with each of the three fungi B. *bassiana* and V. *lecanii,* respectively, at tested concentrations 106 and 5 x 106 then sharply decreased for M. *anisopliae* at 10 x 10^6, 50 x 10^6 and 100 x 10^6 spores/ml. However, the last three concentrations of the other two fungi also decreased the capacity of the predator feeding but not as much as that of M. *anisopliae.*

Thus, from the foregoing results it could be concluded that *B. bassiana* had the least direct effect on the predator *E. scutalis* adult as well as its feeding capacity on treated prey *T. urticae.* Consequently, it was chosen to be tested in the field, for not causing great harm to the predator, which could play a part in spider mite control.

Field Application

The Effect of fungus B. bassiana on T. urticae. Application test was undertaken using the fungus *B. bassiana* in the field to control T. *urticae* on soybean plants. Obtained results (Table7) indicated that the fungal pathogen was highly effective in controlling the mite T. *urticae.* The mean numbers of T. *urticae* were reduced significantly as a result of fungus *B. bassiana* application (Table9). The treatment of fungus gave a high rate of reduction of adults reaching about 75 %, 10 days after application (Table 8). It seemed from these results that, the fungus *B. bassiana* was highly effective in controlling adult stage of T. *urticae*

Influence of the Fungus B. bassiana Application on the Predaceous Mite E. scutalis'(A.-H.). Table (9) showed that the number of predator *E. scutalis* was slightly affected by fungal application. The reduction percentage of predator population ranged from 32 to 10.34 %, 3-21 days after fungal application with an average of 20.43 % throughout the whole period (Table 8). The results also showed that the predator numbers were reduced 3 and 5 days after fungal application, while no significant reduction occurred from the 14th to the 21st day of application. From results of field experiment on the effect of the fungi B. *bassiana* on both the two spotted spider mite *T. urticae* and the phytoseiid predator *E. scutalis,* it was obvious that it greatly affected the prey, while slight effect was caused to the predator. Population reduction ranged from 86.7 to 92.5 % with 75.11% average for the prey, and from 32.6 to 20.0 with 20.43 % average for the predator after 3 and 28 days, respectively. Thus, although the fungi affected the predator to some extent, yet its effect against the pest (prey) was very much larger. This result may throw some light on the use of this fungus as a biocontrol agent against the harmful two-spotted spider mite.

Discussion

The biological activities of tetranychoid mites that were usually associated with various symptoms on several host plants attracted the attention of different authors. Accordingly, several trials have been made by various Acarologists to clarify its role on field and truck crops, ornamentals and fruit trees together with the appropriate approaches towards its biological control. Fungal pathogens as biocontrol agent against injurious plant mites have been growing on. Therefore, it has been necessary to make some studies on the control of the two-spotted spider mite that is worldwide and considered an important pest of this group of mites by using some indigenous fungi. Present work proved the occurrence of about five fungal pathogens associated with mite *T. urticae* Koch individuals on soybean, maize and broad bean plants throughout Kafr EI-Sheikh, Menoufia and Giza Governorates. These are:

1- *Neozygites* sp.

This fungus was isolated from the two-spotted spider mite *T. urticae* Koch sampled from broad bean plants in Menoufia during January 2001 and it was a first record in Egypt. Its diagnostic characters have been shown.

2- *Verticillium lecanii* {Zimm.)

It was isolated from T. *urticae* cadavers on maize plants sampled in Giza during September 2000 and bioassay laboratory study has been done on different stages of the mite at 25±2°C temperature. The obtained results is nearly similar to that previously mentioned by Sewify and Mabrouk (1991), on *Eutetranychus orientalis* Klein under two different temperatures (20 and 27 °C). Their studies proved that the fungus was considered as a biocontrol agent against the mite as treated eggs failed to develop and died. In the present study, *V. lecanii* has an obvious effect on the egg stage of *T. urticae,* as hatching percentage was 11.25 % at 100 x 10^6 spores/ml, while 15 % hatchability *E. orientalis* eggs was obtained at 27°C by Sewify and Mabrouk (1991) studies.

3- *Alternaria* sp. and *Aspergillus* sp.

The isolated fungi *Alternaria* sp. and *Aspergillus* sp. from mites sampled from maize plants in Kafr E1-Sheikh showed that these fungi have a potential control for the mite *T. urticae.* Saminakova (1966) reported that *Aspergillus* sp. was associated with different stages of this mite.

4- *Cephalosporium roseo-griseum*

This fungus was associated with mummies of *T. urticae* collected from maize in Kafr E1-Sheikh and has been a first record in Egypt and its diagnostic characters have been shown.

Susceptibility of *T. urticae* Stages to Pathogenic Fungi *Beauveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae*

Fungus *V. lecanii* under laboratory conditions was highly virulent to adult *T. urticae* at 100 x 10^6 spores/ml at 25±2°C. The calculated LC_{50} = 4.4 x 10^6 spores/ml and LT_{50} was 3.50 days. Also, it was the most virulent against larva under the same conditions, the calculated LC_{50} = 1.28 x 10^6 spores/ml. *M. anisopliae* was the second and *B. bassiana* the third with LC_{50} was 9.5 x 10^6 & 3.35 xl0^6 for adult and larva for the former and 19 x 10^6 & 3.76 x 10^6 for the Latter respectively. This may be attributed to thinner cuticle of immature resulting in making it easier to be wounded (attacked) by fungus infection. This agrees with the findings and interpretation of Susilo *et al.* (1994) who added the female adults of T. *urticae* were more susceptible to fungi *Neozygites fioridana* infection than males. The letters have stronger cuticle compared with that of females, which have a more elastic episthosomal cuticle needed for ovarial development, or being different in chemical composition. However; this difference in susceptibility of mite stages and sexes may be partially attributed to presence or absence of anti fungal substance in the host cuticle.

In case of egg stage, fungus *B. bassiana* was the most pathogenic than the two other fungi V. *lecanii* and *M. anisopliae.* The hatchability of treated eggs was 0 % at 10^6 spores/ml after 5 days at 25±2°C, but it was 11.5 % in the case of *V. lecanii* and 2.5 for *M. anisopliae.* Sewify and Mabrouk (1991),and Carreck *et al* (1998) considered *V. lecanii* to be a good agent to con-

trol *E. orientalis* stages. None of the fungal treated eggs hatched at 20°C while only 15 % hatchability was obtained at 27°C, moreover, newly hatched larvae from treated eggs failed to develop and died soon after emergence.

The Effect of Pathogenic Fungi *B. bassiana*, *V. lecanii* and M. *anisopliae* on Predator *Euseius scutalis* (A.-H.)

V. lecanii proved to be more pathogenic with the direct way at 25°C. Percentage reduction increased 10% at 10^6 to 45% at 100 x 10^6 spores/ml. ranged from 2.5% to 10% at the same concentrations respectively, while M. *anisopliae* was mid-way between the two other fungi. Our results are supported by the findings of Carreck (1998) who reported that M. *anisopliae* killed representatives of different suborders of Acari including the families Tetranychidae and Eriophyidae. In the indirect way, laboratory experiments indicated that *M. anisopliae* was the most effective to *E. scutalis* adult stage than other fungi.

Field Application

In a field application, *B. bassiana* was more virulent than other fungi reduced *T. urticae* adult population by 88.66 % through 14 days and 90 % through 21 days. On the contrary, the predator adult population was reduced by 12.25 % through 14 days and 10.34 % through 21 days. The possible high susceptibility of the fungus *B. bassiana* had been firstly recognized as a disease-causing organism by Agostion Bossiin-1835 and used as a biological control agent from about 100 years. It is non-specific entornogenous fungus with abroad spectrum (Kenneth *et al.,* 1971).

Dresner (1949), Shigeo (1978) and Goettel (1994) recorded the fungus infecting and causing mortality of *T. urticae* Koch in the field population reported that a toxin named Bassianolide has been isolated from *B. bassiana* bodies.

B. bassiana is evidently a promising biocontrol agent against mite T. *urticae* as well as other pest insects. This particular entomopathogenic fungus has the following advantages:

1. It is easily cultured on artificial media, hence, can be propagated in large quantities.
2. It has a relatively wide range of pest hosts, which increase its importance as a biocontrol candidate.
3. It has a long half-life compared to other fungi pathogen species.
4. High stability of the fungus on a mixture of some chemical insecticide group (Sewify, 1998) and pheromones (Hockland *et al,* 1986).

The application of fungi, in general, is evidently faced with a major obstacle, which is the necessity of high level of humidity required for spore germination. This level is estimated to near 100 % R.H. for at least 14 hours (Hall, 1980). The high humidity is apparently not required, once the successful penetration occurred; body's moisture of the host may provide the necessary humidity. In this respect, Hall (1981) and Milner and Lutton (1982) found that, low humidity did not affect the progress of fungal infection in the aphids, one the infection occurred. Furthermore, Ferron (1978) demonstrated that, the infection of insects can be obtained independently of the environmental humidity and suggested a physical phenomenon of insect integument, which could facilitate infection in the absence of humidity. Indeed, a continuous high humidity is probably an asset for the fungal sporulation, which in turn may increase epizootic of the disease among the population of the target pest. It could be concluded that the absolute necessity of high humidity is only required to initiate fungal infection. Thus, the humidity may not be a major obstacle in using fungal biocide. Proper management of using the fungus would suggest a high volume spray, as well as probably evening application, in order to secure the necessary high humidity during the critical 14 hours or so. In this respect, frequent spray treatments may also be useful.

Temperature has both direct and indirect effects on fungi. The indirect effect is mainly the effect on the host, which may alter the development of infection. For instance, high temperature speeds the rate of metamorphosis, *i.e.,* molting of mite instar, which in turn reduce the chance of successful infection. The direct effect of temperature on fungi is related to the species of fungus and even to the different strains or isolates of the same fungal species. In other words, each fungus has optimal value of temperature as well as certain limits of Temperature-range for its activity. *B. bassiana* has been reported to tolerate temperature up to 36°C depending upon the isolate of fungus (Sewify, 1999). However, the optimal range would be 20-25°C. This temperature range is mostly available under field conditions, at least overnight, which may allow the initiation of successful infection.

The control timing using fungal application, may well differ from that when using chemicals. In other words, the utilization of fungal insecticide may be more effective with pest density, slightly lower than the known threshold for this pest. This particular speculation would require to be put into test and investigation.

In Egypt, the extensive use of chemical insecticides urged the need for promising biocontrol agent. The fungus *B. bassiana* could be a reasonable candidate for controlling mites that create major pest problems, especially in glasshouse crop production.

Although the climate in Egypt is relatively dry, the humidity in glasshouses could be manipulated to reach an adequate level of fungal activity. The optimal temperature-range of activity for this fungus is guaranteed, at least overnight, during autumn,

winter and probably spring. The validity of fungal application, in Egyptian fields, certainly requires experimentation that takes into account the proper management necessary for such a control element.

In field application, *B. bassiana* was applied as it has slight effect on the phytoseiid predator *E. scutalis* that together with other predators play a good role as biocontrol agents. Moreover this fungus gave considerable effect against the two-spotted spider mite.

Finally, this work is considered one of the first steps in using fungal pathogens in controlling injurious mites to economic plants. The results obtained will encourage acarologists to do more research in this safe direction to avoid using acaricides that cause pollution and hazards to man and his domestic animals.

References

Aregger, E. (1992). Conidia production of the fungus *Beauveria brongiarii on* barley and quality evaluation during storage at 2°C. J. lnvertebr. Pathol., 59: 2-10.

Bliss, C.I. (1952). Statistics in Biology. Vol. 2, Academic Press, New York.

Burgess, H.D. (1981). Microbial Control of Pests and Plant Diseases, 1980-1981. 949 pp., Academic Press, London, blew York, Toronto, Sydney, San Francisco.

Dresner, E. (1949). Culture and use of entomophagous fungi for the control of insects. Contr. Boyce Thompson Inst., 15: 319-335.

EI-Hady, Mona (1995). Studies on tetranychoid mites and their predators in Egypt. M. Sc. Thesis, Fac. Agric., Cairo UniV., 184 p.

Ferron · P.(1978): Biological control of insect pests by entomogenus fungi. Ann. Rev. Entomol.,23:409- 442.

Ferron, P. (1980). Pest Control by the Fungi *Beauveria* and *Metarhizium.* In: "Microbial Control of Pests and Plant Diseases 1970-1980. Edited by H.D. Burgess, p. 765-498, Academic Press London, New York, Toronto, Sydney, San Francisco.

Ferron, P. (1983). Fungal Control. In : Comprehensive Insect Physiology, Biochemistry and Pharmacology, Edited by G.A. Kerkut and L.L. Gilbert, p. 1-129.

Fisher,F.E. (1950). Two new species of *Hirsutella* Tatouillard. Mycologia, 42: 13 - 16.

Fisher, F.E. (1951). An entomophthora attacking citrus red mite. Fla. Entomol., 34: 83-88.

George, O. Painter, Jr. and Gerard M. Thomas (1984). Laboratory guide to insect pathogens and parasites. Plenum Press, New Yolk and London, pp. 105-378.

Goettel, M. S. (1994). Host range and specificity in relation to safety of exotic fungi. In 6th imitational colloquium of invertebrate pathology, microbial control, 27 Ann. Meeting soc. Invertebrate pathology, Montpellier, France 28 August - 2 September 1994: 325-329.

Hall, R.A. (1980). Control of Aphids by the Fungs *verticillium lecanii.* Effect of spore concentration entomology and experimentalist applicate, 27, 1-5.

Henderson, C. and Tilton, W.F. (1952). Tests with acaricides against the brown wheat mite. J. Econ. Entomol., 48: 157-161.

Hockland, S. H., Dawson, G. W., Griffiths, D.C., Marpies, B., Pickett, J. A. and woodcock, C. M. (1986): The use of Aphids alarm phermone ((Ec)- - franesene) to increase effective of the entomophilic fungus *Verticillium lecanii* in controlling aphids under grass house.

McCoy, C.W. (1975). Large-scale production of the fungal pathogen, *Hirsutella thompsonii* in submerged culture and its formulation for application in the field. Entomophaga, 20 (3): 229-240.

McCoy, C.W. and Ekanavei, R.F. (1969). Isolation of *Hirsutella thompsonii* from the citrus rust mite, *Phyllocoptruta oleivora* and its cultivation on various synthetic media. J. Invertebr. Pathol., 14:386-390.

Pelagatti, O.; Frate, G.del; Caretta, G. and Del Frate, G. (1988). Fungi isolated from insect and mites. Redia, 71: 255-266.

Pena, J.E. and Osborne, L.S. and Duncan, R.E. (1996). Potential of Fungi as bio controlagent of Polyphagotarsonemus latus (Acari: trasonemidae). J. Entomophaga,41 (1): 27-36

Sewify, G.H. (1998). Evaluation of *Verticillium lecanii* entomopatho-genic fungus and its prospects in controlling aphid pests. Ph.D. Thesis, fac. Agric., Cairo Univ., 134 pp.

Sewify, G.H. (1999). Evaluation of the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin for controlling three lepidopterous pests infesting maize in Egypt. J. Appl. Biol. Cont., pp. 203 -210.

Sewify, G.H. and Mabrouk, A.M. (1991). The susceptibility of difefemt stages of the citrus brown mite, *Eutetranychus orientalis* Oudemans (Acarina: Tetranychidae) to the entomopathogenic fungus *Verticillium lecanii* (Zimm.) Viegas. Egypt. J. Biol. P. Control, 1 (1): 89-92.

Shigeo, M. 0979): Presence of toxic substance in fungus bodies of the entomopathogenic fungi, *Beauveria bassiana* and *Verticillium lecanii.* Appl. Ent. Zool., 13(2): 97-102.

Snedecor, G.W. (1956). Statistical Methods. *5th* Ed., Iowa State Univ. Press.

Susilo, F.X.; Nordin, G.L. and Brown, G.C. (1994). Age-specific and inter-sexual susceptibility of two-spotted spider mite, *Tetranychus urticae* Koch, to *Neozygites fioridana* Weiser and Muma. J. Kansas Entomol. Soc., 67: 293-296.

Table 1. Survey on pathogenic fungi associated with the two-spotted spider mite in different regions in Egypt

Fungi	Original host	Host plant	Location	Date
Neozygites sp.	*T. urticae*	*Vicia faba*	Menoufia	January, 2001
Verticillium lecanii	*T. urticae*	*Zea maize*	Giza	September, 2001
Cephalosporium	*T. urticae*	*Zea maize*	Kafr El-Sheikh	September, 2001
roseo-griseum	*T. urticae*			
Alternaria sp.	*T. urticae*	*Zea maize*	Kafr El-Sheikh	2000-2001
Asperillus sp	*T. urticae*	*Zea maize*	Kafr El-Sheikh	2000-2001

Table 2. Percentage mortality of *T. urticae adult* stage treated with fungi pathogens and results LC_{50} (spores/ml) and LT_{50} (days), 5 days after treatment.

	% Mortality at indicated concentrations							
Fungi	**0**	**10^6**	**$5x10^6$**	**$10x10^6$**	**$50x10^6$**	**$100x10^6$**	**LC_{50}**	**LT_{50}**
V. lecanii	7.50	37.50	47.50	60	70.00	77.50	$4.4x10^6$	3.5
M. anisopliae	7.50	35.00	42.50	50	57.50	72.50	$9.5x10^6$	3.60
B. bassiana	7.50	32.00	38.00	48	55.00	62.50	$19x10^6$	5.40

Mean no. of treated adults =40 individuals.

Table 3. Percentage mortality of *T. urticae larval* stage treated with fungi pathogens and results LC_{50} (spores/ml) and LT_{50} (days), 5 days after treatment

	% Mortality at indicated concentrations							
Fungi	**0**	**10^6**	**$5x10^6$**	**$10x10^6$**	**$50x10^6$**	**$100x10^6$**	**LC_{50}**	**LT_{50}**
V. lecanii	7.50	47.50	70.00	77.50	82.50	87.50	$1.28x10^6$	4.40
M anisopliae	7.50	40.00	62.50	65.00	75.00	87.50	$3.35x10^6$	4.03
B. bassiana	7.50	35.00	57.50	62.50	67.50	77.50	$3.76x10^6$	5.75

Mean no. of treated adults =40 individuals.

Table 4. Hatching percentage of *T. urticae* eggs (3 days - old) treated with the three fungi *V. lecanii, M. anisopliae* and *B. bassiana.* Five days after treatment.

Concentrations	No. of Treated egg	% Hatching		
		V. lecanii	*M. anisopliae*	*B. bassiana*
Control	20	98.75	96.75	97.50
10^6	20	45.00	36.25	30.00
$5x10^6$	20	40.00	31.25	16.50
$10x10^6$	20	21.25	10.00	7.50
$50x10^6$	20	17.50	5.00	2.50
$100x10^6$	20	11.25	2.50	0.00

Table 5. Percentage mortality of *E. scutalis* adult stage treated with fungi *M. anisopliae, V. lecani*i and *B. bassiana,* 5 days after treatment.

Fungi	% Mortality at indicated concentrations						
	0	10^6	$5x10^6$	$10x10^6$	$50x10^6$	$100x10^6$	LC_{50}
V. lecanii	5.0	10.0	20.0	30.0	35.0	45.0	$176x10^8$
M. anisopliae	5.0	10.0	12.5	20.0	25.0	25.0	$388x\ 10^7$
B. bassiana	0.0	2.5	2.5	5.0	7.5	10.0	$121x10^8$

Table 6. The feeding capacity of the predator *E. scutalis* adult on Treated *T. urticae*.

Concentrations of fungi	Treated prey individuals/day		
	B. bassiana	*M. anisopliae*	*V. lecanii*
0	5.5±2.19	5.8±1.3	5.4±1.4
10^6	4.7±1.7	5.3±1.3	4.75±1.3
$5x10^6$	4.0±1.4	4.5±1.28	3.5±1.3
$10x10^6$	3.7±1.4	2.3±1.3	2.9±1.3
$50x10^6$	3.5±1.45	1.25±1.4	2.8±1.3
$100x10^6$	3.2±1.46	0.65±1.5	2.7±1.3

Table 7. Effect of the pathogenic fungi *B. bassiana* at $100x10^6$ spores/ml on adult *T. urticae* adults in the field.

Treatment	No. of mites per leaf during dates after treatment						
	Pre Treatment	3 days	5 days	7 days	14 days	21 days	28 days
Cont. I	4.55 b	7.250 a	3.75 a	4.100 a	3.45 a	3.93 a	4.000 a
Treat. I	2.825 c	1.225 c	1.000 b	1.800 b	0.905 b	0.600 b	0.45 b
Cont. II	5.88 a	3.600 b	4.100 a	4.45 a	3.400 a	3.35 a	3.95 a
Treat. II	2.50 c	0.500 c	0.88 b	1.650 b	0.48 b	0.33 b	0.28 b

a , b, c : Means followed by the same letter are not significantly different at 5.% level of probability.
Cont. I : Mites treated with fungus, water and oil.
Treat. I : Mites treated with fungus, water and oil.
Cont. II : Mites treated with water, oil and Tween-80.

Table 8. Reduction percentage of *T. urticae* and predator *Euseius scutalis* caused by treatment (Fungus *B. bassiana* at 100 x 10^6 spores*/ml under field conditions.

Mites species	Reduction percentage at indicated days						
	3	5	7	14	21	28	Mean
Prey							
T. urticae	86.70	76.25	57.10	88.66	90.00	92.50	75.11
Predator							
E. scutalis	32.60	25.00	20.00	12.50	10.34	20.00	20.43

• *Beauveria* + Tween-80.

Table 9. Effect of the pathogenic fungi *B. bassiana* on adult stage of predatory mite *E. scutalis* in the field.

	No. of mites per leaf during dates after treatment						
Treatment	**Pre Treatment**	**3 days**	**5 days**	**7 days**	**14 days**	**21 days**	**28 days**
Cont. I	4.675 b	5.200 a	0.500 b	0.300 a	1.100 bc	2.95 ab	2.25 b
Cont. II	6.075 a	1.000 c	1.050 a	1.300 a	0.650 c	2.8 ab	2.85 a
Treat. I	5.05 c	1.75 b	0.600 ab	1.400 a	1.850 a	3.05 a	2.200 b
Treat .II	3.300c	1.200 c	0.900 ab	1.400 a	1.325ab	2.500 b	3.l00a

a,b,c: Means followed by the same letter are not significantly different at 5 % level of probability.
Cont. I :Mites treated with water and oil.
Treat. I :Mites treated with fungus, water and oil.
Treat. II :Mites treated with fungus, water, oil and Tween-80