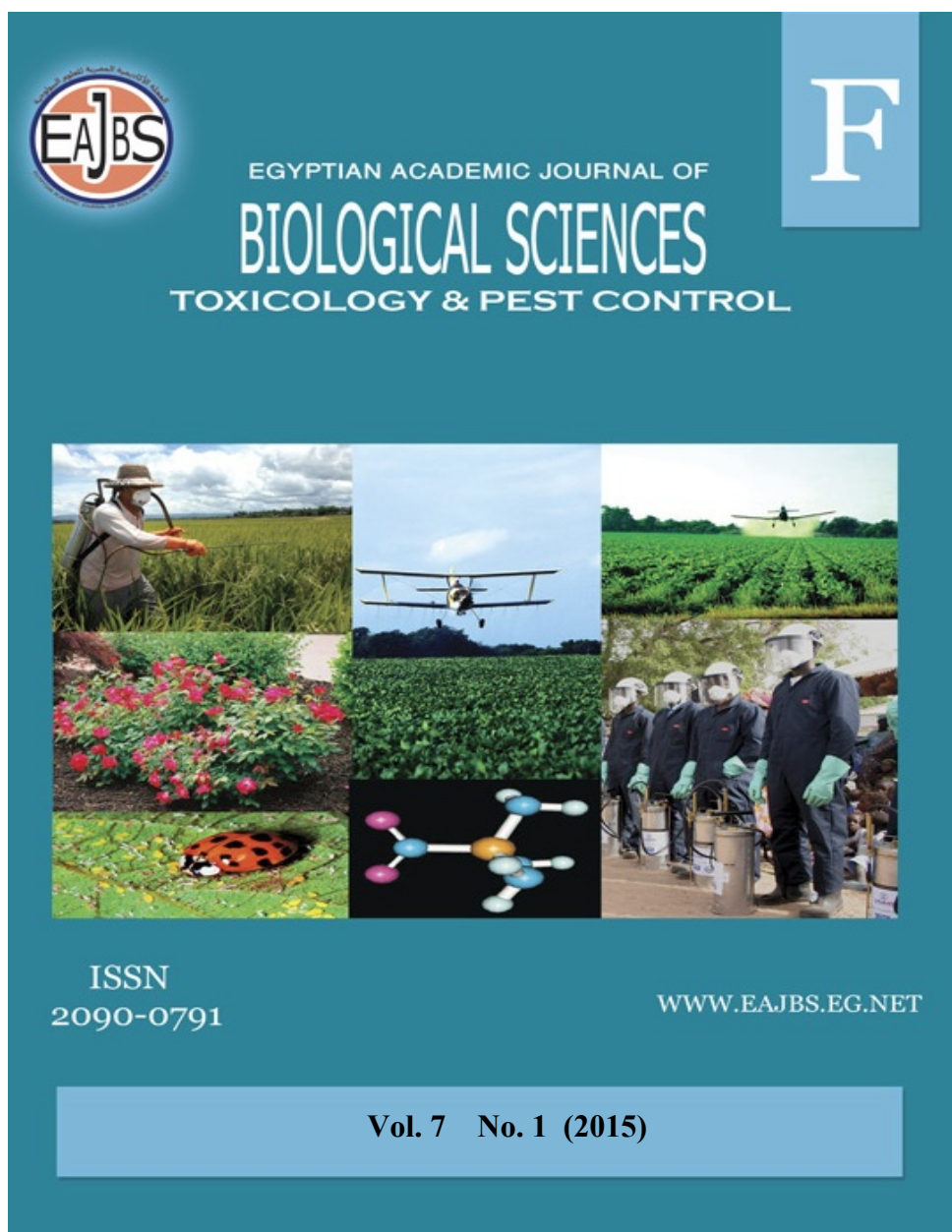


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Using a Biological Control Method For Controlling Red Spider Mite.

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ABSTRACT

The study revealed the pathogenicity of entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) against egg and adult stages of red spider mite *Tetranychus urticae* (Koch). Results showed that *M. anisopliae* was more pathogenic against egg stage than *B. bassiana*, while *B. bassiana* was more pathogenic against adult stage. The results of Scanning Electron Microscope (SEM) described the development cycle of *B. bassiana* on *T. urticae*.

INTRODUCTION

Spider mite is considered the major pest of many crops worldwide in green houses as well as fields (Walter and Proctor 1999). One of the most economically important spider mites is the two-spotted red spider mite *T. urticae*. It was reported infesting over 200 species of economic plants in the field such as cotton (Leigh *et al.* 1968) tomatoes (Rodriguez *et al.* 1972), cucumber (De-Ponti 1980), ornamental plants and fruits (Penman and Chapman 1980). It spreads in temperate zone, subtropical regions and all around the world in green houses. (Tuttle and Baker 1968). The infestation by mites causes great damage to the plants followed by secondary infestation with different pathogens such as virus, bacteria and fungi (Flechmann 1985).

As a result of long term overuse of chemical insecticides for insect control, spider mite showed resurgence and subsequent high level resistance to common pesticides (Ambikadevi and Samarjit 1997).

In recent years, more attention has been paid to find an effective control method of mites that does not involve chemicals to circumvent the problems of acaricides resistance, which should include a fast acting. Entomopathogenic fungi are the most promising microbial control agents against mites which invade them by growing through the cuticle (Chandler *et al.* 2000).

Therefore, the present work was carried out as an attempt to investigate and suggest some alternative agents to be incorporated into integrated control programs to control *Tetranychus urticae* on cucumber plant by determining the pathogenicity of entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff) against egg and adult stages of *T. urticae*.

MATERIAL AND METHODS

Rearing of *T. urticae*

The original colony of the red spider mites *T. urticae* in this study was supplied from Acarology Laboratory in Plant Protection Research Institute, Agriculture Research Center at Dokki. It was reared as a sensitive races test mite for several years at $25 \pm 0.5^\circ\text{C}$ away from any pesticide contamination. *T. urticae* was maintained on detached mulberry leaves with the lower surface upwards placed on moist cotton wool pads in fiber-dishes (20cm in diameter). The cotton pads were moistened daily to avoid disc dryness, and to prevent mite escaping. Mulberry leaves were changed by fresh one from time to time when necessary.

a. Entomopathogenic fungi isolate

Two isolated entomopathogenic fungi were used in this study, *B. bassiana* from *Sesamia cretica* lederer and *M. anisopliae* from soil in 1995 and 2001 respectively, at Giza Governorate by Prof. Dr. Gamal H. Sewify, Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University.

b. Culturing of entomopathogenic fungi

The two tested entomopathogenic fungi *B. bassiana* and *M. anisopliae*, were grown using autoclaved Sabouroud Dextrose Agar Yeast media (SDAY) (10g/L peptone+40g/L Dextrose+ 2g/L yeast extract + 15g/L Agar + 1L. Distilled Water), then incubated at $25 \pm 0.5^\circ\text{C}$ for 10 days. (Uma Devi *et al.* 2005).

The susceptibility of egg and adult female *T. urticae* to the entomopathogenic fungi *B. bassiana* and *M. anisopliae*

The spores of the two incubated entomopathogenic fungi *B. bassiana* and *M. anisopliae* were harvested by rinsing with sterile distilled water of 0.1% TritonX-100, and then filtered through cheesecloth to reduce mycelium

clumping. The spores were counted in the suspension using a heamocytometer (Neubauer improved HBG, Germany $0.100 \text{ mm}^2 \times 0.0025 \text{ mm}^2$). Five concentrations of each isolate were prepared: 10^6 , 5×10^6 , 10^7 , 5×10^7 , and 10^8 spore/ml, as well as the control (Distilled Water of 0.1% TritonX-100).

a. Treatment of eggs

Twenty adult females were placed on a mulberry leaf disks (2.5cm in diameter) and kept on moist cotton wool in fiber dishes with cotton around each disk in circle way to prevent mite escaping. Each dish contained 5 disks as replicates. Adult female were left for 24 hours to deposit egg then adults were removed from the disks and deposited egg were counted. The eggs were sprayed using direct spray technique (Abo-Shabana 1980) by a glass atomizer at 30cm high with 2ml spore suspension for each treatment and 2ml sterilized distilled water of 0.1% TritonX-100 as control.

Eggs were incubated at $25 \pm 0.5^\circ\text{C}$ and the number of hatched and non hatched eggs was counted daily for 9 days of oviposition. The percentage of mortality was determined and corrected by Abbott's formula (1925) as follows:

$$\text{Percentage of mortality} = \frac{\% \text{tested mortality} - \% \text{control mortality}}{100 - \% \text{control mortality}} \times 100$$

LC₅₀, LC₉₀ and slope values were calculated according to Finney (1971), using "Ldp line" software by (Bakr 2000).

b. Treatment of adult females

Twenty fertilized adult female mites were placed on a single leaf-disk of mulberry (2.5cm in diameter) and were kept on moist cotton wool in 5 fiber dishes; each dish contained 5 disks as replicate. The direct spray technique was applied as above (Abo-Shabana 1980). The treated adult females were incubated at $25 \pm 0.5^\circ\text{C}$. Mortality was assessed daily for 9 days Ayoub (1984). The

percentage of mortality was determined and corrected by Abbott's formula (1925). LC_{50} , LC_{90} and slope values were calculated as previous.

Scanning electron microscope

Direct osmium tetroxide vapour fixation was used according to Brey *et al.* (1985). Inoculated mite specimens were transferred to watch glass, which was placed in a closed Petri dish over night. After fixation in OSO_4 vapour, the mites were directly mounted, coated with 15nm of gold and examined by a JEN-JSM-5200 scanning electron microscope (Sewify 1989).

RESULTS AND DISCUSSION

The present results showed the efficiency of the two entomopathogenic fungi *B. bassiana* and *M. anisopliae* against egg and adult stages of *T. urticae* in laboratory experiments.

1. Susceptibility of *T. urticae* egg and adult stages to entomopathogenic fungi *B. bassiana* and *M. anisopliae*.

A. Egg stage

The obtained results in Tables (1,2) showed susceptibility of *T. urticae* eggs to the entomopathogenic fungus *B. bassiana* and *M. anisopliae* after exposing to series of concentrations of 10^6 , 5×10^6 , 10^7 , 5×10^7 and 10^8 spores/ml. The hatchability gradually, decreased along with increasing spore concentration. The lowest concentration (10^6 spores/ml) revealed 93.7 % and 78.2 % hatchability respectively, 9 days after treatment. While hatching at highest concentration (10^8 spores/ml), the hatching decreased to reached 79.86 % and 61.8 % respectively, compared with the control which reached to 94.24 % and 95.8 % respectively.

Table 1: Effect of entomopathogenic fungus *B. bassiana* treatment on egg hatchability of *T. urticae* at different concentrations

Con	Hatchability percentage at indicated days								
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th
0	0	0	3.88	60.16	78.24	86.96	91.25	94.24	94.24
10^6	0	0	2.96	51.70	72.95	84.80	90.57	92.95	93.70
5×10^6	0	0	2.48	51.40	71.23	82.58	86.44	89.62	89.95
10^7	0	0	2.21	51.06	69.52	77.64	82.49	84.38	84.38
5×10^7	0	0	2.09	47.13	68.86	76.41	80.38	81.48	81.48
10^8	0	0	1.88	44.12	66.38	75.85	79.14	79.86	79.86

Table 2: Effect of entomopathogenic fungus *M. anisopliae* treatment on egg hatchability of *T. urticae* at different concentrations.

Con	Hatchability percentage at indicated days								
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th
0	0	0	2.71	48.85	70.72	83.88	92.52	95.80	95.80
10^6	0	0	1.72	44.51	66.67	73.09	76.44	78.22	78.22
5×10^6	0	0	1.51	42.71	62.23	68.47	73.34	75.21	75.21
10^7	0	0	1.46	41.74	55.48	60.69	63.75	64.61	64.61
5×10^7	0	0	1.39	41.09	50.96	56.32	59.21	60.18	60.56
10^8	0	0	1.03	36.13	47.06	54.61	58.32	61.89	61.89

Results in Table (3), and Figure (1) proved that *M. anisopliae* was more effective against *T. urticae* eggs compared with *B. bassiana*. The LC_{50}

value of *M. anisopliae* was 1.05×10^9 spores/ml while *B. bassiana* revealed greater LC_{50} value of 4.77×10^9 spores/ml.

Table 3: Comparison of pathogenicity between *B. bassiana* and *M. anisopliae* against *T. urticae* egg stage.

Line name	LC ₅₀ (Limits)	Significance	Index	Slope	LC ₉₀
<i>M. anisopliae</i>	1.05 x10 ⁹ (1.84 x10 ⁸ - 3.83 x10 ¹¹)	a	100	0.29	2.50 x10 ¹³
<i>B. bassiana</i>	4.77 x10 ⁹ (9.06 x10 ⁸ - 3.23 x10 ¹¹)	a	21.96	0.56	9.47 x10 ¹¹

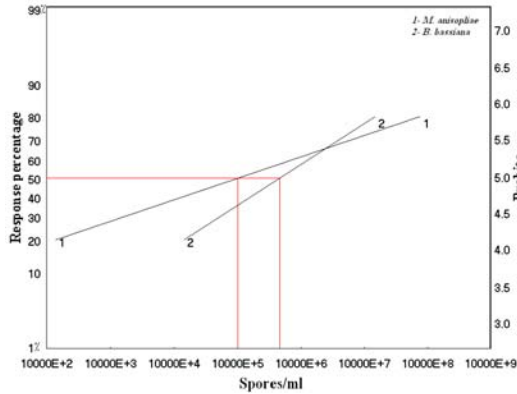


Fig. 1: Percentage Mortality regression lines of *B. bassiana* and *M. anisopliae* against *T. urticae* egg stage.

B. Adult stage

The susceptibility of adult females of *T. urticae* to entomopathogenic fungi *B. bassiana* was conducted. The percentage of mortality, LC₅₀, LC₉₀, LT₅₀ and LT₉₀ values were tabulated with their corresponding slopes for 6 days after treatment in Table (4).

The percentage of mortality gradually increased along with both spore concentrations and time elapse. The Lowest concentration of 10⁶ spores/ml revealed 34.5, 51.8, 61.0, 69.1, 75.9 and 87.4% when mortality was assessed after 1, 2, 3, 4, 5 and 6 days, respectively.

Table 4: Percentage mortality of *T. urticae* adult females treated with series concentrations of *B. bassiana* after given days.

Applied Con.	Days after treatment						LT ₅₀ (Days)	LT ₉₀ (Days)	slope
	1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day			
0	10.00	20.00	32.00	45.00	57.00	74.00	-	-	-
10 ⁶	34.50	51.80	61.00	69.10	75.90	87.40	6.15 (4.21 - 17.36)	422.98	0.70
5 x 10 ⁶	36.20	52.90	62.20	70.40	79.70	87.30	4.77 (3.47 - 9.19)	243.24	0.75
10 ⁷	42.30	60.30	70.60	74.50	80.90	91.10	2.41 (1.58 - 3.23)	95.22	0.80
5 x 10 ⁷	50.10	64.70	72.30	78.00	84.50	92.20	1.47 (0.60 - 2.11)	80.03	0.74
10 ⁸	60.40	72.70	77.20	82.60	88.00	93.30	0.51 (0.01 - 1.11)	79.99	0.58
LC ₅₀	7.59 x 10 ⁷	1.08 x 10 ⁷	5.61 x 10 ⁶	5.19 x 10 ⁶	3.16 x 10 ⁶	1.22 x 10 ⁶			
(Limits)	(3.40x10 ⁷ – 3.72x10 ⁸)	(4.25x10 ⁶ – 2.55x10 ⁷)	(1.42x10 ⁶ – 1.30x10 ⁷)	(1.30x10 ⁶ – 1.19x10 ⁷)	(7.25x10 ⁵ – 6.93x10 ⁶)	(1.16x10 ⁵ – 3.28x10 ⁶)			
LC ₉₀	1.99 x 10 ¹¹	9.10 x 10 ¹⁰	9.27 x 10 ¹⁰	7.12 x 10 ¹⁰	1.73 x 10 ¹⁰	1.00 x 10 ¹⁰			
slope	0.38	0.33	0.30	0.31	0.34	0.33			

While the highest concentration of 10⁸ spores/ml revealed 60.4, 72.7, 77.2, 82.6, 88.0 and 93.3% when mortality was assessed after the same consecutive days, respectively. The obtained results in

Figure (2) showed the mortality time regression line at concentration of 10⁸ spores/ml. The data showed that *B. bassiana* caused high mortality in shortest time, LT₅₀ value was 0.51 days.

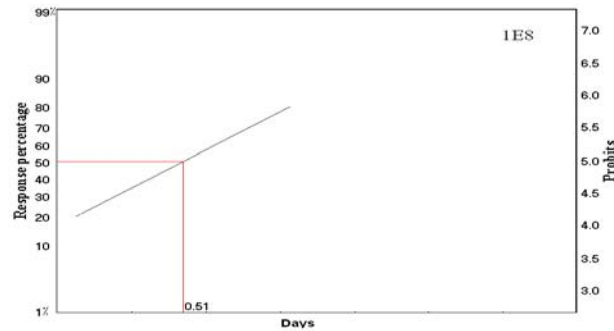


Fig. 2: Mortality-time regression line of *B. bassiana* at concentration of 10^8 spores/ml against *T. urticae* adult females.

Symptoms in Photo (1C) showed sporulating of *B. bassiana* on cadavers of adult stage of *T. urticae* which prove the high susceptibility to the tested entomopathogenic fungi.

The susceptibility of adult females of *T. urticae* to entomopathogenic fungi *M. anisopliae* was examined. The percentage of mortality, LC_{50} , LC_{90} , LT_{50} and LT_{90} values were tabulated with their corresponding slopes for 6 days after treatment in Table (5). The percentage of

mortality gradually increased along with both spores concentrations and time elapse, The Lowest concentration 10^6 spores/ml revealed 7.0, 16.8, 28.6, 39.5, 52.9 and 66.5% percentage of mortality. When mortality was assessed after 1, 2, 3, 4, 5 and 6 days, respectively. While the highest concentration (10^8 spores/ml) revealed 14.4%, 26.7%, 46.7%, 66.0%, 78.5% and 87.0% when mortality was assessed after 1, 2, 3, 4, 5 and 6 days, respectively.

Table 5: Percentage mortality of *T. urticae* adult females treated with series concentrations of *M. anisopliae* after given days

Applied con.	DAYS AFTER TREATMENT						LT_{50} (Days)	LT_{90} (Days)	slope
	1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day			
0	5.2	12.3	23.7	33.9	46.1	59.3	-	-	-
10^6	7.0	16.8	28.6	39.5	52.9	66.5	29.98 (14.77 - 223.61)	230.52	1.45
5×10^6	8.2	17.3	30.4	47.9	61.3	70.6	11.22 (8.36 - 19.34)	50.23	1.97
10^7	9.0	23.0	36.0	49.0	62.0	76.0	9.07 (7.11 - 13.79)	43.53	1.88
5×10^7	12.8	25.5	40.1	55.5	68.5	81.2	6.31 (5.34 - 8.08)	27.96	1.98
10^8	14.4	26.7	46.7	66.0	78.5	87.0	4.18 (3.78 - 4.70)	13.55	2.51
LC_{50}	1.43×10^{11}	4.70×10^{10}	1.33×10^9	1.62×10^8	6.10×10^7	2.68×10^7			
Limits	(3.58×10^9 - 7.68×10^{19})	(2.10×10^9 - 1.87×10^{16})	(3.53×10^8 - 2.57×10^{10})	(8.27×10^7 - 4.98×10^8)	(3.72×10^7 - 1.26×10^8)	(1.81×10^7 - 4.33×10^7)			
LC_{90}	1.83×10^{14}	1.96×10^{14}	4.68×10^{11}	2.22×10^{10}	6.78×10^9	2.02×10^9			
slope	0.41	0.35	0.50	0.60	0.63	0.68			

The obtained results in Figure (3) showed the mortality time regression line at concentration of 10^8 spores/ml. the result showed that LT_{50} value of *M.*

anisopliae was 4.18 days. Symptoms in Photo (2) and (3) showed *M. anisopliae* conidial covered the cadavers of adult stage of *T. urticae*.

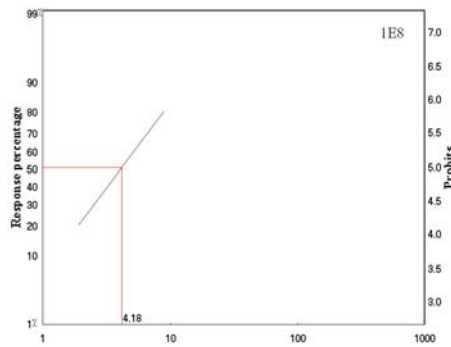


Fig. 3: Mortality-time regression line of *M. anisopliae* at concentration of 10^8 spores/ml against *T. urticae* adult females.

The obtained results in Table (6), Fig (4) proved that adult female of *T. urticae* was high susceptible to both isolates of entomopathogenic fungi. It is also proved that *B. bassiana* was more effective than *M. anisopliae*, against *T.*

urticae adult stage. The LC_{50} value of *B. bassiana* was 1.22×10^6 spores/ml 6 days after treatment while *M. anisopliae* revealed greater LC_{50} value of 2.68×10^7 spores/ml.

Table 6: Comparison pathogenicity between *B. bassiana* and *M. anisopliae* against *T. urticae* adult females.

Line name	LC_{50} Limits	Significance	Index	Slope	LC_{90}
<i>B. bassiana</i>	1.22×10^6 ($1.16 \times 10^5 - 3.28 \times 10^6$)	A	100	0.33	10^{10}
<i>M. anisopliae</i>	2.68×10^7 ($1.81 \times 10^7 - 4.33 \times 10^7$)	B	4.54	0.68	2.02×10^9

Index compared with *B. bassiana*

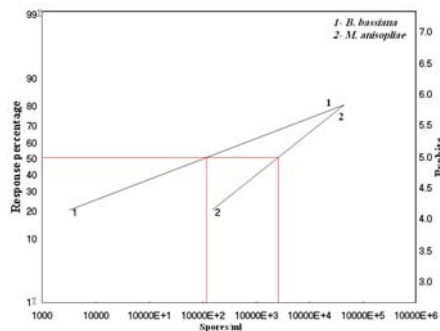


Fig. 4: Percentage mortality regression lines of *B. bassiana* and *M. anisopliae* against *T. urticae* adult females 6 days after treatment.

These findings were agreed with Yousri (1994) who studied the effect of series of concentrations of *B. bassiana* from 1.26×10^6 to 1.26×10^9 spores/ml on the adult female of *T. urticae* at 23.4°C and approximately 100% R.H. The mortality increased along with increasing spores concentrations. Also Yassin (1997) reported that *B. bassiana* had toxic effect on *T. urticae* three days

after treatment. The effect was due to the toxin secretion of the fungus, whereas after five days might be due to the action of fungal hyphae which destroyed the internal tissues of the mite. He also stated that the effect increased accumulatively with concentration, while Tamai *et al.* (1998) examined the pathogenicity of *B. bassiana* against *T. urticae* at $25 \pm 2^\circ\text{C}$, $70 \pm 5\%$ R.H and 12 h photo phase,

using newly emerged *T. urticae* individuals treated with different concentrations 5×10^6 , 10^7 , 5×10^7 , 10^8 and 10^9 conidia/ml. Progressively higher values of accumulated mortality were observed with increasing concentration of conidial spores. At all concentration, corrected mortality was <50% on the sixth day. Total mortality > 50% was observed only at the concentration of 10^9 conidia/ml.

These results indicated by, Pena *et al.* (1996) who tested the pathogenicity of *B. bassiana* against the broad mite, *Pseudotarsonimus latus* in the laboratory under controlled temperature and moisture condition. Results showed that infection started in the 2nd day and reached a peak on the 6th day. LC₅₀ was 1.16×10^6 conidia /ml and percentage of mortality reached 88% after 6 days of treatment. Similarly Barreto *et al.* (2004), When applying *B. bassiana* against *T. urticae* infesting cowpea, (*Vigna unguiculata*), the obtained results revealed that fungus reduced the population growth of mite by 77.59% mortality. Also Simova and Dragnova (2003) found that *B. bassiana* were the most pathogenic isolates and LT₅₀ varied from 1.3 to 1.4 days. EL-Safty (2003) studied the effect of *M. anisopliae* as biological control agent to control the two-spotted spider mite *T. urticae*, (egg, immature stages and adult) under laboratory conditions. Results indicated that fungal suspension 3.6×10^{11} spores/ml. gave high effect against immature stages and adult females than different ages of eggs; also 3-days old eggs were more susceptible than 1 and 2 days old eggs, respectively.

2. Scanning Electron Microscope:

The objective of this work was to describe the development cycle of *B. bassiana* on *T. urticae* using Scanning Electron Microscope (SEM). Conidia of *B. bassiana* adhered to the mite integument within 24 hours after inoculation Photo (4 A). Initiation of

conidial germination of *B. bassiana* was happened between 24 and 48 hours after inoculation Photo (4B), Photo (5) the different phases of fungal infection were noticed after fungal inoculation. Photo (5A) *B. bassiana* formed germ-tubes and penetration structures between 24-72 hours after inoculation fungal.

A thickening of the extremity of the germ-tube, characterizing the formation of appressoria, was observed during penetration by the fungus Photo (5B). Fungal sporelation was noticed 120 hours after treatment. Conidiogenous cells are densely clustered, colour less, with flask-like base extending into denticulate rachis. Conidia is nearly globose carried singly on each denticle Photo (5 C, D). Mycelial extrusion from the cadavers happened between 96 and 120 hours after inoculation, mainly in the intersegmental areas and later in the areas with stronger cuticle, inducing complete cuticle degradation, Photo (5 E, F).

The data from SEM proved the previous results, which showed the highly susceptibility of *T. urticae* to the entomopathogenic fungi *B. bassiana*. Whereas the process divided into ascending steps, adhesion of conidia to the cuticle, conidia germination, formation of appressoria and penetration through the cuticle. Conidia were adhered and germination started on the mite surface forming appressoria after 24 – 48 hours post-infection. The phase of host colonization occurred between 72 and 120 hours, and the most of the mites died between 72 and 96 hours after inoculation. The whole mite body was covered by *B. bassiana* conidia 120 hours after inoculation. In similar study with *Cornitermes cumulans* carried out by Neves & Alves, (2000) and with termite *Heterotermes tenuis*, by Menno *et al.* (2001) greater amounts of mycelia extrusion points and conidiogenesis were observed in both insect legs and head, and also in the membranous area of the labrum. Alves *et al.* (2002) were the first

to report that *B. bassiana* is pathogen for citrus rust mites *Phyllocopetrotia Oleivora* conidia were found to adhere all over the mite body surface, especially at the anal region, where vegetative mycelium was found entering the mite body. The formation of small crystals was noticed inside the mite's bodies that were produced during colonization of the body cavity by the fungus.

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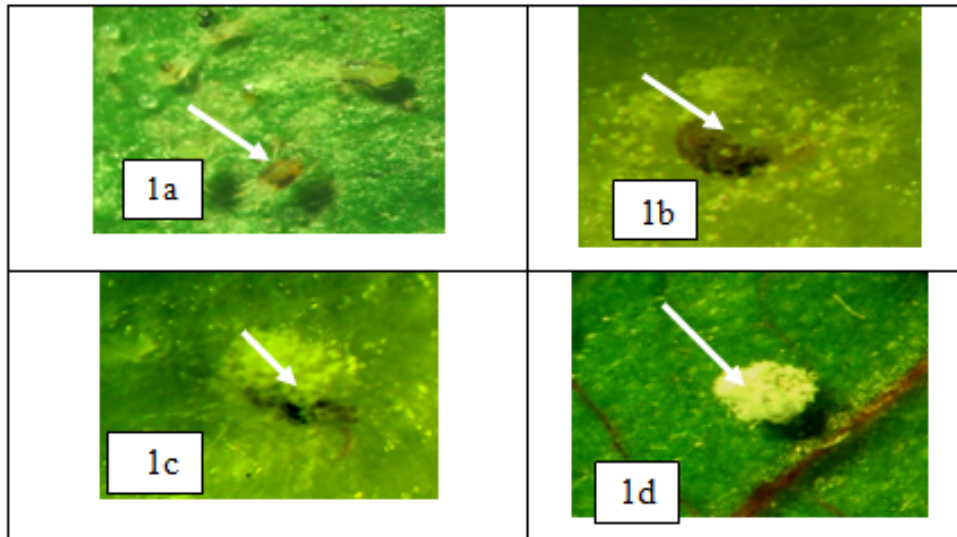


Photo 1: (a) Healthy *T. urticae* adult (b) Freshly killed *T. urticae* with fungus *B. bassiana* (c) Sporulating of *B. bassiana* on Tetranychus cadavers (d) *T. urticae* cadavers covered by a dense white mycelial growth of fungus *B. bassiana*.

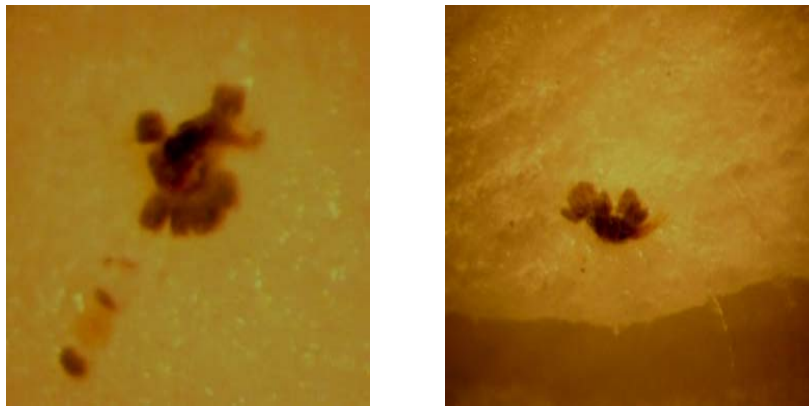


Photo 2: Light micrograph showing conidial columns of *M. anisopliae* covering *T. urticae* cadavers x 400.

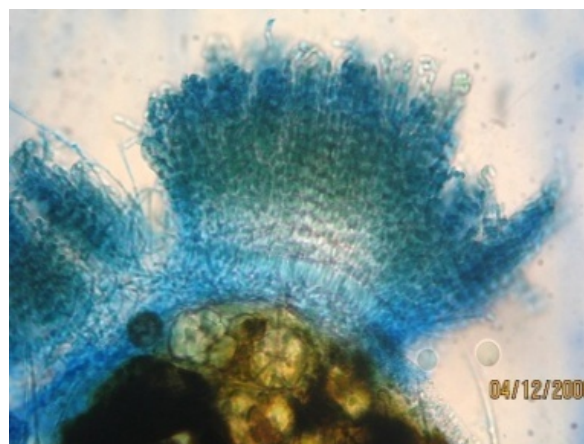


Photo 3: Light micrograph showing fungus *M. anisopliae* branched conidiophores, thickened, blunt tips of conidiogenous cells and laterally adherent conidial chains on *T. urticae* cadavers x400.

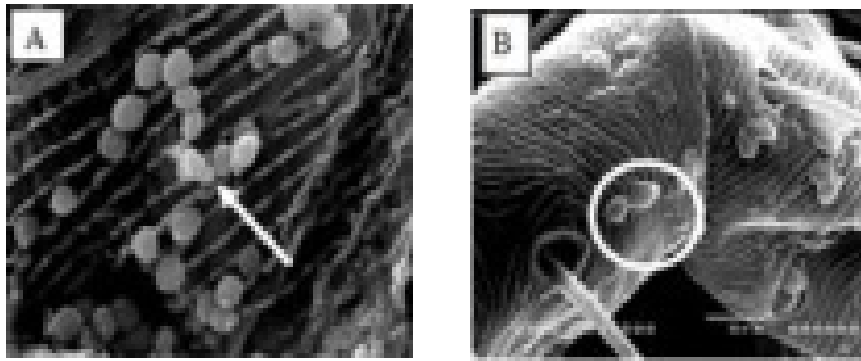


Photo 4: Scanning electron micrograph of the development of *B. bassiana*. (A) Conidiospore adhered to the integument, 24h after inoculation (X 2000) (B) Germinated conidia of *B. bassiana* with extends germ-tubes, 48h after inoculation (X 3500).

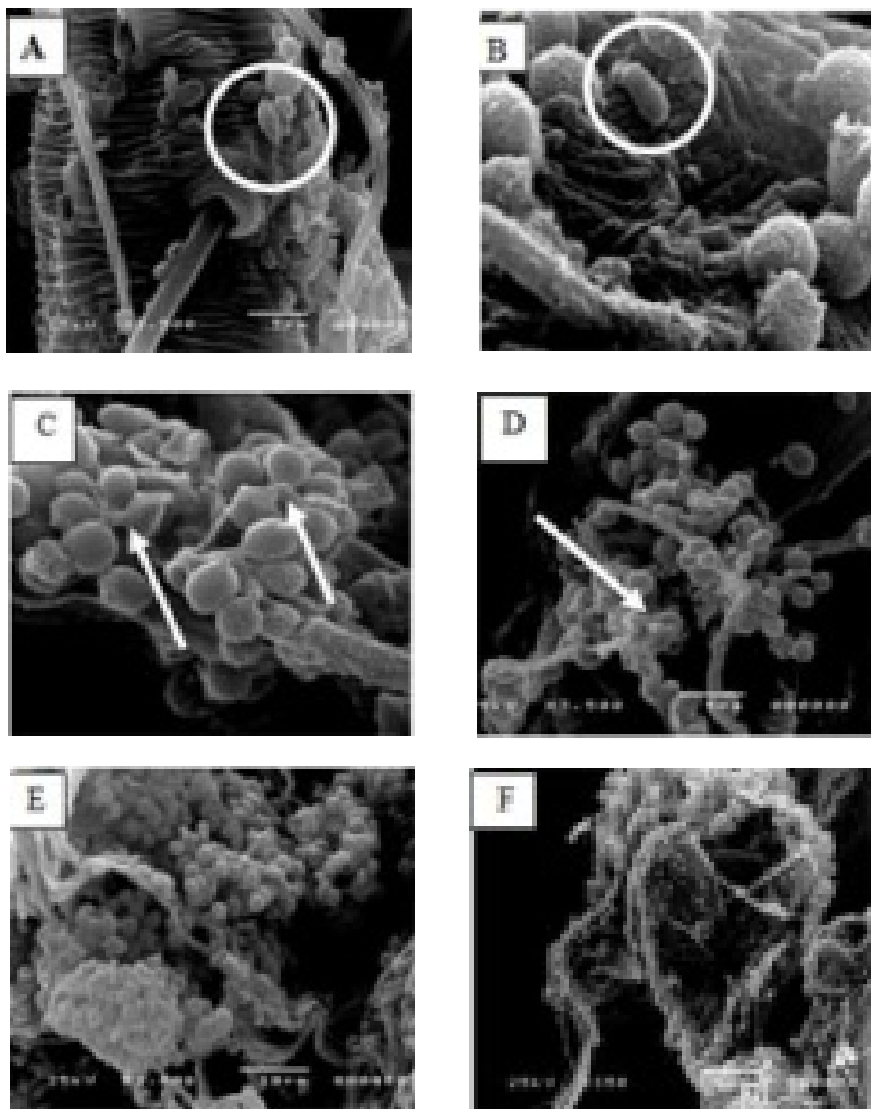


Photo 5: (A-B) Germinated conidia produced appressoria, 72h after inoculation (X3500, X7500, respectively). (C) Conidiogenous cells with globosely bases and extended denticulate rachis, 72h after inoculation (X 3500). (D) Conidia carried singly on conidiogenous cells with flask-like base extending into denticulate rachis, 72h after inoculation (X 3500). (E) Spores balls representing dense clusters of large numbers of conidiogenous cells and conidia, 120h after inoculation (X 2000). (F) Forming a dense white covering on host exoskeleton, occasionally synnematosus (forming erect fascicles of hyphae), 120h after inoculation (X 350).

ARABIC SUMMERY

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تم إجراء مجموعة من التجارب المعملية لتقييم فاعلية استخدام بعض الفطريات الممرضة للحشرات العنكبوت الأحمر (*Tetranychus urticae* (Koch) و *Metarhizium anisopliae* (Metschnikoff) و *Beauveria bassiana* (Balsamo) لمكافحة أظهرت النتائج أن الفطر *M. anisopliae* كان أكثر مرضية على طور البيضة من الفطر *B. bassiana*، بينما كانت النتائج مختلفة في الإناث البالغة للعنكبوت الأحمر حيث كان الفطر *B. bassiana* أكثر مرضية من *M. anisopliae*. الفطر *B. bassiana* كان له تأثير فعال في مقاومة الأطوار المتحركة للعنكبوت الأحمر وكانت نسبة الخفض % 86.3 .