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Virulent Entomopathogenic Fungi against The Two-Spotted Spider Mite *Tetranychus urticae* and some Associated Predator Mites as Non Target Organisms

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ABSTRACT

   Entomopathogenic fungi and predatory mites can independently contribute to control the two-spotted spider mite *Tetranychus urticae* Koch. It is important to assess the risk of possible fungal infections in predators when a combination of them are being considered as a tandem control strategy for *T. urticae* in IPM program. The first part of this study tested 4 *Beauveria bassiana* isolates and 2 *Metarhizium anisopliae* for virulence against *T. urticae*, egg and adult stages. Strains B4 was found to be the most potent toward egg and adult stages, causing 88.5% mortality for the adult stage at a concentration of 10^8 spores/ml. and the LC_{50} was 6.61×10^6. When applied on the egg stage the hatchability was 25.2% compared with the control which reached 99% and the LC_{50} was 1.14×10^7. The second part evaluated the pathogenicity of the most effective isolates B4, three concentrations were applied LC_{25}, LC_{50} and LC_{90} against the adult of the two predator mites *Phytoseiulus persimilis* and *Neoseiulus californicus*. The bioassay results indicated that the isolate B4 was harmless against *P. persimilis* and slightly harmful against *N. californicus*.

   No viable fungal hyphae were found on predator cadavers. Observations with scanning electron microscopy revealed that conidia were attached to the cuticle of predatory mites within 24 h after spraying with strain B4, and had germinated within 24–48 h. After 48 h, conidia had gradually been shed from the mites, after none of the conidia had penetrated the cuticular surfaces. In contrast, the germinated conidia successfully penetrated the cuticle of *T. urticae*, and within 72 h the fungus colonized the mite's body. Our study demonstrated that although several *B. bassiana* strains displayed a high virulence in *T. urticae* there was no evident pathogenicity to phytoseiid mites. These findings support the potential use of entomopathogenic fungus in combination with predatory mites in *T. urticae* control programs.

INTRODUCTION

The two-spotted spider mites (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), are major agricultural pests, which often cause severe damage to a variety of crops (Gotoh *et al.*, 2004). Spider mites become serious pests in a wide range of protected crops across the world. It is one of the pests responsible for yield losses of many horticultural, ornamental and agronomic crops, causing considerable crop damage and economic loss (Puinean *et al.*, 2010). A major problem in the control of *T. urticae* is its ability to develop rapid resistance to many acaricides. Among arthropods, it has the highest incidence of pesticide resistance (van Leeuwen *et al.*, 2010). Another explanation for this increase in mites populations that widespread insecticide usage has eliminated many of the mites' natural enemies, resulting in a reduction in predation pressure on the mite (Choi *et al.*, 2004; Prischmann *et al.*, 2005).

Consequently, using biological control agents such as entomopathogenic fungi and predatory mites has been recommended to control insect pests in integrated pest management programs (IPM), because their use is considered to be more environmentally friendly (Jacobson *et al.*, 2001; Zhang, 2003; Maniania *et al.*, 2008).

Microbial control of pests is an important approach to reduce the dependence on chemical pesticides for increased agricultural sustainability. *Beauveria bassiana* has been tested in the laboratory and applied in the field to control numerous insect pest species (Legaspi *et al.*, 2000) and has shown potential as an effective agent in controlling pest mite species (Maniania *et al.*, 2008; Geroh *et al.*, 2015).

Predatory mites are often used as an alternative to conventional pest management on a variety of plants (Gerson and Weintraub, 2007). The predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and *Neoseiulus californicus* were used in integrated pest management programs for *T. urticae* suppression (Cote, 2001; Skirvin and Fenlon, 2001; Fitzgerala and Easter, 2003).

In order to increase efficiency in controlling *T. urticae*, it has been suggested that using predatory mites accompanied by applications of *B. bassiana* may be an alternative to traditional *T. urticae* management (Chandler *et al.*, 2005). Considering that there are a lot of insect and mite species that are susceptible to *B. bassiana*, there is a risk that the fungus may be harmful to predatory mites (Jacobson *et al.*, 2001). Therefore, there is a must to evaluating the compatibility of *B. bassiana* and predatory mites to the success of potential IPM programs designed to control *T. urticae*. Several studies have evaluated the effects of pathogens on predators by investigating the predator mortality after exposure to spray residues (Flexner *et al.*, 1986). Previous studies on the interaction between fungal pathogens and predatory mites have focused on the fungal infectivity to predators (Donka *et al.*, 2008; Vergel *et al.*, 2011), or on the sub lethal effects of ingesting pathogen treated prey on predators (Seiedy *et al.*, 2012a; Wu *et al.*, 2015).

The aim of this study is to evaluate the virulence of four *B. bassiana* and two *Metarhizium anisopliae* (Metschnikoff) isolates against egg and adult stages of *T. urticae*. The highly virulent isolate of entomopathogenic fungi on *T. urticae* was then tested against the adult stage of two predators mites *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and *Neoseiulus californicus*; mortality and fecundity of the predatory mites were tested. Also by using scanning electron microscopy (SEM), a comparison study take place between the infection of *T. urticae* and the predator mites by entomopathogenic fungi.

MATERIALS AND METHODS

Rearing of *Tetranychus urticae* (Koch):

The original colony of the red spider mites *T. urticae* in this study was supplied from Acarology Laboratory in Plant Protection Research Institute, A.R.C at Dokki. It was reared as a test mite for several generations at 25 ± 1ºC and 70± 5 R.H. 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 escape. Mulberry leaves were changed by fresh one from time to time when necessary (Hassan, 2008; Sewify *et al*., 2015).

To obtain adult *T. urticae* of uniform age, 25 adult females were taken from the mite culture and put on leaf discs placed on wet cotton in Petri dishes (20cm in diameter), and allowed to lay eggs for 24h. After which the females were removed and the eggs remained till adult (Seiedy *et al*., 2012).

Rearing of the predaceous mites:

The predaceous mites, *Phytoselies persimilis* and *Neselious californicus* were obtained from rearing lab, Kaha Research Station, Agriculture Research Center. The predatory mites were maintained at 25± 1°C and 70 ± 5% R.H with a photoperiod of 16:8h (L:D) and away from any pesticide contamination. The predatory mites were 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 escape. The colony of the predator was fed on a mixture of various stages of *T. urticae* day-to-day. Also Mulberry leaves were changed by fresh one from time to time when necessary (Nadimi *et al.,* 2008; Seiedy *et al.,* 2012).

To obtain females of the same age, about five gravid females from the stock colony were transferred to *T. urticae* infested mulberry leaf in each of a series of Petri dishes. The females were allowed to lay eggs for 24h and then removed. Newly emerged predators were of the same age (Seiedy *et al*., 2012).

Fungal culture and preparation of conidial suspension:

Six isolates of entomopathogenic fungi were used in this study, four isolates of *Beauveria bassiana* (Balsamo) (B1- B2- B3- B4) and two isolates of *Metarhizium anisopliae* (Metschnikoff) (M1-M2). They were obtained from the soil. Virulence of all isolates against *T. urticae* was studied. All the six tested entomopathogenic fungi were cultured on autoclaved sabouraud dextrose agar with yeast extract (SDAY) media (4 % Dextrose , 0.1 % peptone + 1.5 % Agar + 0.2 % yeast extract dissolved in 1L of Distilled Water). The media were then incubated at 25 ± 1°C, 60 –70 % RH and in darkness for 10–14 days to obtain conidia (Uma Devi *et al.*, 2005; Sewify *et al*., 2015).

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

Spores of the six incubated entomopathogenic fungi isolates were harvested from the culture medium by rinsing with sterile distilled water containing 0.01% Tween 80, then scrubbing the surface with a glass bar, and filtered the conidia suspension through sterile layer of cheesecloth to remove conidial clumps and mycelial debris. The spores were counted in the suspension using a heamocytometer (Neubauer improved HBG, Germany 0.100 mm^2 x 0.0025 mm^2) under a phase-contrast microscope. 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 0.01%

Tween 80) and were used on the same day after preparation and shaken before use (Hassan, 2008; Sewify *et al.*, 2015).

Treatment of eggs:

Ten vigorous adult females taken from the population were transferred to 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 allowed to lay eggs freely 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; Hassan, 2008; Sewify *et al.*, 2015) by a glass atomizer at 30cm high with 2ml spore suspension for each treatment and 2ml sterilized distilled water of 0.01% Tween 80 as control.

Eggs were incubated at 25 ± 1°C to favor conidial germination, and the number of hatched and non-hatched eggs were daily examined for 7 days of oviposition. The unhatched eggs were transferred to moist Petri dishes for observing fungal out growths. Final egg mortalities in all treatments were computed based on the last-day counts of the hatched and non-hatched eggs. The percentage of mortality was determined and corrected by Abbott's formula (1925) as follows:

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

LC$_{50}$, LC$_{90}$ and slope values were calculated according to Finney (1971), using "Ldp line" software by (Bakr, 2000).

Treatment of adult females:

Ten fertilized adult female of *T. urticae* were placed on a single leaf-disk of mulberry (2.5cm in diameter) and were kept on moist cotton wool in fiber dishes; each dish contained 5 disks as replicates. The direct spray technique was applied directly to the body surface of the mites according to Abo-Shabana (1980) and Sewify *et al.* (2015).

The treated adult females were incubated at 25 ± 1°C. Mortality was assessed daily for 7 days (Ayoub, 1984). Cadavers were transferred into moist Petri dishes and considered as "mycotised" if fungal out growths were visible after three days of incubation at same laboratory conditions. The percentage of mortality was determined and corrected by Abbott's formula (1925). LC$_{50}$, LC$_{90}$ and slope values were calculated according to Finney (1971), using "Ldp line" software by (Bakr, 2000).

The susceptibility of predaceous mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* to the selective strain of entomopathogenic fungi *B. bassiana*

This part of the investigation was undertaken to evaluate the side effect of using the selected entomopathogenic fungus *B. bassiana* (B4) on mortality and fecundity of the predatory mites, *P. persimilis* and *N. californicus*. The prey used in the present study was adults of *T. urticae*.

Two factors were our reason to choose adults' stage, because of their relatively big size, disease diagnosis in adults of *T. urticae* is easier in contrast with the other stages. Also, the adults of two predators aggressively attack to adults of *T. urticae*. (Cote, 2001; Seiedy *et al.*, 2014).

Experimental Procedures:

The most effective isolate of the previous tested entomopathogenic fungi was *B. bassiana* (B4). Three concentrations of the fungus were tested (LC_{25}, LC_{50} and LC_{90}) against the adult stage of both predaceous mites *P. persimilis* and *N. californicus*. By using direct spray technique (Abo-Shabana, 1980; Hassan, 2008; Sewify *et al.*, 2015). A glass atomizer at 30cm high with 2ml spore suspension for each treatment and 2ml sterilized distilled water of 0.01% Tween 80 as control. The sprayed predators were transferred by the aid of fine brush to small circular leaf disc (2 inch in diameter) punched from mulberry leaves were placed in Petri dishes (12 cm diameter) lined with water saturated cotton wool. Ten dishes for each concentration, each dish contains two adult females of the tested predator as a replicate.

After spraying the treatments were kept under constant conditions of 25 ± 1℃ and 70 ± 5 % relative humidity with a photoperiod of 16:8 (L:D). The predators were fed on a mixture of various stages of uninfected *T. urticae* each day. Mortality of predators recorded daily for 7 days until no more mortality could be observed and reproduction per female during the first 7 days of the adult stage were assessed. All dead and living mites were counted, and dead mites were removed daily. Mites were considered dead when they failed to move after repeated gentle prodding with a brush. Cadavers were transferred into moist Petri dishes and incubated at same laboratory conditions to observe any fungal out growths. Predator eggs were counted and removed daily for 7 days after spraying (Nadimi *et al.,* 2008; Gaber, 2016).

Statistical analysis:

Based on total effects, rating of toxicity of the entomopathogenic fungi was evaluated according to International Organization for Biological Control (IOBC) guideline (Blumel and Hausdorf, 2002).

$Er = Rt/Rc$

Where: Er = Effect on reproduction

Rt = Reproduction in treatment

Rc = Reproduction in control

Subsequently effect on survival and effect on reproduction were combined using the following formula (Overmeer and van Zon, 1982):

$E = 100\% - (100\% - Ma) \times Er$

Where: Ma = Mortality corrected according to Abbott

E = Total effect

Class 1: E < 30% (harmless)

Class 2: 30 < E< 80 (slightly harmful)

Class 3: 80 < E < 99 (moderately harmful)

Class 4: E >99% (harmful)

Decreasing of total eggs/females =

(total eggs in treatment – total eggs in control) / total eggs in control × 100

Selectivity ratio = LC_{50} of the compound against the predator/ LC_{50} of the compound against the spider mite (El- Adawy *et al., 2000*; Gaber, 2016).

Scanning electron microscope:

This work carried out in the electronic microscope unite, central laboratory, National Research Centre. The adults of *T. urticae* and *P. persimilis* were killed by chloroform solvent, cleaning manually and freezing. Freezing of the sample very quickly was instead of fixing it. This technique providing the sample stays cold enough, this 'locks up' the water and prevents it from evaporating inside the microscope. After that the adults were coated by gold. Coating of samples with gold

is required in the field of electron microscopy to enable or improve the imaging of samples. Creating a conductive layer of metal on the sample inhibits charging, reduces thermal damage and improves the secondary electron signal required for topographic examination in the SEM. All images were taken under low vacuum scanning electron microscope. (Jeol-JSM-5600LV in SEM). Ragaei and Sabry (2017)

RESULTS AND DISCUSSION

The present results show the efficiency of six entomopathogenic fungi four isolates of *Beauveria bassiana* (Balsamo) (B1-B2-B3-B4) and two isolates of *Metarhizium anisopliae* (Metschnikoff) (M1-M2), against egg and adult stages of *T. urticae* in laboratory experiments.

Also the effect of using the selected isolates of entomopathogenic fungus *B. bassiana* (B4) on mortality and fecundity of the predatory mites, *P. persimilis* and *N. californicus* were tested. A SEM was used to observe and document the micro morphological processes that occurred due to fungal conidial inoculation in each of the *T. urticae* and the predatory mite species.

Susceptibility of *T. urticae* egg and adult stages to entomopathogenic fungi
B. bassiana and _M. anisopliae_

Egg stage:

The obtained results in Table (1) showed susceptibility of *T. urticae* eggs to the six isolates of 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 (93.29%, 81.67%, 85.93%, 80.20 %, 92.2 % and 82.5 %) for (B1, B2, B3, B4, M1 and M2) respectively, 7 days after treatment. While hatching at highest concentration (10^8 spores/ml), decreased to reach (68.07%, 33.9 %, 56.66%, 25.20%, 63.3% and 46.2%) for (B1, B2, B3, B4, M1 and M2) respectively, 7 days after treatment. Compared with the control which reached to 97.34, 96.58, 97.78, 99.08, 96.2 and 96.4 respectively.

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

	Hatchability percentage of all tested isolates at 7th day					
	B1	B2	B3	B4	M1	M2
	7th day	7th day	7th day	7th day	7th day	7th day
con	H%	H%	H%	H%	H%	H%
0	97.34	96.58	97.78	99.08	96.2	96.4
10^6	93.29	81.67	85.93	80.2	92.2	82.5
5×10^6	92.36	72.31	83.79	68.18	90.1	75.8
10^7	87.26	58.75	71.94	36.38	85.2	62.2
5×10^7	80.99	46.3	68.85	33.5	74.1	57.2
10^8	68.07	33.9	56.66	25.2	63.3	46.2

Results in Table (2), Fig (1) proved that *B. bassiana* (B4) was more effective against *T. urticae* eggs compared with all other isolates. The LC_{50} value of B4 was 1.14×10^7 spores/ml while (B2, M2, B3, M1 and B1) revealed greater LC_{50} value (3.33×10^7, 9.33×10^7, 3.82×10^8, 4.57×10^8 and 9.82×10^8 spores/ml) respectively.

Table (2): Comparison of the pathogenicity among six isolates of *entomopathogenic fungi B. bassiana and M. anisopliae* against *T. urticae* egg stage.

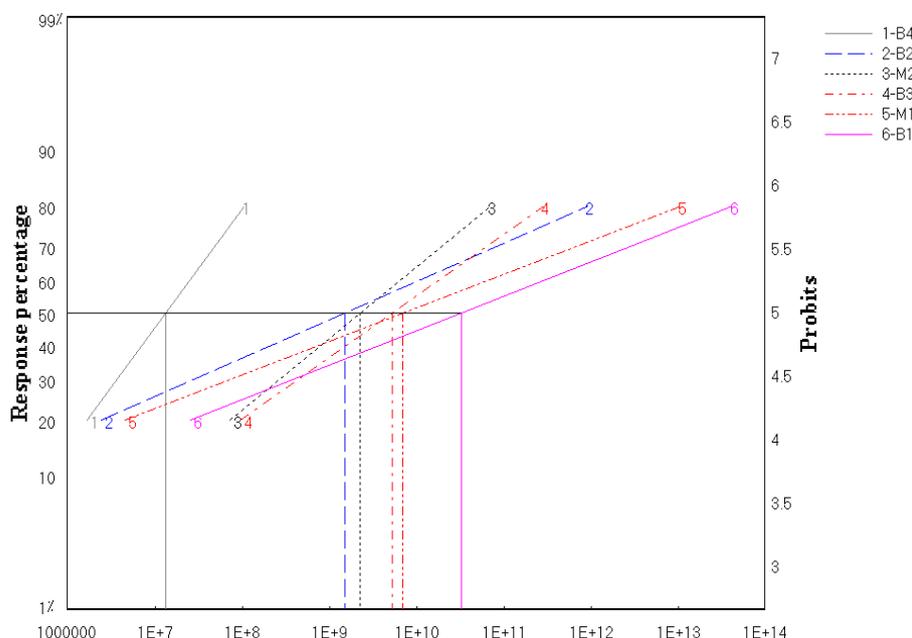| No.	Line	LC_{50} (Limits)	Slope	LC_{90}
1	B4	1.14×10^7 $(4.33 \times 10^6 - 5.67 \times 10^8)$	0.77	5.24×10^8
2	B2	3.33×10^7 $(2.23 \times 10^7 - 5.53 \times 10^7)$	0.69	2.36×10^9
3	M2	9.33×10^7 $(4.98 \times 10^7 - 2.62 \times 10^8)$	0.54	2.24×10^{10}
4	B3	3.82×10^8 $(1.40 \times 10^8 - 2.93 \times 10^9)$	0.48	1.86×10^{11}
5	M1	4.57×10^8 $(2.02 \times 10^8 - 1.98 \times 10^9)$	0.71	2.85×10^{10}
6	B1	9.82×10^8 $(3.30 \times 10^8 - 8.86 \times 10^9)$	0.64	9.86×10^{10}

Fig. (1) Percentage Mortality regression lines of six isolates of entomopathogenic fungi *B. bassiana* and *M. anisopliae* against *T. urticae* egg stage.

Adult stage:

The susceptibility of adult females of *T. urticae* to entomopathogenic fungi *B. bassiana* and *M. anisopliae* were conducted. The percentage of mortality values after exposing to series of concentrations of 10^6, $5x10^6$, 10^7, $5x10^7$ and 10^8 spores/ml. were tabulated for 7 days after treatment in Table (3).

The percentage of mortality gradually increased along with spores concentrations**.** The Lowest concentration of 10^6 spores/ml revealed (29.89, 40.23, 36.67, 45.45, 40.04 and 46 %) for (B1, B2, B3, B4, M1 and M2) respectively 7 days after treatment. While the highest concentration of 10^8 spores/ml revealed (65.63, 56.00, 62.79, 88.52, 54.00 and 80.85%) for (B1, B2, B3, B4, M1 and M2) when mortality was assessed after the same consecutive days, respectively.

Table (3): Percentage mortality of *T. urticae* adult female treated with series concentrations of *B. bassiana* and *M. anisopliae* after seven days.

Mortality percentage of all tested isolates against adult female of *T. urticae* seven days after treatment						
Isolates	B1	B2	B3	B4	M1	M2
	7th day	7th day	7th day	7th day	7th day	7th day
Con.	Mortality %	Mortality %	Mortality %	Mortality %	Mortality %	Mortality %
0	27.38	26.12	22.89	24.64	27.2	24.00
10^6	29.89	40.95	36.67	45.45	40.04	46.00
5×10^6	54.05	46.30	47.73	60.00	45.01	50.00
10^7	55.95	49.15	51.95	64.00	48.03	55.77
5×10^7	64.38	50.00	53.33	80.36	50.02	72.00
10^8	65.63	56.00	62.79	88.52	54.00	80.85

Results in Table (4), Fig (2) proved that *B. bassiana* (B4) was more effective against *T. urticae* adult females compared with all other isolates. The LC_{50} value of B4 was 6.57 x 10^6 spores/ml while (M2, B1, B3, B2 and M1) revealed greater LC_{50} value (1.39 x 10^7, 4.43 x 10^7, 1. 01 x 10^8, 1.20 x 10^9 and   1.92 x 10^9 spores/ml) respectively.

Table (4): Comparison of pathogenicity among six isolates of enomopathogenic fungi *B. bassiana* and *M. anisopliae* against *T. urticae* adult stage.

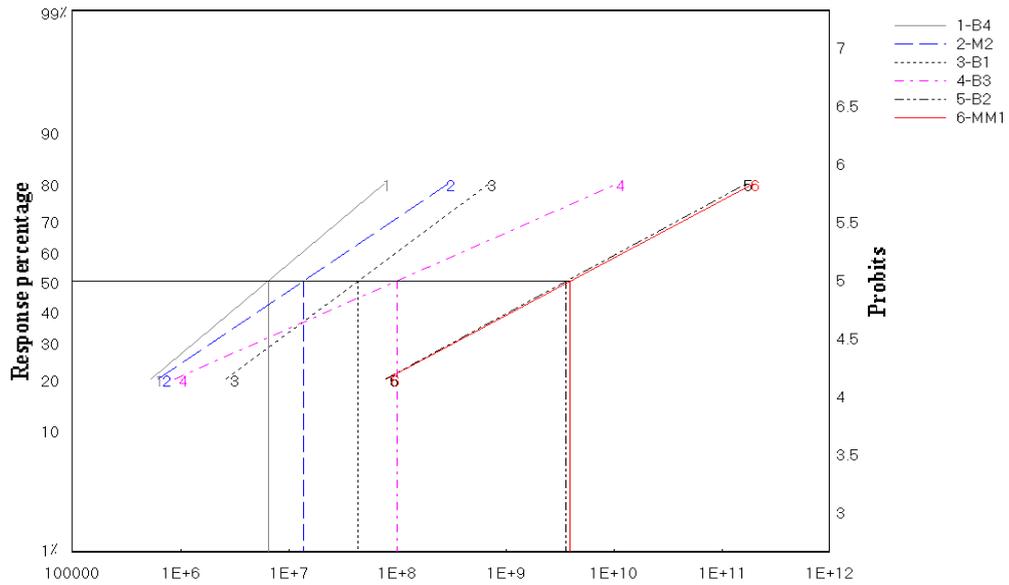| No.	Line name	LC_{50} (Limits)	Slope	LC_{90}
1	B4	6.57 x 10^6 (4.42 x 10^5 – 9.30 x 10^7)	0.78	2.88 x 10^8
2	M2	1.39 x 10^7 (9.04 x 10^5 –2.16 x 10^8)	0.62	1.54 x 10^9
3	B1	4.43 x 10^7 (6.39 x 10^6 –2.25 x 10^8)	0.69	3.17 x 10^9
4	B3	1.01x 10^8 (4.56 x 10^7 – 4.77 x 10^9)	0.41	1.34 x 10^{11}
5	B2	1.20 x10^9 (1.78 x 10^8 – 2.85 x 10^{12})	0.26	9.31 x 10^{13}
6	M1	1.92 x10^9 (2.50 x 10^8 – 6.13 x 10^{12})	0.27	9.72 x 10^{13}

Fig. (2) Percentage Mortality regression lines of six isolates of entomopathogenic fungi *B. bassiana and M. anisopliae* against *T. urticae* adult stage.

The LT_{50} and LT_{90} values of the six isolates entomopathogenic fungi *B. bassiana* and *M. anisopliae* were tabulated with their corresponding slopes for 7 days after treatment in Table (5).

The obtained results in Table (5) and Fig. (3) showed the mortality time regression line at concentration of 10^8 spores/ml. The data showed that isolate B4 caused high mortality in shortest time, LT_{50} value was 3.26 days. While for the other isolates (M2, B1, B3, B2 and M1) the LT_{50} values were (3.55, 3.90, 5.16, 5.93 and 6.00) respectively.

Table (5): Comparison of the mortality time among six isolates of entomopathogenic fungi *B. bassiana* and *M. anisopliae* against *T. urticae* adult stage.

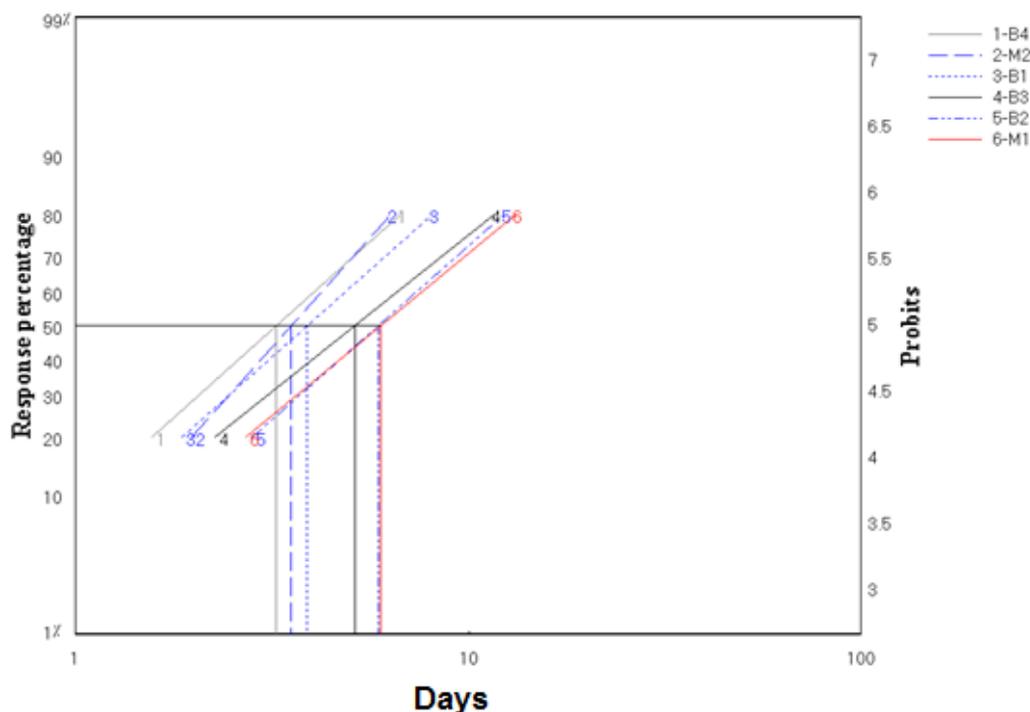| No.	Line name	LT_{50} (Limits)	Slope	LT_{90}
1	B4	3.26 (2.57– 3.94)	2.67	9.86
2	M2	3.55 (3.28– 3.85)	3.31	8.66
3	B1	3.90 (3.55– 4.33)	2.65	11.90
4	B3	5.16 (4.57– 6.04)	2.37	17.91
5	B2	5.93 (5.22– 7.06)	2.62	18.27
6	M1	6.00 (5.25– 7.25)	2.45	20.02

Fig. (3) Mortality-time regression line of six isolates of entomopathogenic fungi at concentration of 10^8 spores/ml against *T. urticae* adult female stage.

Results revealed that application of entomopathogenic fungi was effective in controlling both egg and adult stages of *T. urticae*. This is in line with the previous study of Nada *et al.* (2012) who recorded that *B. bassiana* and *M. anisopliae* had rapid mortality against mites and correspondent LC_{50} for *B. bassiana* and *M. anisopliae* was 2.98 x 10^6 and 1.82 x 10^6 spores/ ml, respectively. The LT_{50} of *B. bassiana* and *M. anisopliae* was 4.08 and 2.2 days, respectively.

Furthermore, Waked *et al.* (2015) reported that isolates of entomopathogenic fungi (EPF) *B. bassiana* and *Paecilomycis fumosoroseus* were highly effective and virulent against females of the *T. urticae* at four different conidial concentrations. The *P. fumosoroseus* isolate had lower LC_{50} (1.8 x 10^7 conidia/ml) than *B. bassiana* (2.6 x107 conidia/ml).

According to Shi and Feng (2004, 2009), some of the tested isolates of *B. bassiana*, *Paecilomyces fumosoroseus* and *M. anisopliae* have high lethal effect on eggs and females of *T. urticae*. The mite mortality observed was 73.1, 75.4 and 69.9 % for *B. bassiana*, *P. fumosoroseus* and *M. anisopliae*, respectively, after 10 days of spraying whereas the control mortality was 15.5%. Most of the infected females died on days 4-8. The infection by the fungus not only killed *T. urticae* females but greatly reduced their fecundity.

Bugeme *et al.* (2009) assesses the virulence of twenty three isolates of *M. anisopliae* and three isolates of *B. bassiana* against the two spotted spider mites, *T. urticae*. Mean mortality in the control was 11.5% 10 days after treatment. At 26°C, all the fungal isolates were pathogenic to adult female of *T. urticae* causing mortality between 36.5 and 100% and the LT_{50} values ranged from 2.2 to 8.2 days.

According to Draganova and Simova (2010), bioassays of five isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.- Criv.), Vuillemin were conducted under laboratory conditions against the two-spotted spider mite

Tetranychus urticae Koch. (Acari: Tetranychidae) that was found susceptible to the examined isolates of *B. bassiana*. On the fifth day, lethal effect reached 100% in the variant with 447 Bb, and $84.87 \pm 19.17\%$ in the variant with 426 Bb. Mycosis caused by the isolate 417 Bb of *B. bassiana* had a less lethal effect $68.89 \pm 7.40\%$ calculated for the fifth post-treatment day. In their study they found that the fast lethal effect of the mycoses to *T. urticae* was due to toxic secondary metabolites and the pigment oosporein produced by fungal isolates. Growing the examined isolates 417 Bb, 426 Bb, 444 Bb, 445 Bb and 447 Bb of *B. bassiana* a red pigmentation of the media was also noticed.

The main characteristic that has an important role in the virulence of entomopathogenic fungi strains from the genera *Beauveria* and *Metarhizium* is the production of enzymes necessary for penetration through arthropod cuticle. The extracellular proteases are considered an important virulence factor in insect disease processes (St. Leger *et al.,* 1986a, 1986b, 1988; Draganova, 1988; Bidochka and Khachatourians, 1990, 1994a, 1994b; Gupta *et al.,* 1992; St. Leger, 1995).

Chandler *et al.* (2005) found in a glasshouse experiment that *B. bassiana* cultured from Naturalis-L (Troy Biosciences, USA) reduced the numbers of *T. urticae* adults, nymphs and eggs (98% reduction in all three cases) on glasshouse tomato crop.

Gatarayiha, *et al.* (2012) screened 62 South African strains of *B. bassiana* for pathogenicity against *T. urticae* in laboratory bioassays on bean leaves, *Phaseolus vulgaris* L., under greenhouse conditions. In the first bioassay, strains of *B. bassiana* were applied at a single concentration of 10^7 conidia/ml. A mortality mite percentage between 4-92.5% was observed, with 40 % of strains causing mortality levels higher than 50%. The LT_{50} ranged between 5.5 - 8.6 days. The six most virulent strains were compared in a second screening, together with the commercial strain PPRI 5339. Five concentrations (2×10^4 to 2×10^8 conidia ml^{-1}) on female mites and three concentrations (2×10^6, 2×10^7 and 2×10^8 conidia ml^{-1}) on eggs were used. Mortality of mites assessed indicated that Strain PPRI 7315 and Strain PPRI 7861 performed similarly and were the most efficient, causing mite mortality of > 80%, 9 days after inoculation, at the highest concentration, with LC_{50} concentration of 1.13×10^6 and 1.22×10^6 conidia ml, respectively. Both strains performed better than the commercial strain (PPRI 5339) in vitro and showed good control of *T. urticae* during greenhouse trials.

Effect of the fungal spores of *B. bassiana* (B4) on mortality and fecundity of the predatory mites *Phytoseiulus persimilis* and *Neoseiulus californicus*:

This part of the investigation was undertaken to evaluate the side effect of using the entomopathogenic fungus *B. bassiana* isolate (B4) in spore suspension formula against predatory mites, *P. persimilis* and *N. californicus* the results are tabulated in Tables (6 and 7).

The results showed that the three tested concentrations of the fungal spore suspensions were safe against the predaceous mites, *P. persimilis* and *N. californicus*. The value of the total effect % for *P. persimilis* were 10.2, 17.9 and 29. 7 for the tested concentrations LC_{25}, LC_{50} and LC_{90}, respectively with IOBC class 1 for the three tested concentrations. While the value of the Total effect % for *N. californicus* were 18.33, 28.6 and 39.4 for the tested concentrations LC_{25}, LC_{50} and LC_{90}, respectively with IOBC class 1 for the LC_{25}, LC_{50} concentration and IOBC class 2 for the LC_{90}.

The most interesting observation is the fact that the highest concentration LC_{90} caused the highest mortality of the adult females of spider mite, *T. urticae* after 7 day

of exposure, was harmless on *P. persimilis* and slightly harmful on *N. californicus.* This means that the entomopathogenic fungus *B. bassiana* in spore suspension formula could be used without any consideration in IPM programs for controlling spider mite, *T. urticae.* These results are in agreement with those of Ludwig and Oetting (2001), who demonstrated that the predatory mite *Ipheseius degenerans* (Acari: Phytoseiidae) was least susceptible to *M. anisopliae* followed by *V. lecanii* and *B. bassiana.*

Table (6) Direct effect of the selective strain *Beauveria bassiana* (B4) on *Phytoseiulus persimilis*

Concentrations Spores/ ml	Females mortality	Fecundity		Decreasing of total eggs no. %	(E) Total effect %	IOBC class*
		Total eggs no./female	Eggs no./female/day			
Control	28.57	10.19	1.46	-		
LC$_{25}$	30	9.8	1.4	8.4	10.2	1
LC$_{50}$	35	8.65	1.38	9.8	17.9	1
LC$_{90}$	42.86	8.95	1.28	12.1	29.7	1

Table (7) Direct effect of the selective strain *Beauveria bassiana* (B4) on *Neoseiulus californicus*

Concentrations Spores/ ml	Females mortality	Fecundity		Decreasing of total eggs no. %	(E)Total effect %	IOBC class*
		Total eggs no./female	Eggs no./female/day			
Control	25	8.8	1.26	-		
LC$_{25}$	30	7.7	1.1	12.5	18.33	1
LC$_{50}$	35	7.25	1.04	17.6	28.6	1
LC$_{90}$	42.86	6.67	0.95	20.5	39.4	2

Scanning electron microscope:

The entomopathogenic fungus *B. bassiana* has been reported as being capable of infecting over 100 species of insects from a wide range of insect orders, although many fungal isolates vary in host range and some isolates have displayed high host specificity (Maurer *et al.*, 1997; McCoy *et al.*, 1988).

For developing entomopathogenic fungi as biocontrol agents, it is crucial to understand their mode of action on their insect pest target. Scanning electron microscopy has been widely used for this purpose (Lopez-Llorca *et al.,*1999, 2002).

The possible effects of pathogens on natural enemies are particularly relevant to biological control of agricultural pests.

If natural enemies are susceptible to infection by pathogens, applying combinations of these two controls for suppression of pests would not be compatible. In contrast, applying multiple species may act synergistically in reducing a pest population if interference between species is minimal or nonexistent (Roy and Pell, 2000).

Scanning electron microscopy are convenient tools to observe the mode of action of entomopathogenic fungi and to study how *B. bassiana* is able to colonize and infect the predator mites and *T. uricae*. It allowed us to observe adhesion and penetration structures on females of *T. uricae* infected with *B. bassiana.* While it was clearly shown that no such structures were found on *P. persmiles* infected with *B. bassiana.*
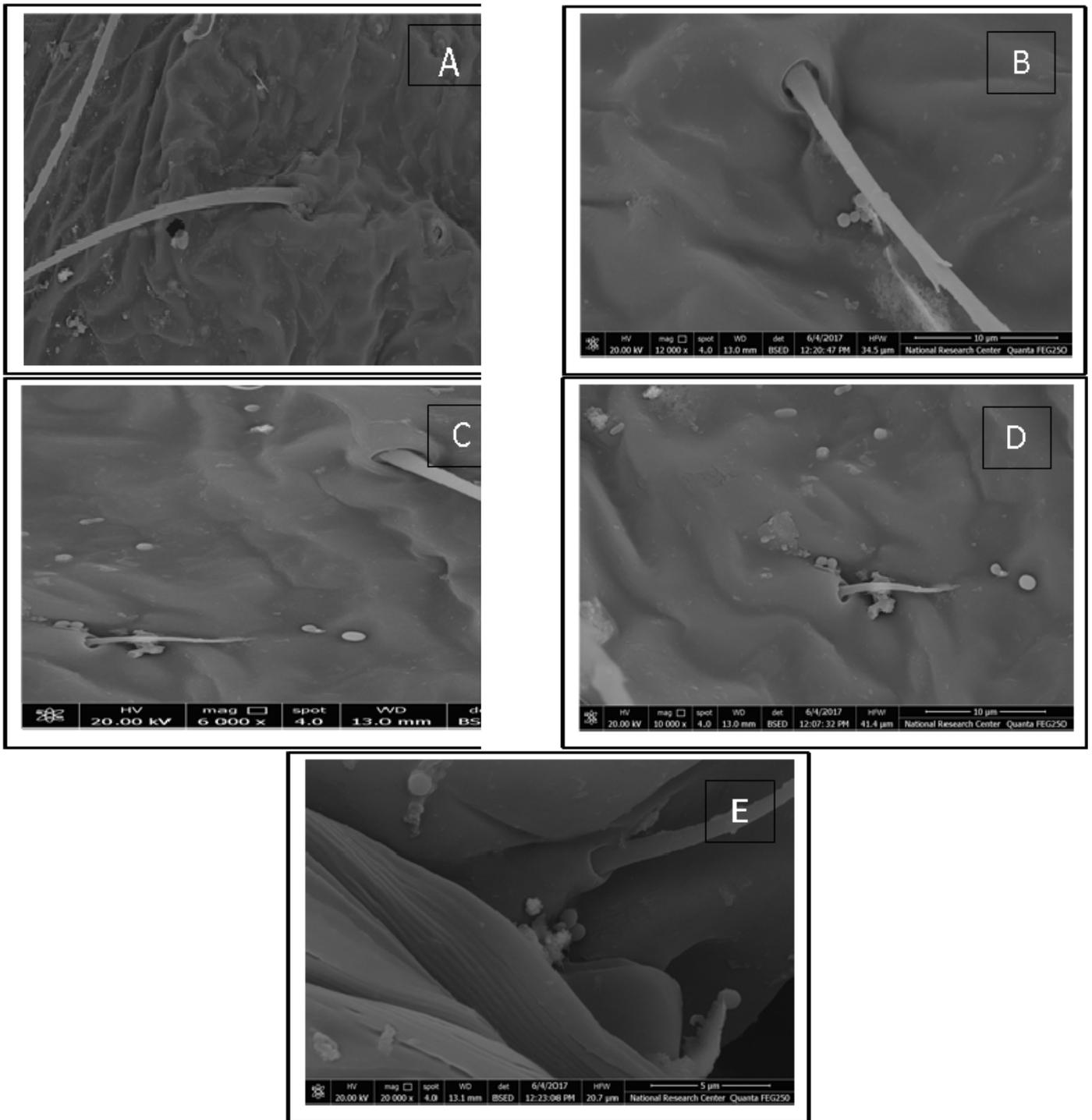
Fig. (4) Inoculation and attachment of *Beauveria bassiana* isolate B4 conidia on the cuticle of *Phytoseiulus persimilis*, (A) 24h. (X6000); (B) 48 h (X12000); (C) 72(X 6000); (D)72 h(X 10000); (E) 72h (X 20000) after inoculation.
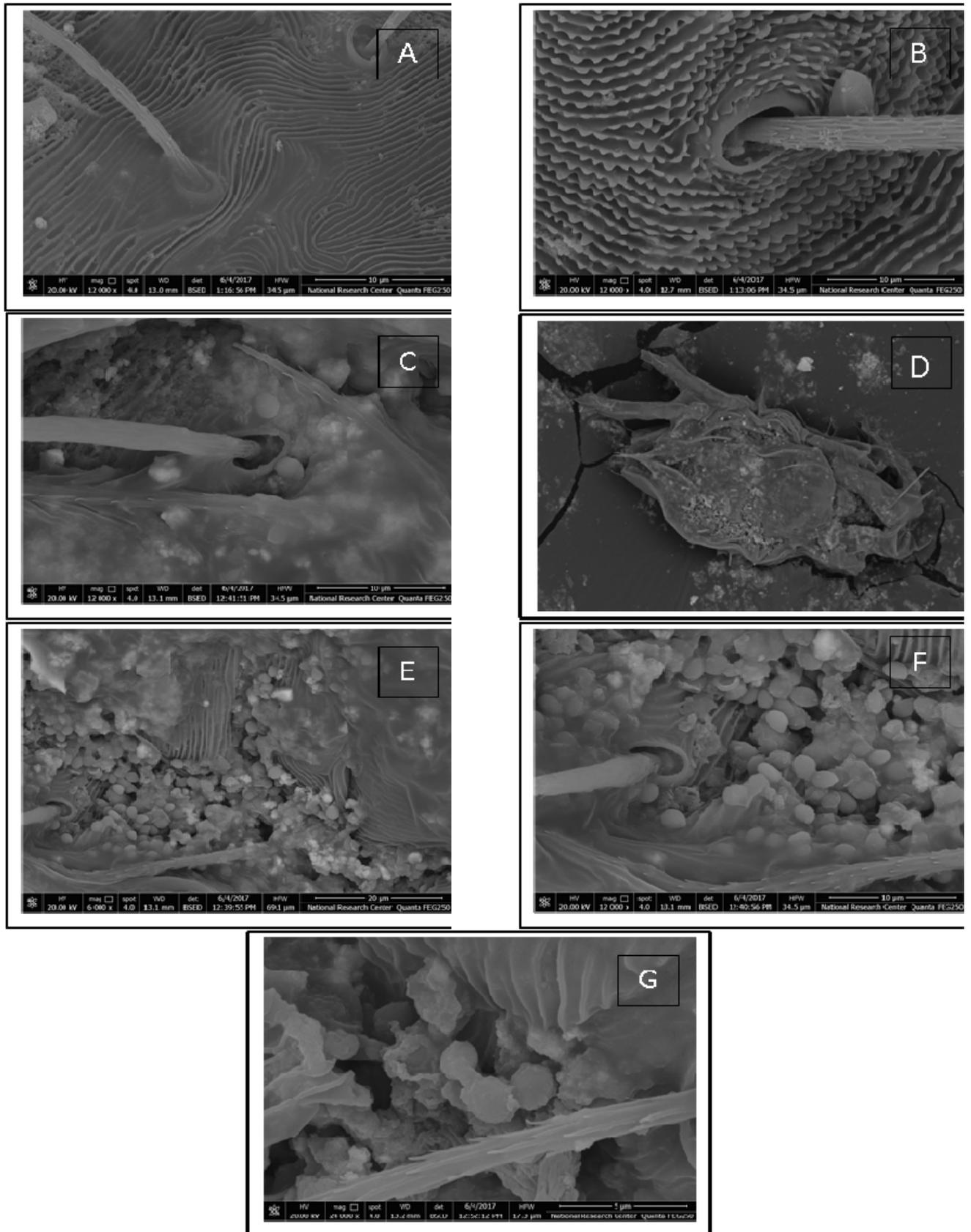
Fig. (5) Germination and infection of *Beauveria bassiana* isolate B4 conidia on the cuticle of *Tetranychus urticae*, (A) and (B) 24h. (X12000); (C) 48 h. (X 12000); (D)72 h (X 1000); (E) 72h (X 6000); (F) 72h (X 12000); (G)72h (X 24000) after inoculation.

Conidia adhered to the cuticle of predatory mites *P. persimilis* within 24–72 h (Figs. 4 A, B, C, D, E). Although conidia germinated within 24–36 h, few conidia were detected on the cuticular surface after 48 h. they apparently did not penetrate the cuticle even after 72h. Figs. (C, D and E) proved that conidia did not penetrate at different magnification scales (X 6000; X 10000 and X 20000).

Similarly, conidia adhered to the cuticle of *T. urticae* within 24–72 h (Fig. a) and germinated within 48 h (Fig. 4 B). However, Germ tubes of conidia successfully penetrated the mite cuticle within 48 h (Fig. 4 C).

In contrast, *B. bassiana* strain B4 conidia penetrated *T. urticae* cuticle soon after germination. These results do raise questions why *B. bassiana* is so virulent to *T. urticae* but shows no apparent infectivity in predatory mite *P. persimilis*.

In this study, although conidia were able to germinate, they apparently were not able to penetrate the cuticle of the predatory mite species (Fig. 5), but were able to penetrate the cuticle of *T. urticae*.

Evaluating the compatibility of entomopathogenic fungi and predatory mites is a critical issue for the successful uses of IPM programs to control pest mite species (Vergel *et al.,* 2011).

This study tested the *B. bassiana* strains (B4) that were virulent to *T. urticae*, but were found to be non infective to the predatory mites *P. persimilis* under laboratory bioassay and SEM observation. The findings here support the potential use of *B. bassiana* in combination with predatory mites to control *T. urticae*. The defence mechanism present in the cuticle of predatory mites that is responsible for this apparent immunity to fungal penetration deserves further study.

The ability of fungal conidia to attach to the insect cuticle is strongly correlated with virulence (Altre *et al.,* 1999). Insect infection occurs following germination of conidia on the cuticle, with subsequent penetration of the cuticle by specialized infection structures (Butt, 1990). Insect cuticle constitutes a defensive barrier to fungal penetration (Samuels and Paterson, 1995).

This result comes in parallel with (Wu *et al.,* 2016) who evaluated the pathogenicity of five strains of *B. bassiana* in five species of predatory phytoseiid mites. The bioassay results indicated that no viable fungal hyphae were found on predatory cadavers. Observations with scanning electron microscopy revealed that conidia were attached to the cuticle of predatory mites within 2–12 h after spraying, and had germinated within 24–36 h. After 48 h, conidia had gradually been shed from the mites, after none of the conidia had penetrated the cuticular surfaces. In contrast, the germinated conidia successfully penetrated the cuticle of *T. urticae*, and within 60 h the fungus colonized the mite's body. The study added that although several *B. bassiana* strains displayed a high virulence in *T. urticae*. There was no evident pathogenicity to phytoseiid mites. These findings support the potential use of entomopathogenic fungus in combination with predatory mites in *T. urticae* control.

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سمية بعض الفطريات الممرضة ضد *Tetranychus urticae* وبعض المفترسات الأكاروسية المصاحبة ككائنات غير مستهدفة.

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٢. معهد البحوث و الدراسات الأفريقة – قسم الموارد الطبيعية – جامعة القاهرة

الفطريات الممرضة و المفترسات الاكاروسية تستخدم كوسيلة للمكافحة البيولوجية للعنكبوت الاحمر ذو البقعتين*Tetranychus urticae* Koch . لذا وجب دراسة تاثير الفطريات الممرضة على المفترسات الاكاروسية الشائع استخدامها فى المكافحة البيولوجية لدراسة امكانية استخدامهما معا كوسيلة للمكافحة البيولوجية ضد العنكبوت الأحمر في برنامج إدارة مكافحة الآفات.

الجزء الأول من هذة الدراسة يتضمن دراسة مرضية لأربع عزلات من *Beauveria bassiana* وعزلتين من *Metarhizium anisopliae* ضد طور البيضة والطور البالغ للعنكبوت الأحمر. ووجد ان العزلة B4 كانت الاكثر مرضية لكلاالطورين، حيث أحدثت %88.5 نسبة موت للطور البالغ عند التركيز المرتفع spores/ml 10^8.ونسبة الفقس فى طور البيضة انخفضت الى %25.2 بالمقارنة بالافراد الغير معاملة التى وصل بها نسبة الفقس الى % 99.

الجزء الثانى من هذة الدراسة تضمن دراسة مرضية الفطر المنتخب من الجزء الأول و الذى أحدث اعلى مرضية على العنكبوت الأحمر (B4) للمفترسين الأكاروسين   *Phytoseiulus persimilis, Neoseiulus californicus*. باستخدم ثلاث تركيزات LC_{25}, LC_{50} and LC_{90} ضد الطور البالغ للمفترسين. واظهرت النتائج ان العزلة (B4) غير ضارة على المفترس الأكاروسى*P. persimilis* وتأثيرها منخفض جدا على المفترس الأكاروسى*Neoseiulus californicus* .

وبدراسة وتحليل العينات المصابة بواسطة الميكروسكوب الأكترونى لم تظهر هيفات للفطر على المفترس الأكاروسى. وضح الميكروسكوب الألكترونى ان كونيديا الفطر تلتصق بالكيوتيكل فى غضون 24 ساعة بعد الرش بالعزلة B4 ويحدث الأنبات فى غضون 48-24 ساعة. ولكن بعد ذلك لا يحدث اختراق لكيوتيكل الحشرة.

وعلى النقيض من ذلك الكونديات المنبتة تخترق بنجاح كيوتيكل *T. urticae* وفى خلال 72 ساعة يحدث استعمار لجسم العنكبوت .

توضح هذة الدراسة أنه على الرغم من أن العديد من سلالات *B. bassiana* أظهرت مرضية عالية على العنكبوت الأحمر ولكن لم تظهر اى اعراض مرضية واضحة على Phytoseiid mites. هذه النتائج تدعم الاستخدام المحتمل للفطريات الممرضة للحشرات مع المفترسات الأكاروسية في برامج مكافحة العنكبوت الأحمر *T. urticae*.