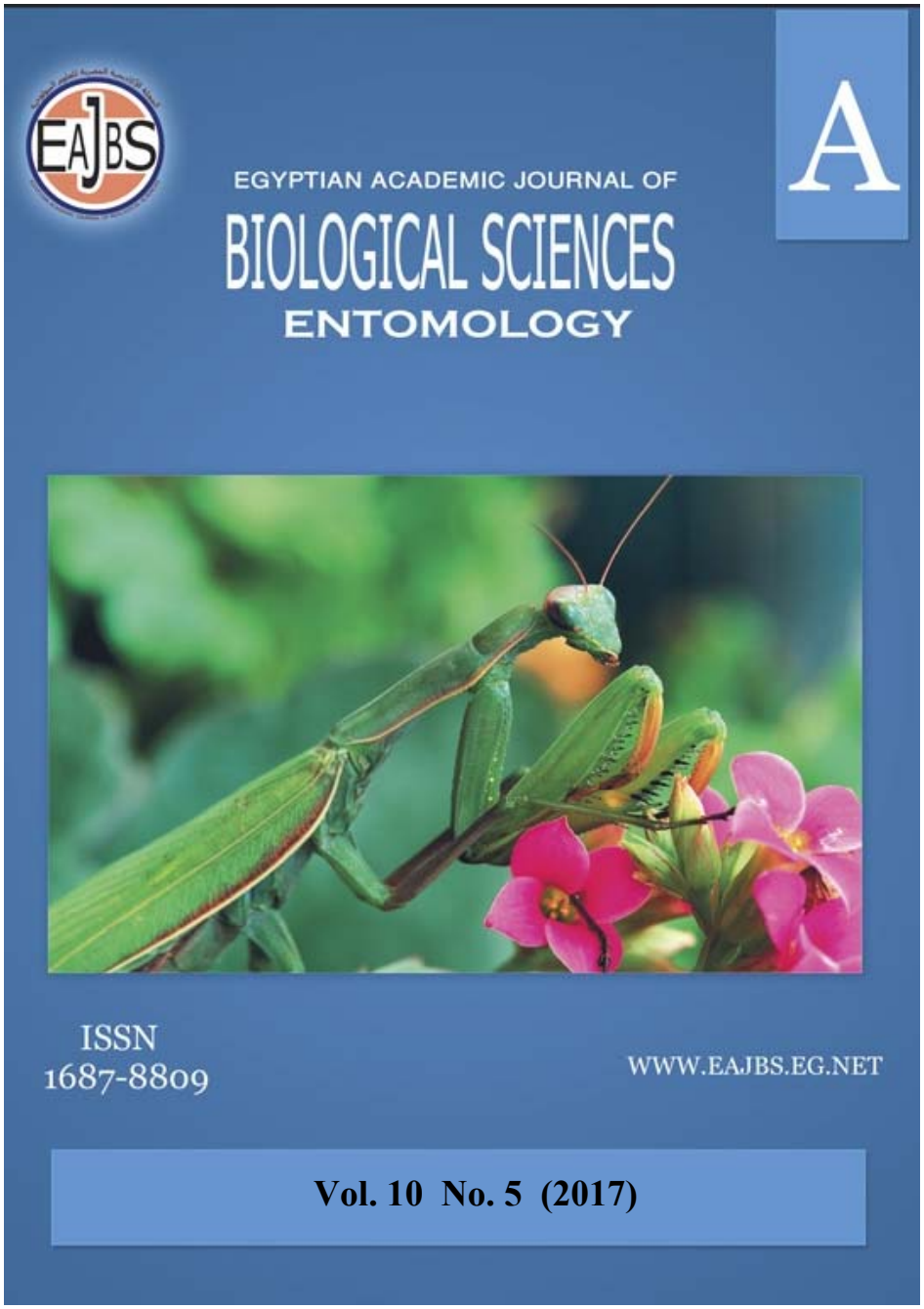

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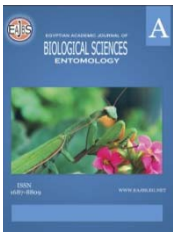

Evaluate the Feeding Preference of Some Predator Mites Towards Red Spider Mites Untreated and Treated With *Beauveria bassiana*

Dalia M. A. Hassan[*1]; Wafai Z. A. Mikhail[2]; Marguerite A. Rizk[1]; Hassan M. Sobhy[2] and Maha S. Nada[1]

1- Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt.
2- Department of Natural Resources, Institute of African Research and Studies, Cairo University.
E.–mail: daliahassan732@gmail.com

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ABSTRACT

Results of the present study are based on the ability of the predator mites *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and *Neoseiulus californicus* that used as biological control agents to recognize if the prey *Tetranychus urticae* (Acari: Tetranychidae) is treated with entomopathogenic fungi, and measure their preference toward untreated and treated *T. urticae* with *Beauveria bassiana.* In the early stages of the fungus infection predators were able to consume *T. urticae* treated with the fungus. While after 48 and 72h. *P. persimilis* and *N. californicus* were able to determine the treated prey with the entomopathogenic fungi *B. bassiana,* and prefer to prey on the untreated mites. This avoidance behaviour of the predator mites permits to use them as complementary biological control agents with entomopathogenic fungi in Integrate Pest Management (IPM) program.

INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), is a major agricultural pests, which often causes severe damage to a variety of crops (Gotoh *et al.*, 2004). It frequently occurs in glasshouse and outdoor crops (Kennedy and Store, 2000). Spider mites are major pests in a wide range of protected crops across the world. It is one of the most serious 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 the response to develop resistance to many acaricides. *T. urticae* is known for its ability to develop rapid resistance to pesticides. Among arthropods it has the highest incidence of pesticide resistance (van Leeuwen *et al*., 2010).

The use of multiple natural enemies has been recommended to control insect pests in integrated pest management programs (IPM) (Jacobson *et al*., 2001). 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).

Microbial control of pests is an important approach to reduce the dependence on chemical pesticides for increased agricultural sustainability. Entomophthoralean is highly virulent, exhibiting fast pathogenesis and epizootics are common in nature, therefore playing an important role in controlling insect and mite populations (Milner, 1997; Lacey *et al.*, 2001; Pell *et al.*, 2001 ; Ravensberg, 2011).

The entomopathogenic fungus *Beauveria bassiana* is known to be effective in controlling many arthropod pests (Ferron 1978), 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).

In the present study, host preference of *P. persimilis* and *N. californicus* were investigated when offered *T. urticae* treated adults by *B. bassiana* vs. untreated *T. urticae* on the leaf. The aim was to understand if the predatory mite was able to detect the presence of an entomopathogenic fungus and avoid feeding on fungus treated prey.

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±1°C 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*., 2012a).

The prey used in the present study was the 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 the two predators aggressively attack to adults of *T. urticae* (Cote, 2001; Seiedy, 2014).

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 fibber-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 after day. Also mulberry leaves were changed by fresh one from time to time when necessary (Nadimi *et al.,* 2008; Seiedy *et al.,* 2012a).

Preparation of conidia

B. bassiana isolate (B4) which was observed to cause non-significant mortality on *P. persimilis* and *N. californicus* (Unpublished data) percentage of mortality for both predators *P. persimilis* and *N. californicus* were recorded as 17.9 and 28.6 %, respectively.

In this study a single concentration was prepared the calculated LC_{50} ($5x10^6$ conidia/ml). It was 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), then incubated at 25 ± 1°C, 60–70 % R.H. and in darkness for 10–14 days to obtain conidia (Uma Devi *et al.*, 2005, Seiedy, 2014; Waked *et al.*, 2015).

Experimental Procedures

Experimental arenas were constructed by placing a mulberry leaf disks (2.5cm in diameter) excised from mulberry plants on top of a layer of cotton in a 12 cm Petri dish filled with sterile distilled water. A layer of cotton put around the leaf in order to prevent predatory mites from escaping.

Adults of the spider mites, *T. urticae* were placed on experimental disks at densities 25 per disk using 5 replicates were conducted for each prey density. Conidial suspension of *B. bassiana* (B4) was prepared at $5x10^6$conidia/ml as calculated LC_{50} (Seiedy, 2014).

Two millilitre of the conidial concentration was applied against the adults of *T. urticae* in Petri dish with different densities by using the direct spray technique a glass atomizer at 30cm high with 2ml spore suspension for each treatment and 2ml sterilized distilled water of 0.1% Tween 80 as control (Abo-Shabana, 1980; Hassan, 2008 ; Sewify *et al*., 2015).

Four time intervals: 0, 24, 48 and 72h post-inoculation of mite adults were considered for introducing predators. A single *P. persimilis* or *N. californicus* female (starved for 24 h) was introduced to *T. urticae* adults. All predator females were in the early stage of adult. Eggs of prey were removed from Petri dishes for the duration of the experiment. The number of consumed *T. urticae* adults in each Petri dish was assessed after 24 h. The experiments were conducted at 25±1 °C, 60–70 % R.H. and a photoperiod of 16:8 h (L: D) (Seiedy, 2014)

Statistical analysis

Predator preference was calculated using Manly's preference index (Manly, 1974). Manly's preference index (β) calculates the number of consumed prey. The sum of Manly's β for all prey species is from 0 to1 and prey species with large values of Manly's β indicates preference for that species.

$$\beta = \frac{\log(\frac{e_1}{A_1})}{\log(\frac{e_1}{A_1}) + \log(\frac{e_2}{A_2})}$$

- β: The preference to prey Type I (*T. urticae* treated adults by *B. bassiana*), Type II (untreated *T. urticae*).
- e_1 and e_2: The number of treated and untreated prey remaining after the experiment.

- A_1 and A_2: The number of treated and untreated prey presented to the predator.

If the preference index is close to 1, the predator prefers Type I prey, if close to 0, it prefers Type II prey. An index value close to 0.5 indicates no preference.

RESULTS AND DISCUSSION

Adults of both untreated *T. urticae* and treated *T. urticae* by *B. bassiana* were offered simultaneously, after zero time, 24, 48 and 72h. *P. persimilis* and *N. californicus* consumed more untreated *T. urticae* than the treated preys. The percentage of predation for *P. persimilis* was (36.8, 28.8, 8 and 4%) after 0, 24 ,48 and 72h respectively compared to the control that was 37.6%, and for the other predator

N. californicus the percentage of predation was (37.6, 27.2, 11.2 and 6.4%) after 0, 24, 48 and 72 h respectively compared to the control that was 38.4%. Table (1) and Table (2).

Table (1): Percentage of predation by *P. persimilis* to untreated and treated *T. urticae* with the selective strain *B. bassiana* (B4).

T. urticae consumed and not consumed				
	not consumed	consumed	Total	%
control	e2=78	47	A2= 125	37.6
0 time	e1=79	46	A1= 125	36.8
24h.	e1=89	36	A1= 125	28.8
48h.	e1=115	10	A1= 125	8
72h.	e1=120	5	A1= 125	4

Table (2): Percentage of predation by *N. californicus* to untreated and treated *T. urticae* with the selective strain *B. bassiana* (B4).

T. urticae consumed and not consumed				
	not consumed	consumed	Total	%
control	e2=77	48	A2= 125	38.4
0 time	e1=78	47	A1= 125	37.6
24h.	e1=91	34	A1= 125	27.2
48h.	e1=111	14	A1= 125	11.2
72h.	e1=117	8	A1= 125	6.4

Data analysis in Table (3) indicated that *P. persimilis* in all treatments exhibited type II response to *T. urticae*. The Manly's preference index (β) for the treated adult *T. urticae* by *B. bassiana* (B4) were (0.5, 0.42, 0.2 and 0.08) that had been treated for zero time, 24, 48 and 72h respectively. These results indicated that *P. persimilis* prefers untreated *T. urticae* and the predator was able to detect the presence of *B. bassiana* in 48 and 72 h treatments, These results are in agreement with Seiedy (2014) who found that *P. persimilis* prefers untreated *T. urticae* rather

than *T. urticae* adults treated by strain DEBI008 of *B. bassiana* with interval 72h after infection.

Table (3): Indirect effect of the selective strain *B. bassiana* (B4) on *P. persimilis.*

β	**for zero time = 0.5**
β	**for 24 h = 0.42**
β	**for 48 h = 0.2**
β	**for 72 h = 0.08**

Results of the present study indicated that *N. californicus* in all treatments exhibited type Π response to *T. urticae* The Manly's preference index (*β*) for treated adult *T. urticae* by *B. bassiana* (B4) were (0.49, 0.4,0.19 and 0.12) that had been treated for zero time, 24, 48 and 72h respectively, Table (4). These results indicated that *P. persimilis* and *N. californicus* prefer the untreated *T. urticae* and the predators were able to detect the presence of *B. bassiana* in 48 h and 72 h treatments.

Table (4): Indirect effect of the selective strain *B. bassiana* (B4) on *N. californicus.*

β	**for zero time = 0.49**
β	**for 24 h = 0.4**
β	**for 48 h = 0.19**
β	**for 72 h = 0.12**

Seiedy (2014) explained that, the quality of the prey seems to have been significantly *P. persimilis*. In the present study we speculated that the presence of fungal conidia on prey body alter the behaviour of *P. persimilis* and *N. californicus* by increasing grooming. This based on other studies by (Pell *et al.,* 2001; Inglis *et al.,*2001), who stated that the fungi primarily enter the host through direct penetration of the cuticle, virulence of fungal entomopathogens involves four steps: adhesion, germination, differentiation and penetration. Each step is influenced by a range of integrated intrinsic and external factors, which ultimately determine the pathogenicity. A successful infection is achieved by the attachment or adhesion of spores to the host. Adhesion is necessary and normally achieved through the secretion of mucilage. Spores attach to the cuticle, germinate and penetrate the integument by means of a combination of physical pressure and enzymatic degradation of the cuticle death of the host usually taking place 4-7 days after infection (Shah and Pell, 2003).

This was a possible reason for the decrease in prey consumption at 48h and 72h treatment, so it was probably that these treated mites had not taste good enough to be eaten in comparison with 0, 24 h after infection and control because after 48 and 72 h, the fungus grew on mites, so predator may be able to recognize the fungus spores and as a result, predators are indisposed for any feeding.

Seiedy *et al.,*(2013) showed that *P. persimilis* was attracted to the control plants that contain untreated *T. urticae* more than plants contain *T. urticae* treated with *B. bassiana* after 72h. This indicated that the predator was able to detect the presence of *B. bassiana* in the 72h treatment. Baverstock *et al.* (2009) showed that entomopathogenic fungi produce a range of volatile chemicals, so it is possible to

assume that the fungus produces one or more compounds that the predatory mite can detect and that trigger the avoidance behaviour. Faraji *et al.* (2001) mentioned that the predatory mite has capability to recognize dangerous conditions based on the odor, and to adjust its foraging behaviour accordingly.

Seiedy *et al*. (2013) reported the avoidance of feeding of the predatory mite on *T. urticae* adults treated by *B. bassiana*, and showed that *P. persimilis* was attracted towards control plants if tested against plants with spider mites treated with *B. bassiana* for 72 h This indicated that the predator was able to detect the presence of *B. bassiana* in the 72 h treatments. Therefore, live *T. urticae* treated with the fungus for 72 h caused avoidance behaviour of the predator.

An insect or mite may gain selective advantage if it is able to detect entomopathogenic fungi from a distance and respond via behavioural avoidance or through post-contact responses such as grooming (Chouvenc *et al.,* 2008 ; Seiedy *et al.* 2012b).

Gao *et al.*,(2012), evaluated the insecticidal activity of strain *B. bassiana* (RSB) which was highly virulent to the western flower thrips *Frankliniella occidentails* and was not insecticidal against the predator *Orius sauteri*, and found that the larva of *F. occidentails* contaminated by *B. bassiana* may be poor quality prey for *O. sauteri* because infection makes the larvae deficient in certain essential nutrients, (Simelane *et al.,* 2008) or creates a build up of fungal toxins or metabolites, which may slow development and shorten adult longevity of *O. sauteri*. (Leckie *et al.,* 2008).

Wekesa *et al.* (2007), demonstrated the effect of entomopathogens on mite behaviour, entomopathogenic fungus *Neozygites floridana* has no ability to affect the predator mite *Phytoseiulus longipes* but the behaviour of phytoseiid can be affected due to the presence of an excessive number of capilliconidia on their bodies. This change in their behaviour can be shown by their self-grooming and reducing their searching activity.

In other study, it has been shown that the behavioural factors such as feeding preference and activity of predator were affected by the presence of fungus on the treated prey , intensive decreasing in feeding of predator *Anthocoris nemorum* on aphid treated with *B. bassiana* was clearly detected (Meyling and Pell, 2006).

Pell and Vandenberg (2002) , observed that the predator *Hippodamia convergens* Guerin-Meneville, did not feed on Russian wheat aphid *Diuraphis noxia* treated with *Paecilomyces* fumsoroseus fungi.

This conclusion comes parallel to Roy and Pell (2000) who stated that fungal control agents have the potential to negatively affect insect natural enemies through direct infection, or indirectly , by depleting the prey population or changing quality extent of prey population. In turn, insect natural enemies could negatively affect fungal control agents by consuming preys that are treated with the fungus, by removing the fungus from the system. While, Seiedy *et al*., (2012a) reported that fungal control agents have the potential to negatively affect natural enemies through their effects on the quality of prey, and *P. persimilis* showed increased handling time of *B. bassiana* treated adults of *T. urticae* if treated for 48 and 72 h.

Therefore, *B. bassiana* strain (B4) and the predators mites *P. persimilis* and *N. californicus* appear to be compatible and complementary biological control agents for spider mite *T. urticae* in the IPM program.

B. bassiana strain (B4) could be used for rapid, short term suppression of spider mites populations, and predator mites could be used for long term suppression of spider mites population.

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ARABIC SUMMARY

تقييم السلوك الغذائى لبعض المفترسات الأكاروسية تجاة العنكبوت الأحمرغيرالمعامل و المعامل بفطر *Beauveria bassiana*

داليا محمد أحمد حسن[١] – وفائى زكى عازر ميخائيل[٢]– مارجريت عدلى رزق[١]– حسن محمد صبحى[٢] – مها صلاح الدين ندا[١]

١. معهد بحوث وقاية النباتات– مركز البحوث الزراعية – الدقى – الجيزة– مصر

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

تعتمد نتائج الدراسة الحالية على قدرة كلا المفترسين الأكاروسيين *Phytoseiulus persimilis* و*Neoseiulus californicus* المستخدمين كعناصر للمكافحة البيولوجية في التعرف على الفريسة *Tetranychus urticae* المصابة بفطر *Beauveria bassiana* وقياس نسبة التفضيل فى التغذية بين الأفراد المصابة وغير المصابة من *T. urticae*.

ففى المراحل المبكرة من العدوى الفطرية، المفترس لديه القدرة على استهلاك *T. urticae* المعامل بالفطريات دون حدوث أى ضرر له. بينما بعد ٤٨ و ٧٢ساعة من العدوى كان المفترسان *P. persimilis* و *N. californicus* قادرين على تحديد الفريسة المعاملة بالفطر الممرض *B. bassiana* ، وفضلوا التغذية على الفريسة غير المعاملة. سلوك التجنب للمفترس الأكاروسى يمكننا من استخدامه كوسيلة للمكافحة البيولوجية التكميليه مع الفطريات الممرضة في برنامج إدارة مكافحة الآفات .