

## **EFFECT OF THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* ON THE TWO-SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* KOCH**

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### **Abstract**

The effect of the entomopathogenic fungus *Beauveria bassiana* on the two-spotted spider mite *Tetranychus urticae* Koch was studied by testing four concentrations of the fungal spore suspensions,  $2 \times 10^5$ ,  $2 \times 10^6$ ,  $2 \times 10^7$  and  $2 \times 10^8$  on the mite adult stage. The percentages of mite mortality increased with the increase of the spore concentration. The spore concentration of  $2 \times 10^6$  spores/ml proved to be the less concentration which gave the best result. Also 80% and 90% R.H. were the suitable for mite mortality. At 90 % Relative humidity, the mortality percentages of eggs, immatures and adults averaged 16.77% ,33.34% and 40.0% after 3 days, respectively, increased to 73.33% ,63.34% and 85.67% after 7 days, respectively. Better control results were obtained when using direct application ( spraying in the presence of the mite individuals) than in indirect application (spraying plant discs before placing mites on it).

**Key words:** Entomopathogenic fungi, *Beauveria bassiana*, spider mite, *Tetranychus urticae*.

### **INTRODUCTION**

The entomopathogenic fungi play an important role in the regulation of natural mite population, and are sometimes able to decimate populations of phytophagous mites (Van der Geest *et al.*, 2000), this can reduce the application of acaricides. The efficacy of fungal pathogens as microbial control agents against mites and insect pests is largely influenced by temperature and relative humidity ( Smitley *et al.*, 1986 and Klubertanz *et al.*, 1991). In Egypt, climatic conditions are considered more suitable for fungal pathogens (Sewify, 1999). Several species of pathogenic fungi were treated and tested against mite pests, *Hirsutella thompsonii* Fisher for citrus rust mite *Phyllocoptruta oleivora* (Ashmed) (McCoy and Ekanavel, 1969, McCoy, 1975 and Latge *et al.*, 1988), and against *Tetranychus urticae* Koch (Hanna and Heikal, 1995), *Neozygites floridana* for controlling *T. urticae* (Brandenburg and Kennedy, 1982, Mietkiewski *et al.*, 1993), *Verticillium lecanii* against *Eutetranychus orientalis*

(Klein)(Sewify and Mabrouk,1991 ) and the fungus *Beauveria bassiana* against *T. urticae* ( Yousri, 1994, Yassin,1997and Hassan,2003). The objective of this work is to study the effect of the entomopathogenic fungus *B. bassiana* on the different stages of the two-spotted spider mite *T. urticae* .

## MATERIALS AND METHODS

Conidia of *Beauveria bassiana* were brought from USDA-ARS Collection of Entomopathogenic Fungal Cultures, Plant Protection Research Unit, Cornell University, Ithaca, New York, USA. Conidia were cultured on autoclaved Potato dextrose agar (PDA) medium. Inoculated PDA medium was incubated for 2 weeks at 25 C°. Fungal spores were harvested by rinsing with sterilized distilled water, then filtered through sterilized cheese cloth to reduce mycelium clumping. Spores were counted using a haemocytometer. The application to infect mite individuals was conducted by using four concentrations,  $2 \times 10^5$ ,  $2 \times 10^6$ ,  $2 \times 10^7$  and  $2 \times 10^8$  spores/ml. Sterilized potato leaf discs (1 inch diameter) were put on moist cotton wool pads placed in glass Petri-dishes, where few drops of water were added daily to cotton wool to maintain suitable moisture content .Ten leaf discs, each containing ten mite individuals of homogenous eggs or immatures or adult females of the two-spotted spider mite *T. urticae* were used. Five replicates were used for each fungal suspension concentration. The control (check) treatments were tested with distilled water. To study the effect of relative humidity (R.H.) on the mite individuals, four levels were checked, 60% , 70% ,80% and 90% . The fungal suspensions were sprayed by a hand atomizer. Plant discs were treated by direct spray (spraying in the presence of the mite individuals) and indirect spray (spraying plant discs then putting the mite individuals on the discs) .The percentages of mortality were determined after 3 and 7 days .

## RESULTS AND DISCUSSION

The Effect of the entomopathogenic fungus *B. bassiana* on the two-spotted spider mite, *T. urticae* Koch was tested to find out the least concentration which can give the high percentage mortality of the adult stage. Data presented in Table 1 showed that , the mortality percentages of the mite at 90% R.H. increased with the increase of the fungal spore suspension concentrations .When mite females were treated with fungal concentrations,  $2 \times 10^5$ ,  $2 \times 10^6$ ,  $2 \times 10^7$ ,  $2 \times 10^8$  the mortality percentages averaged 26.7%,40.0%, 46.7% and 43.3% after three days and 74.1%, 85.67%, 86.86% and 85.8% after 7 days of the previous treatments respectively.

When using the fungal spore suspension  $2 \times 10^7$  and  $2 \times 10^8$ , the percentage mortality after 7 days of treatment, proved to be nearly equal to those obtained by the concentration  $2 \times 10^6$ . Thus, the fungal spore concentration of  $2 \times 10^6$  spores/ml. Seemed to be the least one which gave the best result for controlling *T. urticae*.

Table 1. Percentage mortality s of *T. urticae* adult stage after treatment by series spore concentration of *B. bassiana* at 90 % R.H.

Spore Concentration	Mortality(%)	
	after 3 days	after 7 days
$2 \times 10^5$ spores/ml.	26.7	74.1
$2 \times 10^6$ spores/ml.	40	85.67
$2 \times 10^7$ spores/ml.	46.7	86.86
$2 \times 10^8$ spores/ml.	43.3	85.8
Control	0.0	9.33

### Disease symptoms

Mite individual was probably infected and killed by the action of fungal hyphae that germinate from spores, penetrate the exocuticle, and subsequently invade and destroy the internal tissues. Infected mite becomes lazy (inactive) after one day of spray. On the other hand, the mite colour becomes darker, swollen with liquid (Plasmolysis), the mite body could be disintegrated when touched. These symptoms appeared after 4 to 5 days, after that the body became dry (loosing moisture) and the fungal mycelium gradually covers the whole body (Fig. 1.) Boucias *et al.*, (1988) stated that the fungal hyphae of *B. bassiana* invade the mite cuticle by direct penetration, Heikal and Hanna (1992) found that fungal conidia of entomopathogenic fungus *Hirsutella thompsonii* var. *synnematos* germinate and penetrate the integument of the mite *T. urticae* individuals, specially through legs and other points of the body, on the other hand, Yassin (1997) reported that the fungus *B. bassiana* had a toxic effect on *T. urticae* at the first three days after treatment but after 5 days fungal hyphae destroyed the internal tissue of the mite body.



Fig. 1. Disease symptoms of *T. urticae* adult stage after spray with *Beauveria bassiana* spore suspension .

The pathogenicity of *B. bassiana* was studied on different stages of *T. urticae* at 60%, 70%, 80% and 90% relative humidity (R.H.) and  $20 \pm 2$  °C .The effect of the fungus increased with the increasing of R.H. (Table 2). After 3 days of treatment, eggs mortality percentage (non hatching) was 0.0% at 60% and 70% R.H. but increased to 13.0% and 16.77% at 80% and 90% R.H. respectively., while after 7 days ,these values increased to 13.35%, 30%, 56.67% and 73.33% at 60% , 70% ,80% and 90% R.H. respectively.

The necessity of high levels of relative humidity is required for spore germination, this level was estimated to near 100% R.H. for at least 14 hours ( Hall, 1980). The absolute necessity of high relative humidity is only required to initiate fungal infection, thus the available of suitable relative humidity may be a major problem in using fungal

biocide. Proper management of applying the fungus would suggest using a high volume spray, as well as probably evening application, in order to provide the necessity of high humidity during the critical 14 hours (Hassan,2003) . The optimal range of temperature would be 20 – 25 °C (Sewify,1999).

Table 2. Effect of *B. bassiana* ( $2 \times 10^6$  spores/ml) on different stages of *T. urticae* at 20 ±2 °C and different R.H.

Relative humidity	Stage	Percentage of Mortality			
		Treatment		Control	
		after 3 days	after 7 days	after 3 days	after 7 days
60 %	Egg	0.00	13.35	0.00	1.50
	Immature	3.34	33.33	0.00	4.00
	Adult	0.00	20.00	0.00	2.00
70 %	Egg	0.00	30.00	0.00	3.06
	Immature	20.00	30.60	0.00	5.00
	Adult	13.34	35.36	0.00	3.00
80 %	Egg	13.00	56.67	2.00	3.50
	Immature	26.67	50.00	0.00	4.00
	Adult	26.87	60.00	0.00	5.00
90 %	Egg	16.77	73.33	4.00	10.00
	Immature	33.34	63.34	3.05	6.07
	Adult	40.00	85.67	0.00	6.67

The effect of fungal spore suspension on different stages of *T. urticae* was studied by using direct spray ( spraying plant discs in the presence of the mite individuals) and indirect spray (spraying plant discs before placing mite individuals). Data presented in Table 3 show that direct spray of fungal suspension had more positive effect on different mite stages. Mortality percentages of eggs ,immatures and adults averaged 16.77%, 33.34% and 40.0% after 3 days respectively , increased to 73.33%, 63.34% and 85.67% after 7 days at 90% R.H. respectively. On the other hand, when indirect spray was applied , no mortality occurred for eggs ,immatures and adults after 3 days, while these parameters reached 26.7%, 16.66% and 17.66% after 7 days, respectively. Adult stage proved to be more susceptible to *B.bassiana* direct spray followed by egg and immature stages. This might be attributed to the the changing and removal of the old exuvium (skin) with fungal spores during the moulting process of immatures which can be undertaken more than once. On the

other hand, Susilo *et al.* (1994) and Hassan (2003) stated that immatures of *T.urticae* were more susceptible to the fungus *Neozygites floridana* than adult stage, they attributed this phenomenon to thickness of cuticle which is thinner in immatures than adults and in female than male .

Table 3. Effect of direct and indirect spray of *B. bassiana* ( $2 \times 10^6$  spores/ml) on *T. urticae* stages at  $20 \pm 2^\circ\text{C}$  and 90% R.H.

Treatment	Stage	Mortality (%)	
		after 3 days	after 7 days
Indirect spray	Egg	0.00*	26.7*
	Immature	0.00	16.66
	Adult	0.00	17.66
Direct spray	Egg	16.77*	73.33*
	Immature	33.34	63.34
	Adult	40.00	85.67
Control	Egg	0.00*	10.00*
	Immature	0.00	6.07
	Adult	0.00	6.67

\*Mortality percentages = No hatching %

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## تأثير الفطر الممرض للحشرات *Beauveria bassiana* على اللحم العنكبوتى ذى البقعين *Tetranychus urticae* Koch.

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تمت دراسة تأثير الفطر الممرض للحشرات *Beauveria bassiana* على اللحم العنكبوتى ذى البقعين *Tetranychus urticae* Koch وذلك بتجهيز أربعة تركيزات من معلق الفطر وهى  $10 \times 10^6$  و  $10 \times 10^5$  و  $10 \times 10^4$  و  $10 \times 10^3$  جرثومة/مل وتم معاملة الطور الكامل بها ووجد ان معدل نسب الموت تزداد بزيادة تركيز جراثيم الفطر وان التركيز  $10 \times 10^6$  يعتبر اقل تركيز يعطى نسبة موت جيدة. كما ان الرطوبة النسبية (R.H.) ٨٠% و ٩٠% كانت اكثر مناسبة للفطر و كذلك تأثيره على اللحم. كذلك تمت دراسة تأثير تركيز الفطر ( $10 \times 10^6$ ) على أطوار اللحم المختلفة عند درجة حرارة  $20 \pm 2$  درجة مئوية ورطوبة نسبية ٩٠%، ووجد ان متوسط نسبة الموت بالنسبة للبيض و الأطوار غير الكاملة والأطوار الكاملة قد بلغت ١٦,٧٧% و ٣٣,٣٤% و ٤٠,٤٠% بعد ثلاثة أيام على التوالي بينما ارتفعت الى ٧٣,٣٣% و ٦٣,٣٤% و ٨٥,٦٧% بعد سبعة أيام على التوالي. كما وجد ان الرش المباشر (فى وجود اللحم) كان افضل كثيراً من الرش الغير مباشر (الرش أولاً ثم يوضع اللحم على ورقة النبات).