PHYSIOLOGICAL AND BIOCHEMICAL EFFECT OF FUNGUS *BEAUVERIA BASSIANA* ON THE ADULT FEMALE OF SPIDER MITE, *TETRANYCHUS URTICAE* (KOCH)

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Abstract

The two-spotted spider mite *Tetranychus urticae* is considered to be one of the most economically important pests , since it infests over 200 species of plants (Johnson 1991). *Beauveria bassiana* is highly control agent to this mite ,and affects its mortality ,biochemistry and physiolology. To test this effect ,the *B. bassiana* was applied at 10^5 spores / ml using dipping leaf -disc treatment method. Mortality, total proteins, carbohydrates and lipids of mite were measured; Mites adult females were susceptible to *B. bassiana*, with high mortality rate recorded. The effect of *B. bassiana* on proteins level in the mycosed extract homogenate appeared decreasing than the non-mycosed during the period of experiment. The means of total protiens are 225,220 and 183 µg/ml after 24,48 and 72hrs respectively,compared with327 µg/1000 individuals in untreated control. Effect of the *B. bassiana* on the level of the carbohydrates decreased in the treated extract homogenate than the untreated one during the period of experiment. The mean of total carbohydrates at 24,48 and 72 hrs after treatment were 142,138 and 115 µg/ml respectively, compared with149 µg/1000 ml, In untreated control. The effect of *B. bassiana* on lipids level in treated extract homogenate appeared decreasing than in untreated one during the period of experiment. The obtained result refer to the mean total lipids were 92,77, and 70 µg/ ml after 24,48 and 72 hrs , respectively compared with 88 µg/1000 individuals untreated adult females of *T. urticae*. This study improves that entomopathogenic fungus *B. bassiana* causes many biochemical and physiological changes in the mite *Tetranychus urticae*. *B. bassiana* is also considered as a safe biological control agent to the environment.

Key words: fungus, *Beauveria bassiana*, mite, *Tetranychus urticae*, physiology, biochemistry, biological control.

INTRODUCTION

Many species of spider mites especially of the genus *Tetranychus* are of economic importance in many regions of the world. *Tetranychus urticae* (Koch), the two-spotted spider mite, infests over 200 plant species worldwide (Johnson 1991).

This species *T. urticae*, has been known as a worldwide pest of a wide range of horticultural crops both outdoors and in the green houses.

Fungi pathogen to mites play an important role in the regulation of natural mite population, and are sometimes able to decimate populations of phytophagous mite (Van der Geest *et al*. 2000).

Scientists all over the world have a growing interest in reducing dependence on chemical pesticides as means of controlling pests. Natural antagonists are considered most promising biological mean of pest control such as entomopathogenic fungi (Hassan 2003).

Shi and Feng (2004) reported that some of the tested isolates of *Beauveria bassiana*, *Metarihizium anisopliae* or *Paecilomyces fumosorseus* are highly effective to the eggs and females of *Tetranychus cinnabarinus*. Chandler *et al*. (2000) reviewed opportunities of exploiting fungal pathogens for biological control of Acari, including mites.

This study aims to evaluate the mortality rate, biochemical and physiological changes in the *T. urticae* (Koch) after exposed to fungus *B. bassiana*. Contributing to a better biological understanding and development of the new technique for microbial control.

MATERIALS AND METHODS

1. Rearing of *T. urticae*

The original colony of the two-spotted spider mite. *T. urticae* in this study was supplied from Acarology laboratory at Plant Protection Research Institute, A.R.C. Dokki, Giza, Egypt.

It was reared as a test mite for several generations at 25 $\pm$ 1°C away from any pesticide contamination. The mite was maintained on detached mulberry leaves with the lower surface upwards placed on moist cotton wool pads in fiber-dishes (20 cm in diameter). The cotton pads were moistened daily to avoid disc dryness, and the prevent mite escape. Castor bean leaves were changed by fresh one form time to time when necessary (Hassan 2009).

2. Entomopathogenic Fungi Strains:

Fungus *Beavuveria bassiana* (Blas) was used in this study isolated from soil in 1995, at Giza Governorate by Maha Salah El-Din Nada, Researcher, Department of Piercering and Sucking Insect, Plant Protection Research Institute, Dokki Cairo, Egypt.

Culturing of entomopathogenic fungi :

Fungus *B. bassiana* was grown using autoclaved Sabouroud Dextrose Agar Yeast media (SDAY (10 g/L peptone + 40 g/L Dextrose + 2g/L yeast extract 15g/L Agar + 1L. Distilled Water) then incubated at 25 $\pm$ 1°C for 10 days. (Devi *et al*. 2005).

3. Bioassay procedure:

Twenty fertilized of mite adult females placed on a single leaf-disc of Castor bean leaves (2.5 cm in diameter) and were kept on moist cotton wool in fiber dishes; each dish contained 5 discs as replicate. The dipping leaf-disc technique was applied. Discs were dip in 2 ml suspension of fungus for 10 sec. then left for drying and then adult females were transferred to treatment discs with five concentrations of fungus *B. bassiana* 10^6, 5 x 10^6, 10^7, 5 x 10^7 and 10^8 spores/ml and 2ml sterilized distilled water of 0.1% Triton-x100 as control. The treated adult females of mite and control were incubated at 25 $\pm$ 1°C. Mortality was assessed daily for 7 days. The percentages of mortality were determined and LC_{25}, LC_{50} and LC_{90} values were calculated according to (Finney,1971) .

4. Biochemical and physiological analysis:

Spider mites are minute so the number of mite needed in order to study these changes were 1000 adult females placed in a 1.5 microtube.

Characterization of biochemical and physiological changes of adult females after infection with *Beauveria bassiana* .

1- Preparation of mites for analysis:

Mites were prepared as described by (Amin, 1998). They were homogenized in distilled water (50 mg/1ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2°C in a refrigerated centrifuge. The deposits were discarded and the supernatants, which is referred as enzyme extract, are stored at least one week without appreciable loss of the enzyme activity when stored at 5°C.

2- Determination of total proteins

Total proteins were determined by the method of Bradford (1976). Protein reagent was prepared by dissolving 100 mg of Coomassie Brilliant blue G-250 in 50 ml 95% ethanol. To this solution , 100 ml of phosphoric acid (85% w/v) were added. The resulting solution (50 µl) or for preparation of standard curve 50 µl of serial concentrations containing 10 to 100 µg boving serum albumin were pipetted into test tubes the absorbance at 595 nm was measured after 2 min. and before 1 hr against blank prepared from 1 ml of phosphate buffer and 5 ml protein reagent.

3- Determination of total carbohydrates:

Total carbohydrates were estimated in acid extract of sample females of *T. urticae* by the phenol-sulfuric acid reaction of Dubois *et al*., (1956). Total

carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967). Sample of mites were homogenized in 0.3 HClO4 (5 ml) at 0 C for 1 min. insoluble matter was removed by centrifugation for 3 min. at 2000 r.p.m and washed twice in ice-cold HClO4 (5 ml) by re-dispersion and centrifugation. Hundred micro-liters of the acid extract were added into a colorimetric tube to 0.5 ml of phenol then 5 ml of concentrated sulfuric acid were added rapidly with shaking.the tube were allowed to stand 10 min. ,then they were shaken and placed for 10 -20 min. in 25 to 30°C before readings . The absorbance of characteristic yellow-orange color is measured by spectrophotometer at 490 nm against blank. Total carbohydrates expressed as: µg glucose/1000 individuals.

4- Determination of total lipids:

Total lipids were estimated by the method of Knight. *et al*. (1972) using phosphovanillin reagent prepared by dissolving of 0.6 mg pure vanillin in 10ml ethanol and completed to 100 ml with distilled water. Then 400 ml conc. Phosphoric acid were added . 250 µl of sample were added to conc. sulphuric acid (5 ml) in a test tube and heated in a boiling water bath for 10 min. After cooling to room temprature, the digestion was added to phosphovanillin reagent (6 ml). The developed color was measured after 45 min, at 525 nm by spectrophotometer against reagent blank. Optical density was compared to that of a reference standard and results expressed as µg lipids/1000 individuals.

5- Statistics:

All experiments were replicated 3 times (mites homogenates), and the results of biochemical determinations were pooled from triplicate determinations. The results were analyzed by one-way analysis of variance (ANOVA) using SAS for regression analysis (SAS Institute,2006) When the ANOVA statistics were significant ($P \leq 0.001$), means were compared by the Duncan's multiple range test. (1955).

RESULTS AND DISCUSSION

1-Bioassay procedure:

Data showed that the mortality rate of the adult females of the red spider mite *T. urticae* infected by *B. bassiana* increased with increasing concentrations of spores suspension. The LC_{25}, LC_{50} and LC_{90} were 10^5, 3.03×10^6 and 2.16×10^9 spores/ml, respectively.

2-Biochemical analysis:

Total proteins, total carbohydrates and total lipids are major biochemical components necessary for an organism development, growth and performance of its

vital activities, thus the mean value of homogenate contents of carbohydrates, proteins and lipids were estimated in adult females of *T. urticae* treated with LC_{25} (10^5 spores/ml) of fungus *B. bassiana* after 24, 48 and 72 hrs.

a- Total protein:

Data in Table (1) showed that the mean total protein reached 255, 220, 183 µg/ml after 24, 48 and 72 hrs, respectively ,compared with 327 µg/ml in untreated control. These results agree with that obtained by Mettaweh *et al*. (2001) who found that total protein in the haemolymph of treated grasshopper *Eurpepocnemis plorans* (5^{th} instar nymph) decreased than untreated ones.

Gillespie *et al*. (2000) observed reduction in total proteins content of the haemolymph of adult *Schistocereca gregaria* during the infection with the *Metarihizium anisopliae*. The losses of soluble protein from the host haemolymph during parasitism may be due to the fungus may secretes proteolytic enzymes into the haemocoel of the host and hydrolyze the host's proteins. Abd El-Kerim (2002) found a decrease in haemolymph protein in adult desert locust *S. gregaria* 3 days after inoculation with the entomopathogenic fungus, *M. anisopliae*. This study agree with the obtained data in this study.

b- Total carbohydrates:

The results obtained for total carbohydrates at 24, 48, 72 hrs after treated with LC_{25} of *B. bassiana* are shown in Table (1). It was obvious that mean total carohydrates were highly reduced after treatment by LC_{25} of *B. bassiana* after 24 , 48 and 72 hrs were 142, 138, 115µg/ml respectively, compared with 149 µg/1000 individuals, in untreated control. Our results revealed actual decrease in total carbohydrates contents of infected hosts after 24, 48, 72 hrs from infection with *B. bassiana* these results are in agreement with (Wright, *et al*., 2004).

El-Banna *et al*. (2012) reported that actual decreased in the total carbohydrates contents of infected *S. gregaria* after 24 hrs from infection with *M. anisopliae* and this agree with our results.

El-Banna *et al*. (2012) demonstrated that decreasing of the total carbohydrates lead to decreasing in mite fitness after infection with the fungus and may the fungus causes physiological imbalance.

C -Total lipid:

Data in Table (1) revealed that the mean total lipids were 92, 77 and 70 µg/1000 individuals after 24, 48 and 72 hrs, respectively treatment with LC_{25} with *B. bassiana* compared with 88 µg/1000 indviduals untreated adult females of *T. urticae*.

Seyoum et al. (2002) found that mycosed locusts have significantly lower haemolymph lipids and carbohydrates concentrations than the control and the present study agree with.

El- Banna et al. (2012) reported that decreasing in total lipids in 5th instar of *S. gregaria* infected with *M. anisopliae* and that may due to metabolite depletion by the fungus (parasite) could cause physiological imbalances in the host that lead to changes in enzymes activities and a reduction in hacmolymph protein, carohydrates and lipid contents.

Table 1. The effect of *B. bassiana* (10^5 spores/ml) on total proteins, total carohydrates and total lipids contents in treated adult females of *T. urticae*

Time -hours	Total proteins	Total carbohydrates	Total lipids
	Means ± SE		
24	255±24^{b}	142±7.5^{ab}	92±7^{a}
48	220±23^{c}	138±5^{b}	77±4^{b}
72	183±11^{d}	115±11^{c}	70±4^{b}
Control	327±23^{a}	149±14^{a}	88±4^{a}
L.S.D	40.107	20.3	9.75
F	124.68	27.8	55.3

Values represent mean of three separated groups ± SE, P≤ 0.001 highly significant

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التاثير الفسيولوجي والبيوكيميائي لفطر المسكاردين الابيض على الحلم العنكبوتي ذي البقعتين

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يعتبر الأكاروس الأخضر ذي البقعتين *Tetranychus urticae* من أهم انواع الحلم اقتصاديا، حيث أن هذا الحلم يصيب أكثر من ٢٠٠ نوع من النباتات ' كذلك فطر المسكاردين الابيض عامل مكافحة حيوي ذات فاعلية جيدة .

ولاختبار تأثير فطر المسكاردين الأبيض على الاكاروس فقد تم استخدام تركيز ١٠^٥ جرثومه/مل وتطبيقه بطريقة الغمر لقرص ورقة نبات التوت و تقدير نسب موت هذا الأكاروس . وكذلك تأثيره البيوكيميائي والفسيولوجي:

حيث تم تقدير نسبة كلاً من: البروتينات الكلية والكربوهيدرات الكلية وكذلك اللبيدات الكلية لهذا الاكاروس. و اظهرت النتائج حساسية هذا الاكاروس للفطر و خاصة الاناث البالغة حيث سجلت معدلات موت مرتفعة نتيجة المعاملة.حيث انخفض متوسط البروتينات الكليه ليصل الى ٢٢٥و٢٢٠و١٨٣ميكروجرام/١٠٠٠انثى بالغه وذلك بعد ٢٤ و٤٨ و٧٢ ساعه بعد المعامله على التوالى مقارنة بالعينه غير المعاملة والتي كان متوسط البروتينات الكليه بها ٣٢٧ ميكروجرام/انثى بالغه.

وكان تاثير الفطر ايضا سلبيا مما ادى لانخفاض كمية الكربوهيدرات في المستخلص المتجانس للاكاروس خلال فترة المعاملة. حيث وصل الى١٤٢و١٣٨و١١٥ ميكروجرام/ ١٠٠٠انثى بالغه بعد ٢٤ و٤٨ و ٧٢ ساعه من المعامله على التوالى مقارنة بالعينه غير المعامله والتي كانت نتيجتها ١٤٩ ميكروجرام / ١٠٠٠انثى بالغه وقد وجد ايضا ان الفطر يسبب انخفاض في مستوى اللبيدات في الافراد المعاملة مقارنة بالافراد غير المعاملة.حيث كانت ٩٢ و٧٧ و٧٠ ميكروجرام/١٠٠٠انثى بالغه بعد ٢٤ و ٤٨ و ٧٢ ساعه بعد المعامله بالفطر على التوالي وذلك مقارنة بالعينه غير المعامله حيث كانت٨٨ ميكروجرام/١٠٠٠ انثى بالغه.

واثبتت الدراسة تاثر الوظائف الحيوية داخل الاكاروس(*T. urticae*) نتيجة المعاملة بالفطر (*B. bassiana*) مما يجعله احد عناصر المكافحه الحيوية و الامنة على البيئة.