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BIOCHEMICAL IMPACTS OF FUNGUS *Metarihizium anisopliae* ON THE ADULT FEMALES OF THE TWO SPOTTED SPIDER MITE *Tetranychus urticae* KOCH.

Mikhail, W.A.[1]; H.M. Sobhy[1]; Maha S. Nada[2]; M.E. El- Naggar[2] and Rania H. Mahmoud[2]

1-Natural Resources Dept. , Inst. African Research & Studies ,Cairo Univ.,Egypt.

2- Plant Protection Research Institute, A.R.C.

ABSTRACT

The two-spotted spider mite *Tetranychus urticae* koch is a very destructive pest causing considerable loss to crops. *Metarihizium anisopliae* fungus strain is highly specific biopesticide to mites. The effect of fungus *M. anisopliae* was studied on the major components of the two-spotted spider mite, adult females reared in laboratory and treated by LC_{25} (4 x 10^6 spores/ml). Mite adult females were susceptible to *M. anisopliae* with high mortality rate recorded. Total mites protein, carbohydrates and lipids were declined in the treatment. . The effect of *M.anisopliae* 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 149,148and85µg⁄1000 individuals after 24,48 and 72hrs respectively,compared with165 µg⁄1000 individuals in untreated control. Effect of the *M.anisopliae* 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 24,48and 72 hrs after treatment were 96,92 and 88 µg⁄1000 individuals respectively ,compared with 95 µg⁄1000 individuals, In untreated control. The effect of *M.anisopliae* 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 56,50,and 39 µg⁄1000 individuals after24,48 and 72 hrs ,respectively compared with59 µg⁄1000 individuals untreated adult females of *T. urticae* . The results showed biochemical changes in the components of adult females of mite *Tetranychus urticae* due to the fungus treatment.

Keywords : biochemical impact fungus , *Metarihizium anisopliae* , mite ,*Tetranychus urticae* .

INTRODUCTION

Spider mites are the most common mites attacking economic plants and the two-spotted spider mite *Tetranychus urticae* koch is the one of the most economically important one. This mite is also a serious pest in greenhouses as well as on field grown crops (Hassan 2003).A number of vegetable crops such as tomatoes ,squash , eggplant ,cucumber are also subjected to the twospotted spider mite infestation and damage. One isolate of the entomopathogens has also proved potential for spider mite. The dipping leaf-disc technique in suspension of fungus *Metarhizium. anisopliae* is highly infective to adult females of *T. urticae* (Shi and Feng 2004).

Wei – Bing Shi and Ming-Guang Ferg (2009) reported that the effect of fungal infection on the reproductive potential of two-spotted spider mite. *T. urticae* was evaluated as part of the full biocontrol potential of *M. anisopliae*,

mite females were exposed to the sprays of *M. anisopliae* at different concentration and the results showed the great affect of the fungus on the adult females and their fecundity. Fungi pathogen to mites play an important role in the regulation of natural mite population, and are sometimes able to decimate populations of phytopagous mite (Van der Geest *et al*. 2000).

The aims of this study were to evaluate the mortality rate, biochemical and physiological changes in the *T. urticae* Koch after treatment with the fungus *M. anisopliae*.

MATERIALS AND METHODS

1- Rearing of *T. urticae*

The original colony of the two-spotted spider mite *T. urticae* koch 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 away from any pesticide contamination. The mite was maintained on detached mulberry leaves with the lower surface up wards 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. Mulberry leaves were changed by fresh one from time to time when necessary (Hassan 2009).

2- Entomopathogenic fungi strains:

Fungus *M. anisopliae* was used in this study isolated from soil in 1995, at Giza Governorate by Dr. Maha Salah El-Din Nada,Researcher on Department of Piercering and Sucking Insect, Plant Protection Research Institute, El-Dokki Giza, Egypt.

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

3. Bioassay procedure:

Twenty fertilized mite adult females placed on a single leaf-disc of mulberry (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 *M. anisopliae* 10^6, 5 x 10^6, 10^7, 5 x 10^7 and 10^8 spores/ml., and 2 ml sterilized distilled water of 0.01 % Triton x-100 as control. The treated adult females of mite and control were incubated at 25 ± 1°C. Mortality was assessed daily for 7 days. The percentage of mortality was 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 micro tube.

a-characterization of biochemical and physiological changes of adult females after infection with *M. anisopliae*.

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 refrigerated centrifuge. The deposits were discarded and the supernatants, which is referred as enzyme extract, can be stored at least on 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 100mg 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 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,t hen 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 digest was added to phosphovanillin reagent (6 ml). After 45 min, the developed color was measured at 525 nm 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 3-4 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.

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 *M. anisopliae* increased with increasing concentrations of spores suspension. The LC_{25},LC_{5o} and LC_{90} were 4 x 10^6, 2 x 10^8 and 6.6 x 10^{11} 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} (4 x 10^6 spores/mL) of fungus *M. anisopliae* after 24, 48 and 72 hrs.

a-Total protein:

Data in Table (1) showed that the mean total protein reached 149, 148 and 85 µg/1000 individuals after 24, 48 and 72 hrs, respectively, compared with 165 µg/1000 individuals in untreated control. These results agreed with that of Mettaweh *et al*., (2001).

Who found that total protein in the haemolymph of treated grass hopper *Eurpepocnemis plorans* (5^{th} instar nymph) with the entomopathogenic fungus, *M. flavovridae* (5 x 10^6 spores/mL) decreased than the untreated ones. Gillespie *et al*. (2000) observed reduction in total proteins content of the adult haemolymph *Schistocereca gregaria* during the infection with the *M. anisopliae*. The losses of soluble protein from the host haemolymph during parasitism may be due to the fungus may secrete proteolytic enzymes into the haemocoel of the host and hydrolyze the host's proteins.

b-Total carbohdyrates:

The results obtained for total carbohydrates at 24, 48 and 72 hrs after treated with LC_{25} of *M. anisopliae* are shown in Table (1).

It was obvious that mean total carbohydrates were reduced after treatment by LC_{25} of *M. anisopliae* after 24, 48 and 72 hrs. were 96, 92 and 88 µg/1000 individuals respectively, compared with 95 µg/1000 individuals in untreated control. Our results revealed actual decreases in total carbohydrates contents of infected hosts after 24, 48 and 72 hrs from infection with *M. anisopliae*. And these results in agreement with (Wright *et al*., 2004). El-Banna *et al*. (2012) reported that actual decreased in total carohydrates contents of infected *S. gregaria* after 24 hrs from infection with *M. anisopliae*. And its agree with our results.

El-Banna *et al*. (2012) demonstrated that decreaseing of 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 lipid were 56, 50 and 39µg/1000 individuals after 24, 48 and 72 hrs respectively, treatment with LC_{25} with *M. anisopliae* compared with 59 µg/1000 individuals untreated adult females of *T. urticae*.

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 causes physiological imbalances in the host lead to changes in enzymes activities and a reduction in haemolymph protein, carbohdyrates and lipid contents.

Table (1): The effect of *M. anisopliae* (4 x 10^6 spores/mL) on total proteins, total carbohydrates and total lipids contents in treated adult females of *T. urticae*.

Hours	Total proteins	Total carbohydrates	Total lipids µ/1000 individuals
	Means ± SE		
24	149 ± 13^a	96 ± 12^a	56 ± 11^a
48	148 ± 6.5^a	92 ± 7^b	50 ± 12^b
72	85 ± 9^a	88 ± 8^c	39 ± 3^c
Control	165 ± 12^a	95 ± 8.8^a	59 ± 4^a
L.S.D.	50.13	17.8	16.7
F	33.3	8.76	17.75

Values represent means of three separated groups ± SE, ≤ 0.001 highly significant.

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التاثير البيوكيميائي لفطر *Metarihizium anisopliae* على الاكاروس الاخضر ذي البقعتين.

وفائي عازر ميخائيل* , حسن محمد صبحى*، مها صلاح الدين ندا، محمود السيد النجار** و رانيا حسن محمود**.**

*** قسم الموارد الطبيعية (الحيوانية)- معهد البحوث والدراسات و الافريقية- جامعة القاهرة.**

**** معهد بحوث وقاية النباتات- مركز البحوث الزراعية- الدقي- الجيزة.**

يعتبر الأكاروس الأخضر ذي البقعتين آفة ذات تأثير مدمر ويسبب خسارة اقتصادية كبيرة لكثير من المحاصيل.

ويعد الفطر الممرض المسكاردين الأخضر *Metarihizium anisodiae* فطر مكافح للأكاروسات الضارة بالنبات وخاصة هذا النوع من الأكاروس.

ولاختبار تأثير هذا الفطر على الأكاروس تم استخدام إناث بالغة من سلالة معملية وتم معاملتها بتركيز ٤ × ١٠^{٦} جرثومه/ملل وذلك بطريقة الغمر لقرص ورقة نبات التوت.

وقد تم تقدير نسب الموت وكذلك تقدير نسبة كلا من:

البروتينات الكلية والكربوهيدرات الكلية واللبيدات الكلية لهذا الأكاروس وأظهرت النتائج حساسية هذا الأكاروس للفطر وسجلت معدلات موت مرتفعة نتيجة المعاملة بالفطر. و خاصة الاناث البالغة حيث سجلت معدلات موت مرتفعة نتيجة المعاملة.حيث انخفض متوسط البروتينات الكليه ليصل الى ١٤٩و١٤٨و٨٥ميكروجرام/١٠٠٠انثى بالغه وذلك بعد ٢٤ و٤٨ و٧٢ ساعه بعد المعامله على التوالى مقارنة بالعينه غير المعاملة والتي كان متوسط البروتينات الكليه بها ١٦٥ ميكروجرام/ ١٠٠٠انثى بالغه.

و كان تاثير الفطر ايضا سلبيا مما ادى لانخفاض كمية الكربوهيدرات في المستخلص المتجانس للاكاروس خلال فترة المعاملة. حيث وصل الى٩٦و٩٢و٨٨ ميكروجرام/١٠٠٠انثى بالغه بعد ٢٤ و٤٨ و ٧٢ ساعه من المعامله على التوالى مقارنة بالعينه غير المعامله والتي كانت نتيجتها ٩٥ ميكروجرام/١٠٠٠انثى بالغه وقد وجد ايضا ان الفطر يسبب انخفاض في مستوى اللبيدات في الافراد المعاملة مقارنة بالافراد غير المعاملة.حيث كانت ٥٦ و٥٠ و٣٩ ميكروجرام/١٠٠٠انثى بالغه بعد ٢٤ و ٤٨ و ٧٢ ساعه بعد المعامله بالفطر على التوالي وذلك مقارنة بالعينه غير المعامله حيث كانت٥٩ ميكروجرام/١٠٠٠ انثى بالغه.وبهذا يتضح التاثير البيوكيميائي السلبي لفطر المسكاردين الاخضر على الاكاروس الاخضر ذي البقعتين.

حيث أدى إلى تغيرات بيوكيميائية وفسيولوجية للأكاروس مما يجعل الفطر أحد عناصر المكافحة الحيوية الفعالة والامنه على البيئة.