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RESPONSE OF GREEN STINKBUG *Nezara viridula* (LINNAEUS), TO THE ACTIVITY OF ENTOMOPATHOGENIC FUNGI *Beauveria bassiana* AND *Metarhizium anisopliae*

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ABSTRACT

Greenbug *Nezara viridula* L. have wide host range more than 30 families and feed on all parts of plant causing high economic damage. Also, it can transmit plant pathogens like bacteria and fungus. Laboratory bioassay of the two entomopathogenic fungi; *Beauveria bassiana* and *Metarhizium anisopliae* were tested against *N. viridula* adult and evaluated their effect of some biological aspects after 24 , 48 and 72 hours from treatment with 10^7 spores/ml. Result revealed that *M. anisopliae* treatment had higher activity than *B. bassiana* treatment. The LC_{50} values were $1.4x10^6$ and $2.05x10^7$ spores/ml, respectively and the LT_{50} values were 4.47 and 5.29 days, respectively. Results obtained showed that *M. anisopliae* caused decrease in each total protein, carbohydrates and lipids contained than *B. bassiana* . In case of *M. anisopliae* treatment the mean of total protein content was 19.67, 15.6 and 19.77 mg/g.b.wt after 24, 48 and 72 hours, respectively. However in case of *B. bassiana* mean of total protein , content recorded 20.83, 24.33 and 25.67 mg/g.b.wt after 24, 48 and 72 hours, respectively whereas the untreated check was 27.3 mg/g.b.wt. The mean of total carbohydrates was 16.63 for the check untreated and recorded 12.47, 10.9 and 12.5 mg/g.b.wt in case of *M. anisopliae* treatment while these values were 15.3, 16.27 and 16.27 mg/g.b.wt after 24, 48 and 72 hours, respectively in case of *B. bassiana* . Total lipids recorded highly reduction in case of *M. anisopliae* content which being were 5.3, 4.63 and 5.1 mg/g.b.wt after 24, 48 and 72 hours compared with 8.63 mg/g.b.wt of untreated. While total lipids showed slight significant decrease after 24 hour in case of *B. bassiana* treatment and no significance after 48 and 72 hours which recorded 6.73,7.93 and 8.7 mg/g.b.wt, respectively. *M. anisopliae* treatment caused higher reduction in trehalase activity of *N. virdula* adults after 24, 48 and 72 hours where the activity levels were 489.67, 390.67 and 161.33 ug glucose/min/g. b. wt, respectively than *B. bassiana* where the enzymatic activity recorded 273.67, 359 and 506.33 ug glucose/min/g. b. wt, respectively. On the other hand, treatment with *M. anisopliae,* induced higher reduction in invertase activity for *N. virdula* after 24, 48 and 72 hours where the enzymatic activity exhibited 401.33, 304 and 258.67 ug glucose/min/g. b. wt, respectively compared with *B. bassiana* (360, 383.67 and 412 ug glucose/min/g. b. wt, respectively). It could be concluded that *M anisopliae* showed more virulence and its effect on biochemical aspects under the study than that of *B. bassiana.*

INTRODUCTION

Phytophagus green stinkbugs *Nezara viridula* L. (Heteroptera: Pentatomidae) are one of the largest families with over 4000 described species. Among the several pentatomid pests of legume crops, the southern green stink bug *Nezara virdula* which is consider most important pest

(Meglič *et al*., 2001). This species feeds on plant species in more than 30 families, and causing a major problem in soybean and other crops (panizzi *et al*., 2000). Green stinkbugs attack host plants by inserting their piercing sucking mouthparts into tissues and introducing digestive enzymes. It feeds on all parts of plant, including stems, leaf veins, growing shoots, immature fruits, seeds and even flowers (Meglič *et al*., 2001). In addition, holes made by their styles may allow the entrance of microorganisms that affect fruit quality (Panizzi *et al*., 2000). Medrano *et al.*, (2009) reported that the southern green stink bug *N. viridula* can transmit *Pantoea agglomerans* (Ewing and Fife), an opportunistic bacterium, into green cotton bolls resulting in plant disease.

Microbial control with entomopathogenic fungi is an alternative to conventional insecticides because it minimized adverse effects on beneficial insects (Sosa-Gómez and Moscardi 1998).

Entomopathogenic fungi are different in pathogenicity than bacteria and virus in that they infected insects by breaching the host cuticle to enter the insect hemocoel, while other microorganisms enter by ingestion through mouth and then caused disease. The cuticle is composed of chitin and protein surrounded by wax, lipid layer or fatty acids. Entomopathogenic fungi secrete extracellular enzymes protease, chitinases and lipases to degrade the major constituents of the cuticle and allow hyphal penetration (wang *et al.,* 2005). The objectives of this study are to evaluate the pathogenicity of *B. bassiana* and *M. anisopliae* against *N. viridula* adults and understanding their effect on some biological aspects, in the treatment insect.

MATERIALS AND METHODS

1- Insect Maintenance

Individuals of the green stink bug, *Nezara viridula* L. insect were collected from the Faculty of Agriculture of Experimental Station, Giza and maintained on the potted broad bean *Vicia fabae* L. that supplemented with fresh green beans *Phaseolus vulgaris* L. These fresh green beans were renewed every two days intervals. Potts were covered with cylindrical glass 15cm in diameter, 22cm in length and covered with muslin. The deposited egg-masses were collected daily and placed in Petri dishes containing pieces of moistened cotton wool, supplemented with fresh green beans until hatching. Finally adult insects were collected and used for the following experiments (Nada 2006).

2- Entomopathogenic fungi isolate

Two isolates of entomopathogenic fungi used in this study *Beauveria bassaina* was originally isolated from soil at Dakhalia Governorate, and *Metrahizium anisopliae* was isolated from soil at Giza governorate, Egypt. According to method described by Nada (2006), the fungus was grown on autoclaved Sabourad dexstrose yeast agar (SDAY), containing 1% peptone, 0.2% yeast extract, 4% dextrose and 1.5% agar dissolved in 1L distilled water and incubated for two weeks at 25±1ºC .

3- Bioassay procedure

Spores were harvested by rinsing with sterilized aqueous solution of 0.02% Tween 80, then filtered through cheese cloth to reduced mycelium clumping. The spores were counted in the suspension using a haemocytometer (Neubauer improved HBG, Germany 0.100 mm X 0.0025 mm^2). Five concentrations of 10^6, $5X10^6$, 10^7, $5X10^7$ and 10^8 spores/ml of each isolate were prepared. Aqueous solution of 0.02% Tween 80 was used as control.

Adults of *N. virdula* were contaminated by dipping method technique for three seconds in one of each fungi conidia suspension and in sterile aqueous solution of 0.02% Tween 80 for control, then maintained in Petri dishes prepared with saturated filter paper, and supplemented with fresh green beans which were renewed every two day intervals. Five replicates from each concentration as well as the control were prepared with 2 individuals of adults for each Petri dish. Mortality was assessed *M. anisopliae* for seven days after treatment.

Biochemical studies:

Adults were treated with 10^7 spores/ml of the two the examined entomopathogenic fungi, *B. bassiana* and *M. anisopliae* individually for determining some biochemical aspectsincluded trehalase, total carbohydrates, phosphates and inorganic phosphorus and total proteins, determination as following:

1- Insects were excised appendages, rinsed with normal saline and homogenized for biochemical analysis using a chilled glass Teflon tissue homogenizer (St-2Mechanic-Preczyina, Poland) as described by Amin (1998).
2- Preparation of insects for analysis: the insect were homogenized in distilled water (50mg/1ml). Homogenates and centrifuged at 8000 r.p.m. for 15min at 5ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants were kept in deep freezer at -20 ºC till use. Double beam ultraviolet /visible spectrophotometer (Spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds.

Determination of total proteins:

Total proteins were determined according to Bradford (1976). Protein reagent was prepared by dissolving 100mg of coomassie Brilliant blue G-250 in 50ml 95% ethanol. 100ml 85% (w/v) phosphoric acid were added to the reagent solution. The resulting solution was diluted to final volume of liter. Five millimeters of protein reagent were added to the test tube and the contents were mixed either by inversion or vortexing. The absorbance at 595 nm was measured after 2 min and before 1hr against blank prepared from 1 ml of phosphate buffer and 5ml protein reagent.

Determination of total carbohydrates:

Total carbohydrates were estimated in acid extract of green bug tissues by the phenol-sulphuric acid reaction of Dubois *et al*., (1956). Total carbohydrates were extracted from the green bug and prepared for assay according to Crompton and Birt (1987).

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 gm pure vanillin in 10ml ethanol and completed to 100ml with distilled water, then 400 ml of concreated. Phosphoric acid was added. 250 µl of sample were added to concreted sulphuric acid (5ml) in a test tube and heated in aboiling water bath for 10min. After cooling to room temperature, the digest was added to phosphovanillin reagent (6ml). After 45min, the development color was measured at 525nm against reagen blank. Optical density was compared to that of a reference standard and results express as mg lipids/ml haemolymph.

Effect of *M. anisopliae* and *B. bassiana* on *N. virdula* on Digestive enzymes:

Digestive enzymes were determined according to the method described by Ishaaya and Swirski (1976) and modification by Amin(1998), using trehalase, Sucrose and soluble starch as substrates for trehalase and invertase.

Statistical analysis

The corrected mortality percentages were statistically computed according to Finney (1971) to determined the LC_{50} values against *B. bassiana* and *M. anisopliae* . Obtained data were statistically analyzed using one way analysis of variances (ANOVA and the means were compared using the least significant differences test (L.S.D.,$p \leq 0.05$) using SAS program (SAS Institute, 1988).

RESULTS AND DISCUSSION

Susceptibility of *Nezara virdula* against *Metarhizium anisopliae* and *Beauveria bassiana*

Obtained results represented in Table (1) showed that, *M. anisopliae* had higher activity than *B. bassiana* against *N. virdula* adults. The correspondents LC_{25}, LC_{50} and LC_{90} were $1.39x10^4$, $1.4x10^6$ and $8.97x10^9$ spores/ml, respectively for *M. anisopliae* and $6.75x10^5$, $2.05x10^7$ and $1.34x10^{10}$ spores/ml, for *B. bassiana* respectively. The results agree with Sosa-Gómez and Moscardi (1998) and Ihara *et al.,* (2001), they reported that *M. anisopliae* was found to be more virulent to the green bug *adults N. virdula* than *B. bassiana.* In contrast Nada (2006) reported that adult of *N. virdula* was more susceptible to *B. bassiana* (isolated from *N. virdula* adults) than *M. anisopliae* (isolated from soil).The lethal time LT_{25} were 2.8 and 3.6 days for *B. bassiana* and *M. anisopliae,* respectively. However the lethal time LT_{50} and LT_{90} were 5.29 and 17.9 days for adult infected with *B. bassiana* compared with 4.47 and 6.71 days, respectively, in case of *M. anisopliae* infection. The results indicate that *B. bassiana* caused rapid mortality after three days than of those caused by *M. anisopliae.* In contrast after three days the mortality was faster when treated with *M. anisopliae.* The obtained results are going in line with those reported by Nada (2006) who reported that *B. bassiana* needs more time to cause mortality than *M. anisopliae.*

Effect of *M. anisopliae* and *B. bassiana* on *the* total Proteins of *N. virdula*:

Proteins are fundamental components of all living cells and include many substance, such as enzymes , hormones, and antibodies, that are necessary for the proper functioning of an organism (Fagan et al., 2002). Data illustrated in Table (2) clear that, total protein levels of adults treated with *M. anisopliae* were decreased significantly than that treated with *B. bassiana*, during 24, 48 and 72 hours after treatments. Total protein levels in case of *M . anisopliae* infection were 19.67, 15.6 and 19.77 mg/g. b. wt, respectively, and were 20.83, 24.33 and 25.67 mg/g. b. wt, respectively in case of the adults infected with *B. bassiana* compared with untreated insects.

Table (1) LC values (spores/ml) and LT values (days) of entomopathogenic fungi *B. bassiana* and *M. anisopliae* against *N. virdula* adults.

Entomopathogenic fungi	LC values (spores/ml)			LT values (days)		
	LC_{25}	LC_{50}	LC_{90}	LT_{25}	LT_{50}	LT_{90}
M. anisopliae	$1.39x10^{4}$	$1.4x10^{6}$	$8.97x10^{9}$	3.6	4.47	6.71
B. bassiana	$6.75x10^{5}$	$2.05x10^{7}$	$1.34x10^{10}$	2.8	5.29	17.9

Reduction rates of protein were fluctuated in case of *M. anisopliae* infection however, it decreased gradually what the adults treated with *B. bassiana.* This findings are agreed with that obtained by Robert *et al*., (2002) and Seyoum *et al*., (2002) they reported that infection of the desert locust *Schistocerea gregaria* by the fungus *M. anisopliae* caused a decline in protein content after two days from infection. The obtained date are accordance with those findings by (Mettaweh *et al*., 2001) they reported that treatments with entomopathogenic fungi *M. anisopliae* and *B. bassiana* to 5^{th} nymps instars of desert locust, *S. gregaria* during all periods post inoculation decreased the protein levels in the haemolymph. They also added that *M. anisopliae* was more active than *B. bassiana* in reducing the levels of the protein gained by insects growth. Decreasing the total protein contents may lead to death for the treated insects and this may be one reason of insect mortality. Elbanna *et al.* (2012) reported that lack of protein content caused retardation of many physiological processes in insects required protein to promote ovulation and egg development.

Effect of *M. anisopliae* and *B. bassiana* on total carbohydrates in *N. virdula*:

Carbohydrates providing the major source of energy production for development and growth living cells (Lee *et al* ., 2002), and serve as structural building blocks of cells and components of numerous metabolic intermediates (Wang *et al*., 2007) . Data represented in Table (3) revealed that total carbohydrates level decreased significantly when the adults of *N. virdula* treated with *M. anisopliae* than those infected by *B. bassiana*. In case of treating adults with *M. anisopliae* during 24, 48 and 72 hours carbohydrates level were 12.47,10.9 and 12.5 mg/g.b.wt, respectively , while there was significant effect in treated adult with *B. bassiana* after 24h, and it

was slight different significant after 48 and 72 hours (15.3, 16.27 and 16.27 mg/g. b. wt, respectively) as compared with untreated.

Table (2) Effect of *M. anisopliae* and *B. bassiana* on total Proteins contain in *N. virdula*

Entomopathogenic fungi 10^7 spores/ml	Entomopathogenic fungi 10^7 spores/ml	Entomopathogenic fungi 10^7 spores/ml	Entomopathogenic fungi 10^7 spores/ml
M. anisopliae	24	19.67 ± 1.69^{C}	27.94
	48	15.6 ± 0.87^{D}	42.85
	72	19.77 ± 2.51^{C}	27.58
B. bassiana	24	20.83 ± 1.59^{C}	23.69
	48	24.33 ± 0.99^{B}	10.87
	72	25.67 ± 1.31^{AB}	5.97
untreated		27.3 ± 0.98^{A}	0
F		2.6572	
L.S.D		21.64	

[a] Mean followed by the same letter are not significantly different at level 5% and L.S.D. was 2.6572

Table (3) Effect of *M. anisopliae* and *B. bassiana* on total carbohydrates in *N. virdula*

Entomopathogenic fungi 10^7 spores/ml	Hours after treatment	Total carbohydrates (mg/g.b.wt)	Reduction%
M. anisopliae	24	12.47 ± 0.89^{C}	25.01
	48	10.9 ± 0.56^{D}	34.46
	72	12.5 ± 0.46^{C}	24.83
B. bassiana	24	15.3 ± 0.56^{B}	7.99
	48	16.27 ± 0.56^{AB}	2.16
	72	16.37 ± 0.87^{AB}	2.16
untreated		16.63 ± 1.14^{A}	0
F		28.34	
L.S.D		1.3227	

[a] Mean followed by the same letter are not significantly different at level 5% and L.S.D. was 1.3227

Effect of *M. anisopliae* and *B. bassiana* on total Lipids in *N. virdula*:

Lipids are important source of energy reserves compared to carbohydrates. Lipids can supply as much as eight time more energy per unit weight (Beenakkers *et al*., 1985 and Ali, 2011). Panizzi and Hirose 1995 mentioned that severe species store lipids which are used during flight hibernation and starvation . The obtained data illustrated in Table (4) showed that total lipids decreased significantly when adults treated with *M. anisopliae* and their no significant differences during 24, 48, 72 hours , the corsponding amounts of toal lipids were 5.3, 4.63 and 5.1. mg/g. b. wt, respectively. In case of adult treatment with *B. bassiana* results appeared slightly decreased during 24h,while there is no significant during 48 and 72 hours compared with the untreated adults (6.73, 7.93, 8.7 and 8.63 mg/g. b. wt, respectively). The results are agreed with Sewify and Moursy (1993), they found that

reduction of total crude lipids among cabbage aphid, *Brevicoryne brassicae* infected with *Verticillium lecanii* and mentained that may be due to their use and consumption by the germinated spores and conidial of fungus which require it for nutrition . Mettaweh *et al.,* (2001) reported that 5th nymphal instar of the grasshopper, *Euprocnemis plorans* after infection with *M. anisopliae* and *B. bassiana* caused reducation haemolymph lipids compared with the untreated one. Also, Seyoum *et al*., (2002) reported that adult of *S. gregaria* at 3 days after inoculation with *M. anisopliae* var. *acridum,* had significantly less carbohydrates and lipids in the haemolymph than control.

In this field of study, Ahmad(2009) reported that the *M. anisopliae* acts mainly on the haemolymph of the inoculated 5th nymphal instar of the desert locusts, *S. gregaria* causing decrease in protein, carbohydrate and lipid levels. *M. anisopliae* could affect the energy reserves in the haemolymph of the desert locust control and kill them. Mikhail *et al*,. (2015a,b) represented that treatment of the two spotted mite *Tetranychus urticae* (koch) with *M. anisopliae* and *B. bassiana* caused actual decreased in the total protein , carbohydrates and lipids .

Table (4) Effect of *M. anisopliae* and *B. bassiana* on total lipids in *N. virdula*

Entomopathogenic fungi 10^7 spores/Im	**Hours after treatment**	**Total lipids (mg/g.b.wt)**	**Reduction%**
M. anisopliae	24	5.3 ± 0.61^{C}	38.59
	48	4.63 ± 0.15^{C}	46.35
	72	5.1 ± 0.46^{C}	40.90
B. bassiana	24	6.73 ± 0.66^{B}	22.02
	48	7.93 ± 0.26^{A}	8.11
	72	8.7 ± 0.59^{A}	-0.81
untreated		8.63 ± 0.59^{A}	0
F		39.40	
L.S.D		0.8381	

[a] **Mean followed by the same letter are not significantly different at level 5% and L.S.D. was 0.8381**

Effect of *M. anisopliae* and *B. bassiana* on Digestive enzymes in *N. virdula*:

Trehalase activity:

The results presented in Table (5) revealed that Trehalase activity significantly decreased in adults of *N. virdula* treated with *M. anisopliae* during 24, 48 and 72 hours the crosponding enzyme activity levels were 489.67, 390.67 and 161.33 ug glucose/min/g.b.wt, respectively as compared with control. Also, there was significance difference in the treated adult with *B. bassiana* during 24 and 48 hours after treated but slight significance was occurred after 72hours where the enzymatic activity being 273.63, 359 and 506.33 ug glucose/min/g.b.wt, respectively as compared with control. Adult treated with *B. bassiana* decreased significants trehalase after 24h compared with treated adult by *M anisopliae* ,while trehalase was increased after 72h in case of *B. bassiana* treatment and decreased sharply with *M anisopliae*

treatment. Seyoum *et al*., (2002)reported that *Metarhizium* spp. can utilize trehalase a the sole of carbon and produced both extracellular and intracellular forms of trehalase.

Table (5) Effect of *M. anisopliae* and *B. bassiana* on Trehalase activity in *N. virdula*

Entomopathogenic fungi 10^7 spores/ml	Hours after treatment	Total Trehalase (mg/g.b.wt)	Reduction%
M. anisopliae	24	489.67 ± 9.07^{B}	6.55
	48	390.67 ± 10.50^{C}	25.44
	72	161.33 ± 19.43^{E}	69.21
B. bassiana	24	273.67 ± 15.18^{D}	47.77
	48	359 ± 13.23^{C}	31.49
	72	506.33 ± 5.50^{AB}	3.37
untreated		524 ± 21.63^{A}	
F		254.73	
L.S.D			

[a] Mean followed by the same letter are not significantly different at level 5% and L.S.D. was 25.409

Invertase activity:

The obtained data summarized in Table (6) showed that the mount of secreted invertase activity varied between the two adult treatments with *M . anisopliae* and *B. bassiana,* where the highest activity was observed for *M . anisopliae* after 48 hours which recorded the highest levels of reduction. The invertase levels recorded after 24, 48 and 72 were 401.33, 304 and 258.67ug glucose/min/g. b. wt., respectively, compared with 360, 383.67 and 412 ug glucose/min/g. b. wt, respectively for *B. bassiana* .

Generaly, in insect bodies, carbohydrates are of vital important since they can be utilized by the insect body for production of energy or conversion to lipids or proteins. Metabolism of carbohydrates is controlled mainly by trehalase, amylase and invertase that play a principle role in the digestion and utilization of carbohydrate by the insects. Trehalase has the important function for librating glucose for energy and was activated during molting to generate glucose for chitin build up.

Table (6) Effect of *M. anisopliae* and *B. bassiana* on Invertase activity in *N. virdula*

Entomopathogenic fungi 10^7 spores/ml	Hours after treatment	Invertase (ug glucose/min/g. b. wt)	Reduction%
M. anisopliae	24	401.33 ± 11.02^{BC}	8.30
	48	304 ± 21.63^{E}	30.54
	72	258.67 ± 7.77^{F}	40.89
B. bassiana	24	360 ± 9.54^{D}	17.74
	48	383.67 ± 7.23^{CD}	12.34
	72	412 ± 11.53^{B}	5.86
untreated		437.67 ± 19.86^{A}	0
F		64.18	
L.S.D		24.051	

[a] **Mean followed by the same letter are not significantly different at level 5% and L.S.D. was 24.051**

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استجابة حشرة البقة الخضراء *Nezara viridula* لنشاط اثنين من الفطريات الممرضة للحشرات *Beauveria bassiana* ، *Metarhizium anisopliae*

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تعتبرالبقة الخضراء عديدة العوائل يضم أكثر من ٣٠ عائلة وتتغذى على كل اجزاء النبات مما يتسبب عنها أضرار اقتصادية عالية. وأيضا، كما انها تنقل مسببات الأمراض النباتية مثل البكتيريا والفطريات. تم اجراء الاختبارات الحيوية باستخدام اثنين من الفطريات الممرضة للحشرات هى *Beauveria bassiana* و *Metarhizium anisopliae* لمعاملة الحشرات الكاملة وتقييم تأثيرها على بعض الجوانب البيوكميائية بعد ٢٤ و ٤٨ و ٧٢ ساعة بعد المعاملة بتركيز $١٠^{٧}$. اظهرت النتائج المتحصل عليها أن *M . anisopliae* اكثر كفاءة مرضية من *B. bassiana* . وكانت قيم LC_{50} $1.4x10^{6}$ و $٢.٠٥X١٠^{٧}$ ء (جراثيم / مل) على التوالي، وكانت قيم LT_{50} 4.47 و ٥.٢٩ (أيام)، على التوالي. اشارت النتائج أن *M . anisopliae* تسبب في انخفاض اعلى فى المحتوى الكلى لكل من البروتين والكربوهيدرات والدهون من الفطر*B. bassiana* . في حالة المعاملة ب *M . anisopliae* كان متوسط البروتين الكلي ١٩.٦٧، ١٥.٦ و ١٩.٧٧ ميكروجرام بروتين / جرام من جسم ميكروجرام الجلوكوز / دقيقة / جرام من وزن الحشرة الحشرة بعد ٢٤ و ٤٨ و ٧٢ ساعة، على التوالي. بينما في حالة *B. bassiana* كان متوسط البروتين الكلي، ٢٠.٨٣، ٢٤.٣٣ و ٢٥.٦٧ ميكروجرام بروتين / جرام من جسم الحشرة بعد ٢٤ و ٤٨ و ٧٢ ساعة، على التوالي، وكان فى الحشرات الغير معاملة ٢٧.٣ ميكروجرام بروتين / جرام من جسم الحشرة. سجل متوسط الكربوهيدرات الكلية ١٦.٦٣ لغير المعاملة بينما كانت سجلت ١٢.٤٧، ١٠.٩ و ١٢.٥ ميكروجرام كربوهيدرات / جرام من جسم الحشرة عند المعا

ملة بالفطر *M . anisopliae* و ١٥.٣، ١٦.٢٧ و ١٦.٢٧ ميكروجرام كربوهيدرات / جرام من جسم الحشرة بعد ٢٤ و ٤٨ و ٧٢ ساعة،عند المعاملة بفطر *B. bassiana* على التوالي. ادت المعاملة بفطر أن *M . anisopliae* انخفاضا معنويافى محتوى الدهون الكلية حيث بلغت ٥.٣، ٤.٦٣ و ٥.١ ميكروجرام دهون / جرام من جسم الحشرة بعد ٢٤ و ٤٨ و ٧٢ ساعة مقارنة بالحشرات غير المعالجةحيث سجلت ٨.٦٣ ميكروجرام دهون / جرام من جسم الحشرة. بينما انخفضت الدهون الكلية بدرجة معنوية طفيفة بعد ٢٤ ساعة من المعاملة بالفطر *B. bassiana* وغير معنوية بعد ٤٨ و ٧٢ ساعة حيث سجلت ٧.٩٣و٦.٧٣ و ٨.٧ ميكروجرام دهون / جرام من جسم الحشرة ، على التوالي. انخفض نشاط انزيم التريهالوز بدرجات معنوية عند الحشرات الكاملة نتيجة المعاملة بالفطر *M . anisopliae* بعد ٢٤ و ٤٨ و ٧٢ ساعة حيث بلغت ٤٨٩.٦٧، ٣٩٠.٦٧ و١٦١.٣٣ ميكروجرام الجلوكوز / دقيقة / جرام من وزن الحشرة ، على التوالي بالنسبة للفطر *B. bassiana* كانت 273،٦٧و 359 و ٥٠٦.٣٣ ميكروجرام الجلوكوز / دقيقة / جرام من وزن الحشرة، على التوالي.اشارت النتائج الى انخفاض نشاط الانفرتيز بدرجة معنوية عند الحشرات الكاملة المعاملة بالفطر *M . anisopliae* بعد ٢٤ و ٤٨ و ٧٢ ساعة حيث سجلت ٤٠١.٣٣ و ٣٠٤ و ٢٥٨.٦٧ ميكروجرام الجلوكوز / دقيقة / جرام من وزن الحشرة ، على التوالي بينما سجلت 383.68 و360 و ٤١٢ ميكروجرام الجلوكوز / دقيقة / جرام من وزن الحشرة ، على التوالي عند المعاملة بفطر *B. bassiana* يتضح من النتائج ان *M . anisopliae* كان أكثر سمية و تاثيرا على بعض النظم الكميائية ضد البقة الخضراءمن الفطر *B. bassiana*.