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Full Length Research Paper

Impact of *Metarhizium anisopliae* on Physiological and Biochemical Responses in the Fifth Instar of *Schistocerca gregaria (Orthoptera: Acrididae)*

Shereen M. Elbanna[1]*, Nabawia M. Elhadidy[2], Fayez M. Semida[1],Tayseer Abdel-Rasool[3]

[1]Department of Zoology, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt.
[2]Department of Biology, Faculty of Education, Suez Canal University, El-Areesh, Egypt.
[3]Department of Botany, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt.

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The desert locust is a very destructive herbivore causing considerable loss to crops and pastures in the arid and semi-arid areas of Africa and Asia. *M. anisopliae* fungus strain is highly specific biopesticides to locusts and grasshoppers, and is safe to non-target invertebrates. To test the effect of *M. anisopliae* fungi on the Locusts mortality, biochemistry and physiology, fungi at doses 10^3, 10^6 and 10^{12} spores/ml were applied with two different treatment methods; fungi spray and fungi soil application. The insects were treated under either non-starvation and/or starvation conditions. Mortality, total proteins, carbohydrates and lipids contents of insects were measured. All the locust nymphs were susceptible to *M. anisopliae*, with high mortality rate recorded. The insect enzymes Phenoloxidase, Peroxidase activities were affected, fluctuated between increasing and decreasing by fungi infection. Total insect proteins, carbohydrates and lipids contents were dramatically declined in all treatments. The results indicated physiological and biochemical changes in the locusts in return to fungi infection.

Keywords: *Metarhizium anisopliae, Schistocereca gregaria,* biopesticides, Biochemical changes, biological control.

INTRODUCTION

The desert locust *Schistocerca gregaria* is one of the most important pests due to its catastrophic damage to crops. The desert locust is characterized by the formation of bands of hopper and swarms of adults, which include millions of individuals which migrate crossing very long distances, invading agricultural areas and causing heavy crops loss. The costs of figurehting this upsurge was estimated by the FAO to have exceeded $400 million and had disastrous effects on the food security situation in West Africa (Ceccato *et al*., 2007). The major control strategy adopted against desert locust is based on the use of insecticides. However, besides the environmental risks associated with this method, it is costly (Steedman, 1990). All the insecticides sprayed had some potentially negative environmental effects Rembold (1994) adverted to the rapidly increasing insect tolerance against any type of neuro-toxic insecticide, and all insecticides given their wide spectrum of action undoubtedly had substantial side-effects on the non-target fauna (Müller, 1988) and alternatives to these harmful insecticides have to be found and used. One of the promising alternatives biopesticides was the fungus *Metarhizium anisopliae,* because of its high specificity and very little adverse effect on the environment (Ignacimuthu, 2008). The soil forms its normal habitat, although it does not grow saprophytically in soil but exist as dormant conidia which infect susceptible hosts on contact. Most isolates grow well between 15-30 C°, although some develop at temperatures as low as 5-10 C° and other grow even at 35-40 C°. The strategy of desert locust control is to prevent the formation of hopper bands and swarms. The aims of this study were to evaluate the mortality rate, biochemical and physiological changes in

*Corresponding Author E-mail: sh_elbana@hotmail.com

the *Schistocerca gregaria* after exposed to a biopesticide fungus *M. anisopliae* native formulated with different doses and applications under laboratory conditions, contributing to a better biological understanding and development of the new technologies for microbial control.

MATERIAL AND METHODS

Pathogen (fungi) source

Entomopathogenic fungus, *M. anisopliae.* Bioranza as commercial name was obtained from Plants Protection Research Institute, El-Dokke, Cairo, Egypt. The active ingredient of Bioranza is *M. anisopliae* (10%) formulated as WP, while the inert ingredient represented 90%; the recommended application dose is 2 gm/L.

Insect culture

Desert locusts, nymphs were obtained from a stock culture bred continuously for several generations in the gregarious phase in the department of Entomology at Faculty of Science, Cairo University. Insects were reared under controlled laboratory conditions. Conditions as described by Vanden Broeck *et al.* (1998). The cages were kept at a controlled temperature of 30°C, under a 12
h: 12 h L: D lighting regime with relative humidity ranged between 70-80%.

Treatments

1. Spray treatment

The bioassay method used in this study by Goettel and Johnson (1992). Locusts were sprayed using method of spray application which involved a direct application of conidia (water-based suspension) on the posterior dorsum of the pronotum. Each locust nymph received 1 ml of fungus suspension with fractional doses (10^3, 10^6 and 10^{12} spores/nymph) respectively. The control were treated with 1 ml of distilled water

2. Soil treatment

Aerated plastic vessels were filled to a depth of 1 cm with autoclaved sand (weight of sand 218 gm). For inoculation with three doses of fungus suspension, pipette in a volume of 200 ml distributed water equally on the sand. Final total water content of the sand was between 9 and 11% (w/w). Sixteen locust 5^{th} instars nymphs were used in each concentration with three replicates per concentration. Locusts were held in the prepared vessels and fed with fresh Clover. The applications were applied with starved and non-starved conditions.

3. Mortality evaluation

Dead insects were recorded every day and continuously removed from the bottles to avoid scavenging. Any morphological abnormalities in inoculated insects were observed. Microscopic observations and the growth of biopesticides on nymphs were confirmed. Dead nymphs were removed and surface sterilized using a method described by Lacey (1997). Dead locusts were soaked in 75% ethanol solution for few seconds and rinsed in a plenty of sterile distilled water. The cadavers were then placed into 0.5% sodium hypochlorite for two minutes, followed by two rinses with sterile distilled water then left to dry for 48h. Locusts were incubated in a petri-dish with moist cotton under sterile conditions inside the clean desiccators at room temperature to examine whether they died because of fungus infection or not.

Biochemical studies

Haemolymph sampling for the biochemical assays

This procedure was carried out only with non-starvation condition. Prior to bleeding, the locusts were chilled for 30 min. at 4 C^{o} a leg was surface sterilized with 70 % ethanol and severed between coax and femur. Twenty-four hours after application of fungus at three fractional doses, the haemolymph samples were taken from treated and control groups. The Haemolymph samples were obtained according to the method of (Abdel El-kawy 1981). Using a disposable micropipette, 5 µL sample of haemolymph was obtained from each locust (treated and untreated) as described before.

Estimation of Phenoloxidase (PO)

Phenoloxidase activity was determined as Ishaaya (1971) in a reaction mixture consisting of 0.5 ml phosphate buffer (0.1 M, pH 7), 200 µl enzyme solution and 200 µl catechol solution (2 %). Prior to the initiation of the reaction, the substrate and other ingredients of the reaction mixture were separately incubated at the optimum temperature of the reaction (25 ^{o}C). Enzyme reaction was initiated by adding catechol solution. The optical density was determined and zero adjustment was against sample blank at 405 nm.

Estimation of Peroxidase (POD)

Peroxidase activity was determined according to Vetter *et*

al. (1958). To the sample (200µl), in which the color is to be formed, the following reagents are added: 1ml of 1% o-phenylenediamine (in 95% ethyl alcohol; fresh every 4 hours) and 1ml of 0.3% hydrogen peroxide (in distilled water). The reaction is allowed to proceed for 5 minutes at which time it is stopped by adding 2 ml of saturated sodium bisulfite. The reagent blank for each sample is prepared by adding a dye, followed by the sulfite, and then by hydrogen peroxide. The enzyme is inhibited by the sulfite so that it is inactive when the hydrogen peroxide is added. The samples are centrifuged at approximately 3000 RPM for 5 minutes. The clear supernatant is decanted into a colorimeter tube and its absorbance recorded at 340µm. the colorimeter is set at 100% transmittance with the corresponding blank for each sample. The enzyme activity was expressed as the change in absorbency at (ΔOD_{340})/min/gm.

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 100ml 85% (W/V) phosphoric acid were added. The resulting solution was diluted to a final of 1 liter. Sample solution (50µl) or for preparation of standard curve 50µl of serial concentrations containing 10 to 100µg bovine serum albumin were pipette into test tubes. The absorbance at 595nm was measured after 2 min. and before 1 hr against blank prepared from 1ml of phosphate buffer and 5 ml protein reagent.

Determination of total carbohydrates

Total carbohydrates were estimated in heamolymph of 5^{th} instars of desert locust nymphs by the phenol-sulfuric acid reaction of Dubios *et al*., (1956). Total carbohydrates were extracted from the heamolymph and prepared for assay according to Crompton and Birt (1967). Samples of haemolymph (five to ten were homogenized in 0.3 HCLO4(5 ml) at 0ºC for 1 min. insoluble matter was removed by centrifugation for 3 min. at 2000 RPM and washed twice in ice-cold HCLO4 (5 ml) by re-dispersion and centrifugation. Hundred micro-liters of the haemolymph were added into a colorimetric tube to 0.5 ml of phenol (20 percent w/v). Blanks were prepared by substituting distilled water for the sugar solution. The absorbance of characteristic yellow-orange color is measured at 490 nm against blank. Total carbohydrate is expressed as: µg glucose/gm fresh weight.

Determination of total lipids

Total lipids were estimated by the methods of Knight *et al* (1992) using phosphovanillin reagent prepared by dissolving of o.6 mg pure vanillin in 10 ml ethanol and completed to 100 ml with distilled water. 250 ml 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 temperature, 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 mg lipids/ ml heamolymph.

Data analysis

All experiments were replicated 3-4 times and results of biochemical determinations were pooled from triplicate determination. The data were recorded, tabulated and subjected to statistical analysis using a software SPSS (2005) test program. The significance of the main effects was determined by ONE-way analysis of variance (ANOVA). The significance of various treatments was evaluated by Range test ($P \leq 0.01, 0.05$).

RESULTS

Effect of *Metarhizium anisopliae* by spray treatment on desert locust.

Metarhizium anisopliae affect all treated locust nymphs significantly in a dose dependent manner (Figure 1a). There was significant difference between % mortality at different three doses with ($F_{(3, 28)} = 12.13$, $P \leq 0.001$) Table 1 and Figure 1 b.

There was significant difference between Starved treated nymphs and Non-starved treated nymphs. Starved nymphs die faster than Non-starved nymphs with ($F_{(3, 28)} = 8.84$, $P \leq 0.003$) Table 1 and Figure (2a and b).

Effect of *Metarhizium anisopliae* by soil treatment on desert locust.

Infections with fungus by soil treatment induce higher and faster percentage mortality than spray treatment (Table 2). Figure 3a show the effectiveness of different three doses of *M. anisopliae* on 5^{th} instar locust nymphs. Highest dose induce mortality faster than low dose. There was significant difference in % mortality of Non-

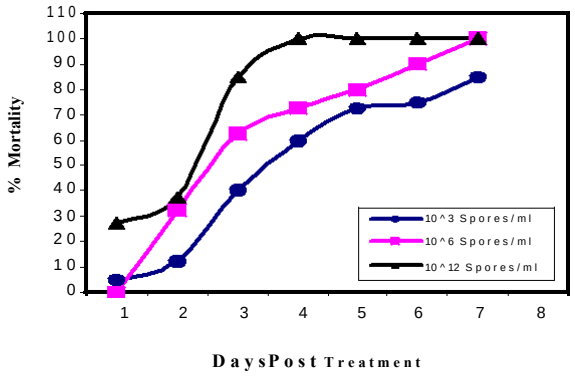

Figure 1a. Effectiveness of M. anisopliae on Non -starved 5th instar nymphs of Sch. gregaria treated as spray treatment.

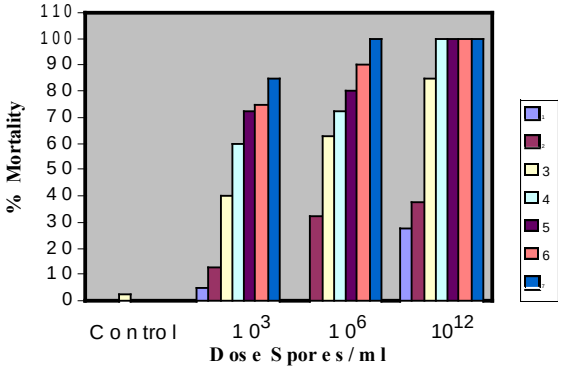

Figure 1b. Effect of M. anisopliae on mortality of Non-starved nymphs of Sch. gregaria treated as spray treatment.

Table 1. Effect of spray treatment of *M. anisopliae* on 5th instar

Application Dose	Spray treatment	
	Non-starvation	Starvation
Control	1.88±0.41	0.00±0.00
10^3 spores/ ml	54.06±11.15	62.50±14.09
10^6 spores/ ml	67.19±12.40	68.75±13.25
10^{12} spores/ ml	81.25±10.84	77.08±13.71

Values represent mean of three separated group's ± SE, P ≤ 0.001 highly significant.

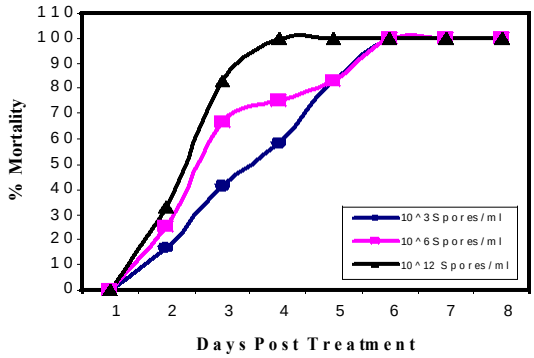

Figure 2a. Effectiveness of M. anisopliae on Starved 5th instar nymphs of Sch. gregaria treated as spray treatment.

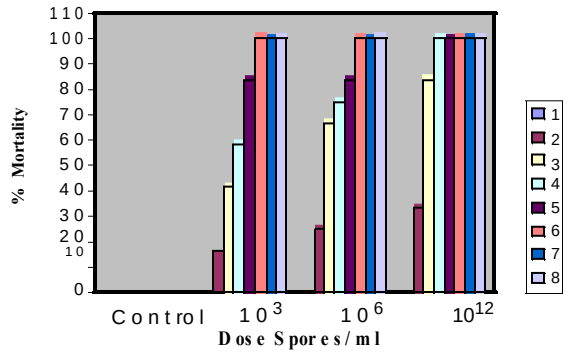

Figure 2b. Effect of M. anisopliae on mortality of Starved nymphs of Sch. gregaria treated as spray treatment.

Table 2. Effect of soil treatment of *M. anisopliae* on 5th instar of

Application Dose	Soil treatment	
	Non-starvation	Starvation
Control	0.00±0.00	3.19±1.50
10^3 spores/ ml	50.00±11. 14	63.54±14.08
10^6 spores/ ml	64.58±14.92	80.21±11.13
10^{12} spores/ ml	81.25±9.28	84.38±9.51

Values represent mean of three separated group's ± SE, P ≤ 0.001 highly significant.

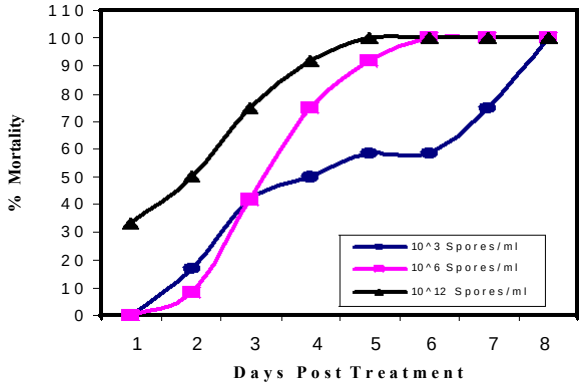

Figure 3a. Effectiveness of M. anisopliae on Non-starved 5th instar nymphs of Sch. gregaria treated as soil treatment.

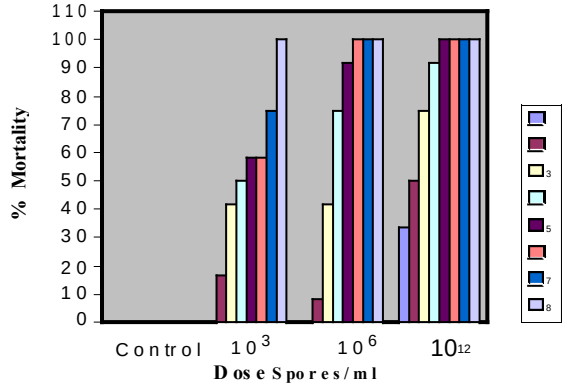

Figure 3b. Effect of M. anisopliae on mortality of Non-starved nymphs of Sch. gregaria treated as soil treatment.

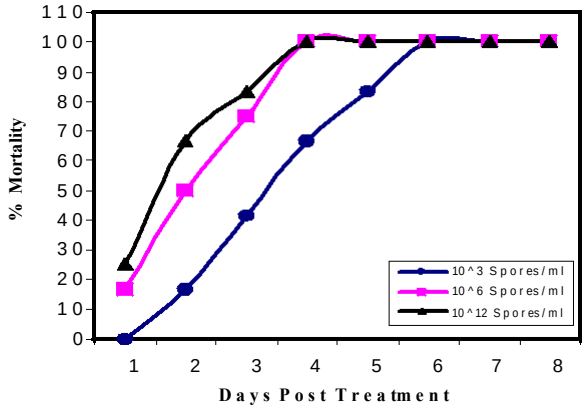

Figure 4a. Effectiveness of M. anisopliae on 5th starved instar nymphs of Sch. gregaria treated as soil treatment

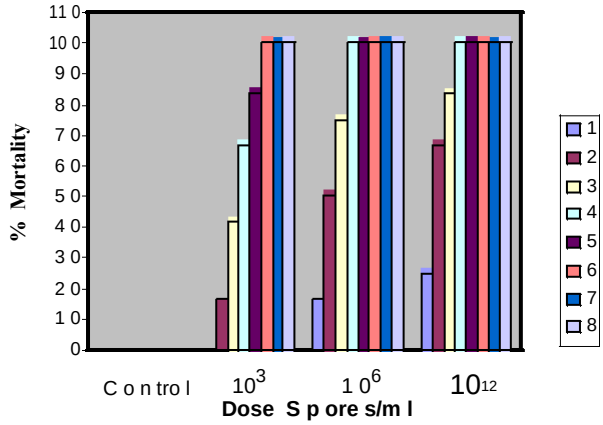

Figure 4b. Effect of M. anisopliae on mortality of starved nymphs of Sch. gregaria treated as soil treatment.

starved treated nymphs at different doses with ($F_{(3, 28)}$ = 11.35, P ≤ 0.001) as in (Table 2 and Figure 3b).

Mortality of Starved nymphs by all doses was significantly different from the control with ($F_{(3, 28)}$ =14.57, P ≤ 0.001) and easily seen in (Table 2 and Figure 4 a and b).

All treated dead nymphs appear with full symptoms of mycosis of *M. anisopliae*.

Effect of *Metarhizium anisopliae* treatment on locust's morphology:

Molting process in treated locusts was faster than control groups. The fungus producing deformed fledging, Fledging was unable to extricate from old cuticle whereas the control larvae showed 100% adult emergence. Deformation at wings, tibia and abdomen also the color turned from the normal pink to the grayish. The malformed fledging was shorter in length with crumpled wings and broken legs. The abdomen was reduced in length and the deformed wings did not reach the ovipositor. Those insects were not able to develop the coloration pattern of sexually mature females (Plate 1a and b).

Biochemical changes.

1. Phenoloxidase (PO) activity

Data represented in Table (3 and 4) Revealed that the fungus causes alteration in PO activity at all doses and both two different treatments. The enzymes activities showed sharply decline at low doses. At low dose, significant decline in PO activities was recorded with ($F_{(3, 8)}$= 175.97, P ≤ 0.001) but elevated at high dose at spray treatment. There was a significant difference in PO activities by soil treatment at dose 10^6 spores/nymph, but enzyme activities prohibit at dose 10^3 and 10^{12} with ($F_{(3, 8)}$ = 75.76, P ≤ 0.001). PO activities changed between increasing and decreasing significantly by doses and type

Table 3. PO and POD changes in 5th instar locust nymphs treated with fungus as spray treatment

Parameters (O.D. U/min/ml)	Control Mean±SE	Spray Treatment 10^3 Spores/ml	10^6 Spores/ml	10^{12} Spores/ml
Phenoloxidase	8.44±0.30	3.69±0.25	6.35±0.21	14.07±0.50
Peroxidase	6.31±0.11	4.91±0.07	6.22±0.07	20.20±0.53

Values represent mean of three separated group's ± SE, P ≤ 0.001 highly significant.
O.D. = Optical Density

Table 4. PO and POD in 5th instar locust nymphs treated with fungus as soil treatment

Parameters (O.D. U/min/ml)	Control Mean±SE	Soil Treatment 10^3 Spores/ml	10^6 Spores/ml	10^{12} Spores/ml
Phenoloxidase	10.77±0.54	5.07±0.19	12.40±0.40	7.43±0.30
Peroxidase	4.90±0.14	3.28±0.11	3.43±0.11	3.87±0.14

Values represent mean of three separated group's ± SE, P ≤ 0.001 highly significant.
O.D. = Optical Density

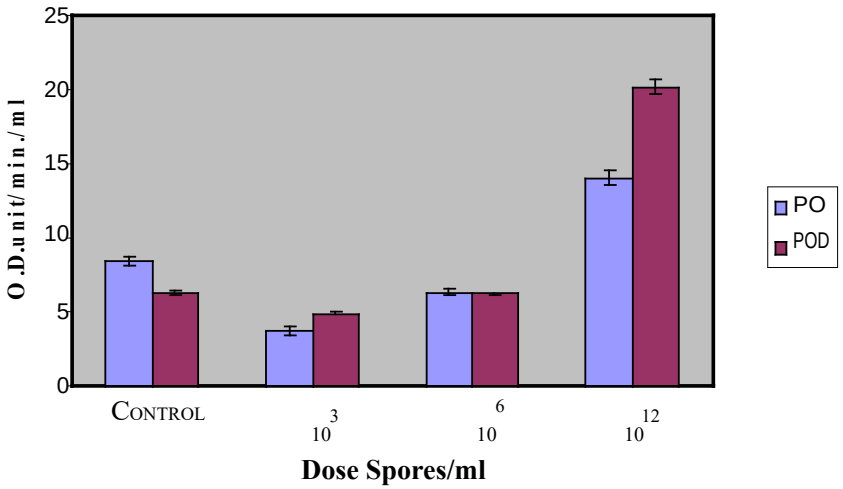

Figure 5. Effect of different doses of M. anisopliae as spray treatment on PO and POD activities of 5th instar locust nymphs, the decline is found to be statistically significant.

of treatment (Figure 5 and 6).

2. Peroxidase (POD) activity

The results revealed that there were significant differences (P ≤ 0.001) in Peroxidase activity at two different treatments and all three doses. The highest elevation in Peroxidase activity was recorded at highest dose of fungus by spray application with ($F_{(3, 8)}$ = 693.36, p ≤ 0.001) (Table 3 and 4). At soil treatment slightly prohibit in peroxidase activities at all doses with ($F_{(3, 8)}$ = 33.59, P ≤ 0.001) as in (Figure 5 and 6).

3. Total proteins, carbohydrates and lipids contents

Total proteins, carbohydrates and lipids contents were independently changed with the dose and changed with treatments manner, (Figure 7 and 8).

Total proteins, carbohydrates and lipids contents were dramatically declined in both soil and spray treatments, Table (5 and 6). Highly significant decline were recorded in protein content at all doses. The infection with *M. anisopliae* at soil treatment induced higher prohibit in total protein content than spray treatment with ($F=_{(3, 8)}$ 186.71, P ≤ 0.001) and ($F_{(3, 8)}$ = 458.21, P ≤ 0.001) respectively, Figure (7 and 8).

There were significant differences of total carbohydrates contents at all doses comparing with control (Figure 7 and 8). Soil treatments induced higher prohibit of carbohydrates contents than spray treatment with ($F_{(3, 8)}$ = 71.44, P ≤ 0.001) and ($F_{(3, 8)}$ = 90.38, P ≤ 0.001) respectively. There were slightly reductions in total lipids contents at all doses and two different treatments, Table (5 and 6). The infection with fungus by soil

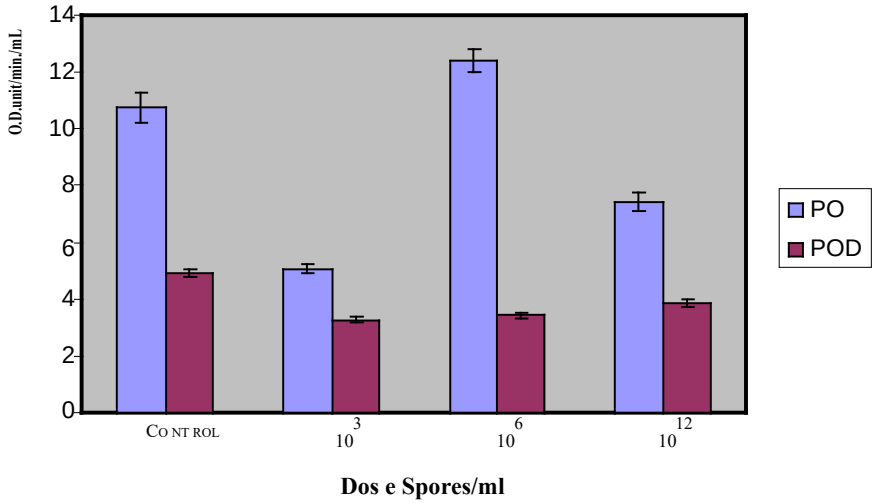

Figure 6. Effect of different doses of M. anisopliae as soil treatment on PO and POD activities of 5th instar locust nymphs, the decline is found to be statistically significant.

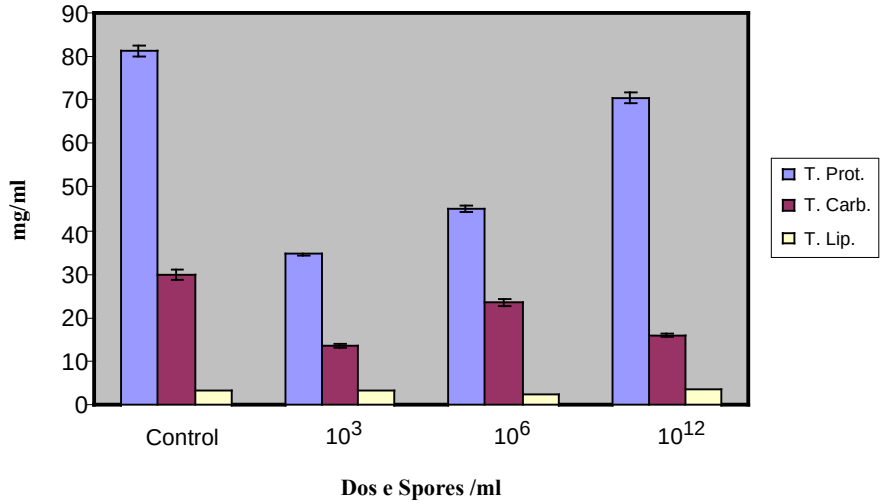

Figure 7. Effect of different doses of M. anisopliae as spray treatment on total proteins,carbohydrates and lipids contents of 5th instat locust nymphs, the decline is found to be statistically significant.

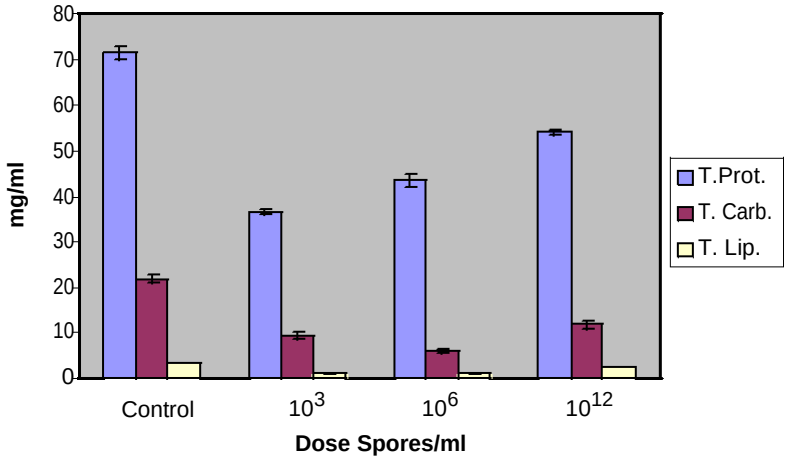

Figure 8. Effect of different doses of *M. anissopliae* as soil treatment on total proteins, carbohydrates and lipids contents of 5th instar locust nymphs, the decline is found to be statistically significant.

Table 5. Biochemical changes in 5th instar locust nymphs treated with fungus as spray treatment

Parameters (mg/ml)	Control Mean±SE	Spray Treatment 10^3 Spores/ml	10^6 Spores/ml	10^{12} Spores/ml
Total Proteins	81.20±1.24	34.50±0.32	44.90±0.85	70.50±1.32
Total Carbohydrates	29.77±1.20	13.70±0.40	23.53±0.77	16.02±0.42
Total Lipids	3.24±0.09	3.08±0.04	2.44±0.11	3.55±0.08

Values represent mean of three separated group's ± SE, P ≤ 0.001 highly significant.

Table 6. Biochemical changes in 5th instar locust nymphs treated with fungus as soil treatment

Parameters (mg/ml)	Control Mean±SE	Soil Treatment 10^3 Spores/ml	10^6 Spores/ml	10^{12} Spores/ml
Total Proteins	71.43±1.62	36.60±0.60	43.47±1.27	53.97±0.58
Total Carbohydrates	21.94±1.05	9.30±0.75	6.10±0.47	11.90±0.86
Total Lipids	3.36±0.12	1.22±0.10	1.27±0.09	2.58±0.13

Values represent mean of three separated group's ± SE, P ≤ 0.001 highly significant.

Plate (1a). Control molted 5th instar locust nymph of Sch. gregaria which sprayed with distilled water without M. anisopliae spores.

Plate (1b). Malformation of molted 5th instar locust nymphs of *Sch. gregaria* which treated with different doses of *M. anisopliae* spores.

treatment induce higher significant prohibit in total lipids contents than spray treatments with ($F_{(3, 8)} = 90.41$, $P \leq 0.001$) and ($F_{(3, 8)} = 32.18$, $P \leq 0.001$) respectively.

DISCUSSION

The present study revealed that fungi *M. anesopliae* treatments significantly suppress locusts' population, and lead to substantial reductions as in other study on *Bemisia tabaci* populations as (Lacey *et al.,* 2009). The above results revealed that the fungi affect the locusts fast in a dose dependent manner in agreement with Shaw *et al*. (2002) and Lomer *et al* (1997) confirm this results, locust mortality after infection with *M. Flavoviride* isolates induced 98-100% mortality. Smith *et al*. (2006) found 90% mortality of *Prostephanus truncatus* observed after a week treated with the highest dose of conidia of *Beauveria bassiana.* The above results indicated that the mortality of *S. gregaria* nymphs at soil treatment were higher than with the spray treatment, due to that the soil is the natural habitats of fungus. These results in agreement with (Wright, *et al.,* 2004) reported that the fungus *M. anisopliae* is a natural component of soil flora world-wide and is a causal agent of the green muscardine diseases of insects. This is in fact indicated that the matter of application method of the same fungi can affect the effectiveness of the fungal treatment. The high effectiveness of fungus at soil treatment may be due to either reduces movement or increase movement of infected hoppers. Spores can either land on locusts directly during application or can be picked up as they move through vegetation. Live spores germinate when they contact the cuticle of the locust or grasshopper. After germination, the fungus penetrates the cuticle of the insect and begins to grow within the body of its host which, once infected, reduces insect feeding and its movements. Findings in our results indicated insects less able to move agreed with Arthurs and Thomas (2001) noted the host behavioral changes of *S. gregaria* locusts infected with *M. anisopliae* increased movements within 3 days of infection. Later in infection they became sluggish and less able to move. The above results revealed starved locusts are more susceptible to fungus treatment than non-starved, this may be explained by starved locust has no food storage to withstand or tolerate the infection. This conclusion is in agreement with (Moore 2002) who noted the infection by pathogens imposes significant fitness costs on hosts, reducing survival and the rate of reproductive output. Coop and Kyriazakis (2001) reported the ability of the host to figureht and withstand infection depends on its nutritional state. This is in agreement with the previous studies have demonstrated that starvation of the host increases parasite virulence (Brown *et al*. 2000). Our results on PO and POD activities were fluctuated between decreasing and increasing at different doses and different treatments methods. This result coincided with the finding of Trudeau *et al*. (2001) reported invasion of the insect body and haemolymph occurs once the fungus has passed through the cuticle of the external insect skeleton. When conidia land on the cuticle of a suitable host, they attach and germinate, initiating cascades of recognition and enzyme activation reactions both by the host and the fungal parasite. The defensive responses to fungi infection lead to activation of the (PO) and other enzymes of cascade. This finding is in accordance (Hagstrum 1983) noted the increase in PO activity may be beneficial to *H. hebetor*, as increased levels of PO may help suppress microbial infection during the time interval, thus preserve the nutritional value of the paralyzed larva as a subsequent food source for the parasitoid. The insect cuticle, fat body and insect proteins were the barriers for insects to defend against microbes. *Metarhizium* infection may result in declining hemolymph protein and PO titers over the course of the infection until death of *Schistocerca gregaria* (Gillespie *et al.,* 2000) and *Locusta migratoria* (Mullen and Goldsworthy 2006) whereas *Beauveria* infection increases active PO levels *Melanoplus sanguinipes* (Gillespie and Khachatourians 1992). Lysozyme activity of *S. gregaria* was declined (Gillespie *et al.,* 2000) or remains unchanged *as Spodoptera exigua* (Boucias *et al.,* 1994). In addition Silva *et al*. (2000) who demonstrated that fungi infection suppress insect phenoloxidase activity. *Metarhizium anisopliae* might affect the hormonal metabolism resulting in receipt of wrong code by cells inducing to perform wrong functions. This disturbed hormonal metabolism induced by the extractive may be attributed to the malformations observed in the adult. At the present study, *M. anisopliae* severely deplete proteins in the locust *S. gregaria* haemolymph at 24 h post infection. This finding in agreement with (Gillespie *et al.,* 2000) observed reduction in total protein content of the adult haemolymph of *S. gregaria* during the course of infection with the *M. anisopliae*. The losses of soluble protein from the host' haemolymph during parasitism may be due to the parasite may secrete proteolytic enzemes into the haemocoel of the insect and hydrolyze the host's proteins. This finding agreed with that obtained by (Mckinstry and Steinaus 1970) who were able to separate electrophoretically septicaemic and fresh plasma of *G. mellonella* infected with *pseudomonas aeurginosa* and detected a reduction in the amount of protein and this agreement with (Highnam and Hill, 1969) who noted protein metabolism and excretion are all regulated hormonally and disturbance of the host's endocrine balance by the parasite could be responsible for reduced protein concentrations in the host haemolymph. Decreasing of total protein contents may lead to death of treated insects and this may be one reason of insect mortality. This conclusion supported by (House, 1963) who reported lack of protein caused retardation of many physiological processes in insects and adult insects require protein to promote ovulation and egg

development. Our results revealed actual decrease in total carbohydrates contents of infected hosts after 24 hr from infection with *M. anisopliae* at all different doses and in both different treatments. This findings in accordance with those of (Rutherford and Webster 1978) who reported reduction in the individual carbohydrates in the haemolymph of infected locusts with nematodes. Lee *et al*., (2002) reported that carbohydrate providing the major source of energy and protein being used for growth. In addition results shown slightly decreasing in total lipids contents at all doses and through two different treatment spray and soil. Hawlitzky and Boulay (1986) reported significant declines in lipids of parasitized larvae *Anagasta kuehniella* by *Phanerotoma flavitestacea,* are a normal feature with the general scope of endoparasite action on host chemical composition. It was concluded from our results that decreasing in total carbohydrates and lipids contents lead to decreasing in locust fitness especially flight capability. This conclusion in agreement with (Seyoum *et al*., 1994) reported that adults of *S. gregaria* infected with *M. anisopliae* had reduced flight capability. Metabolite depletion by the parasite could cause physiological imbalances in the host that lead to changes in enzyme activates and a reduction in haemolymph protein, carbohydrates and lipid contents. The observed reduction in insect survival and suppression in biochemical parameters explain the reason of high mortality rate caused by *M. anisopliae* and a drastic effect of this fungi on the population density of *S. gregaria* nymphs.

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