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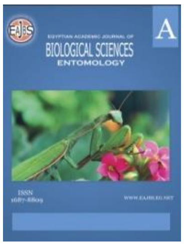

The Effect of an Entomopathogenic Fungus, *Beauveria bassiana* on Flight Muscles of The Desert Locust, *Schistocerca gregaria* Forskal

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ABSTRACT

The infecting flight muscles of adult desert locust *Schistocerca gregaria* with the entomopathogenic fungus *Beauveria bassiana* were studied. The entomopathogenic fungus caused mortality percentages 85.56 and 81.11% in 5th nymphal instar and adults after 15 days of treatment; also, LT_{50} was 9.17 and 10.07 days.

The histopathological studies of treated muscles showed that the nucleus, mitochondria, myofibrils and vacuoles were highly deformed. Estimation of total carbohydrates, total lipids and total protein were carried out. The results showed that the Haemolymph content of treated insects was highly affected and all the studied parameters were lower than in the control insects. The decrease in the Haemolymph contents increased with increasing the post-inoculation period.

INTRODUCTION

Migratory insect commonly relays on lipids as main energy supply for flight muscle during their migration. In Orthopteran species, such lipid consumption during continued flight has been studied. (Haunerland, 1997). Huge amount of energy utilized during locust migration flight. One of the most dynamic muscles known is the locust flight muscles, it seems suitable for such sustained muscular motions adequately. These muscles contain huge number of mitochondria, which express their high oxidative capacity (Crabtree and Newsholme, 1975). Van der Horst *et al*. (1993), described in detail the processes explain the lipid release from the fat body and their passage through the aqueous haemolymph. During its start, the flight is fuelled mainly by carbohydrates, but after 30 min, lipids quickly become the leading energy source (Beenakkers *et al*., 1984; Van der Horst, 1990). β-oxidation of fatty acids is very important process for continuous flight during migration, such fatty acids transported through the haemolymph in form of lipoprotein bound diacylglycerol. After hydrolysis, fatty acids moved over the sarcoplasm to the mitochondria probably by small intracellular fatty acid-binding (Haunerland and Chisholm, 1990). High amount of the binding protein and other enzymes involved in lipid oxidation express the high metabolic rate of flight muscle. The fatty acid-binding protein is the most abundant soluble muscle protein in *S. gregaria* comprising about 18% of the total cytosolic proteins (Haunerland *et al*., 1992).

Beenakkers *et al*., (1975) showed that the muscle tissue is also full of in enzymatic activity required for carbohydrate metabolism. The aim of the present work, is to study the effect of the entomopathogenic on the flight muscles through it is histological and biochemical effects.

MATERIALS AND METHODS

Experimental Insect:

Experimental Insect was desert locust, *Schistocerca gregaria* which took from the culture of department of locust and grasshopper, Plant Protection Research Institute, Agricultural Research Centre. The insects were reared according to Hassanein (1965). Fresh clover leaves, *Trifolium alexandrinum* were presented during the period of study.

Treatment:

Adults were treated by treating entomopathogenic fungus, *Beauveria bassiana* topically (2.5µl/insect). Entomopathogenic fungus was dissolved in water (2.5gm/ 1 litre water). Mortality counts were recorded daily until 15 days post-treatment. Mortality data were summarized as estimates of the Medium Lethal Time (MLT).

Samples Collection and Preparation:

Adults were taken for experiments where the biochemical effects of *Beauveria bassiana* on haemolymph components were evaluated. The experiments were carried out by treatment of 1-day old after molting under laboratory conditions. Samples of the haemolymph were taken at different intervals of 2, 4, and 6 days after treatment. The haemolymph was collected through a fine puncture in the hind leg membrane and transferred into clean dry Eppendorf tubes 1.5 ml. Few crystals of phenylthiourea were added to prevent melanisation before analysis. A known volume of the collected haemolymph was centrifuged at 13000 rpm to 15 min. to remove blood cells and pigments. Then the supernatant was collected for analyses (El-Gawhary, 1997).

Histological Preparations:

Electron Microscope:

Recently emerged adults were treated with *B. bassiana* spores and dissected in insect saline solution after 3days post treatment. For transmission electron microscopy (TEM) investigation muscles were dechorionted by dipping for 3 min in 6% sodium hypochlorite then rinsed in distilled water three times. Then the muscles were immersed in 5% glutaraldehyde by prepared in 0.1M sodium cacodylate buffer for 4 hours at room temperature, then washed in 0.1M cacodylate buffer for the first fixation. Post fixed by 1% osmium tetroxide then washed in 0.1M cacodylate buffer three times, after fixation its dehydration by using graded ethanol series and then embedded in an epoxy resin. Embedded muscle sectioning by glass knives which cut into 25-mm squares plastic tape was used to form a boat at the edge of the glass knife, and then the tape sealed to the glass with dental wax. And then viewed with a Zeiss 10C transmission electron microscope at 80 kV JOEL JEM-1400 cx TEM at 120KV (Biserova and Pflüge, 2004)

Determination of Total Carbohydrates:

Total carbohydrates were determined: according to Singh & Sinha (1977).

Determination of Total Lipids:

Total lipids were estimated by the method of Knight *et al*. (1972).

Determination of Total Protein:

The total protein is determined according to the method described by Bradford (1976).

Statistical Analysis:

Data have been subjected to analyze variance (ANOVA). Means were compared using LSD according to IPM SPSS Statistics 26.

The percentage of nymphal mortality was corrected according to Abbott's formula (Abbott, 1925).

LT_{50}, values and slope of regression lines were calculated by using (Ldp line) software www.ehabsoft.com for calculating and drawing toxicity lines according to Finney (1971).

RESULTS AND DISCUSSION

The present results show the effect of the entomopathogenic fungus *Beauveria bassiana* on the flight activity of *Schistocerca gregaria*. Data in (Table 1) showed that the entomopathogenic fungus caused mortality percentages 85.56 and 81.11% in 5th nymphal instar and adults after 15 days of treatment; also, LT_{50} was 9.17 and 10.07 days.

The ultrastructure of untreated muscles is shown in (Figs. 1 a & b). The fibrils are clearly constructed of filaments and the distribution of those filaments is related to the alternating light and dark bands. From the several bands in the striation pattern, the Z-line is commonly selected as a marking for the limits of the sarcomere. This line is comparatively denser, especially in contracted fibrils, and maybe correctly regarded as a kind of septum that is continuous transversely across the fibril. Other bands are an isotropic band I which is bisected by Z-line and anisotropic which is denser and is bisected by the narrow light band (H band). The histopathological examination of muscles of insects showed different determinations after treatment with the entomopathogenic fungus *B. bassiana*. Fungal infected muscles 72hrs. After infection showed great differences in the nucleus compared with control. Nuclei of treated insects appeared to be greatly damaged and irregular in shape containing small areas of heterochromatin and large vacuoles (Fig. 1). The nucleus appeared smaller in size, deformed in shape, and is surrounded by fewer numbers of mitochondria and SR.

Muscle fibers from infected insects treated showed degeneration and disorganization of muscle fibers. The mitochondria were clustered and their size was somewhat smaller than those of the control muscle fibers. Some mitochondria were elongated while others were swollen with irregular shapes. In the electron micrograph of the longitudinal section, myofibrils were more indistinct, I and A-bands were less defined, Z-line was diffused and indistinct. H zone is completely unclear and undefined. These results agree with (Nation, 2002 and Soltan, 2009). The muscle of insect consists of many of long fiber. Multinucleate cells commonly extend along the length of the muscle. In hemimetabolous insects, such as the desert locust *S. gregaria*, a complete set of muscles is present in the nymphal stage. In nymphes, the flight muscle functionless then developed rapidly before molting to adult.

In the present study, the available electron micrographs revealed severe effects of *B. bassiana* on the flight muscles, (Figs. 2, a&b), such as malformation in nuclei and distortion of Z-bands, disorganisation of A, I and H bands, the appearance of gaps and vacuoles in the sacromere and the mitochondria appeared irregular shapes, clustered and their size was somewhat smaller than those of control. Similar results were obtained when using plant extracts and synthetic pyrothroid on muscles of different locust species and the effect of *Metarhizium anisopliae* var. *acridum* on flight muscles of *S. gregaria* (Shinga *et al*., 2002; Biserova and Pflüger, 2004 and Soltan, 2009), they found that the remarkable affected muscles treated by different insecticides may be explained the disturbance of proteins in treated nymphs which appeared abnormal in shape and failed to completely shed their exuvia due to hormonal balance changed. So these nymphs metamorphosed to adults with many morphological aberrations.

Ghoneim, *et al*. (2008) Indicated disrupted in I, H and A bands and degeneration in Z disk for thoracic muscle after treated the desert locust with tebufenozide. Also, Al-Zeeb, *et al*. (2018) reported distribution in the adult muscles of desert locust after treatment with Lufenuron whish displayed a breakdown of the fibers of these muscles, where I band became like a grid intermingled with other fibers components.

These effects may be due to the reestablishment of muscle cells by treatments affecting auto disintegration of tissue, like the programmed cell death of many larval muscles seen during metamorphosis (Lockshin, 1985). The increase in the hemolymph Juvenile hormones caused decadent in the indirect muscles in the cricket *Acheta domestica*. And the fine structure of such degenerating muscles resembles that seen in flight muscles from methoprene treated locusts (Chudakova, 1978). Hyphae were able to invade various cells and tissues directly such as muscles and caused damage for them by enzymatic activity so that the flight increased.

Tab. 1: The mortality percentages of desert locust after treated with *Beauveria bassiana*.

Days / Instar	7	10	15	Total	Slope	LT25	LT50	LT90
5th	31.11	53.33	85.56	85.56	4.6586+/- 0.6057	6.57	9.17	17.27
Adult	25.56	44.44	81.11	81.11	4.65+/- 0.59	7.21	10.07	18.99

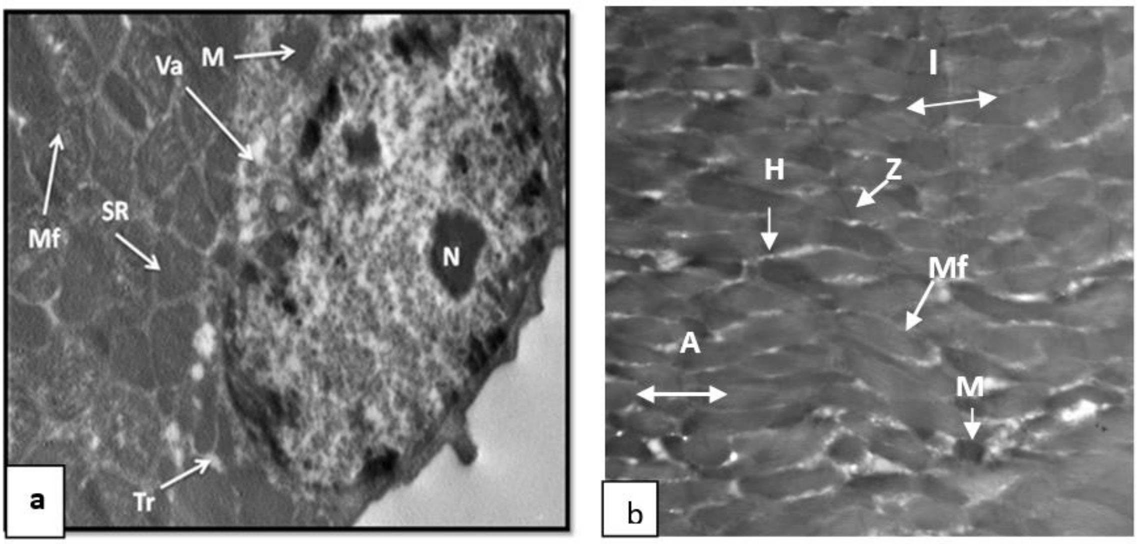

Fig. 1: Electron micrographs of longitudinal sections of untreated muscles fibres of adult *S. gregaria* (a) showing Myofibrils (Mf), Nucleus (N), oval-shaped mitochondria (M), Sarcoplasmic reticulum (SR), Vacuole (Va) and Tracheae. (b) Showing Z- line (Z), I, H and A-bands of sacromere and Mitochondria (M). (RX. 10000).

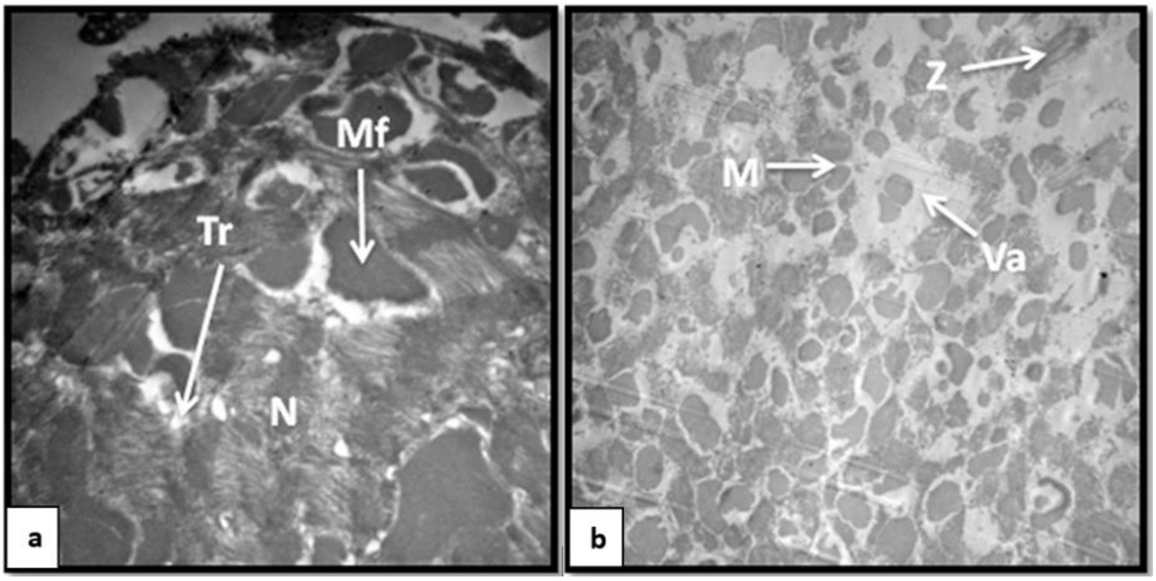

Fig. 2: Electron micrographs of longitudinal sections through flight muscle fibres from infected insects with *B. bassiana*, 48 hrs after infection. (a) Showing the malformation in nucleus (N); Trachea (Tr) and myofibirls (Mf) were more indistinct. (b) Showing the mitochondria (M) smaller than those control muscle fibers and irregular in shape and large vacuoles (Va); Z-line was more indistinct. (RX, 10000).

Effect of *Beauveria bassiana* on the Total Carbohydrates, Protein and Lipid of The Desert Locust:

Results in table (2) showed that total carbohydrates decreased during treatment periods and reached to lowest value 311.67mg/100ml haemolymph after 6 days of treatment with entomopathogenic fungus, *Beauveria bassiana* LSD= 12.77 $F_{cal.}$ =373.98 F_{tab} = 3.11. The mean difference is significant at the 0.05 level. P-value=9.78-13.

Total lipid values were 16.37, 10.13 and 6.5 mg/100ml haemolymph after 2, 4 and 6 days of treated with *B. bassiana* LSD=1.1 F_{cal}= 225.1, F_{tab}= 3.11 P-value = 1.99-11.

Total protein in treated insects was lower than total protein in control insects during the treatment period LSD= 18.02, F_{cal} = 34591.549, F_{tab}= 3.11 and P-value =1.63-24.

Tab. 2. Effect of *Beauveria bassiana* on the total carbohydrates, protein and lipid of the desert locust.

Treat. / Days after app.	Total carb.		Total lipids		Total protein	
	Treat.	Control	Treat.	Control	Treat.	Control
2nd days	315.67b	328.67 a	16.37b	21.20a	3008.67b	3110.00a
4th days	330.30b	370.00a	10.13b	15.13a	4296.30b	5965.67a
6th days	311.67b	521.00a	06.50b	09.83a	4182.30b	4622.00a

[b] significant; [a] non-significant

Means, within row, bearing different subscripts are significantly different (p<0.05%).

The day-to-day activities of an insect require a constant supply of energy. Most adults need food intake to support their activities (dispersal, reproduction). Flight, in particular, is a very energy-intensive activity, requiring rapid mobilization of energy sources, transport, and transformation of food energy into ATP. Those metabolic reactions are directly involved in mobilizing stored energy reserves and in releasing that energy for flight (Chapman, 1971). Quantitative assays of protein in the haemolymph and reproductive organs are of considerable importance for the understanding of the different physiological processes associated with reproduction. In insects, changes in proteins are prominent during stages undergoing marked development and tissue differentiation such as during metamorphosis (Bakr *et al*., 2007) and sexual maturity of the reproductive organs (Engelmann, 1970). Polanowski *et al*. (1997) reported that treating the desert locust, *S. gregaria* with the entomopathogenic fungus, *M. anisopliae*; caused a decrease in the total protein level of the haemolymph during the course of infection. But Gurwattan *et al*. (1991) found that vegetative growth of *B. bassiana* in the haemocoel of *Spodoptera exergue* did not alter the protein profile.

Lepidopteran and Orthopteran insects depend on lipid as fuels in flight due to release of huge amount of energy during metabolism. During flight fatty acids provide the tracheal system in insects with oxygen by rapid metabolism of lipids, therefore some insects able to migrate and fly for long - distances. (Chapman, 1971). Gillespie, *et al*. (2000) found that in the desert locust, *S. gregaria* when topically inoculated with *M. anisopliae* var. *acridum* the total proteins and total lipids of the haemolymph decreased during the course of infection. Moreover, The percentage of carbohydrates and lipid in the hemolymph lower than control after treated *S. gregaria* with *M. anisopliae* with concentration of (4 x 107spores/ml), this was not due to the reduction in the amount of food where (Seyoum *et al*., 2002) explained that 3 days of full starvation showed no effect on titers of energy reserves in control haemolymph. Some insects use carbohydrates in the haemolymph as the only source of fuel to fly for 30 minutes then they switch to another source like proline or fatty acids (Nation, 2002). Hua *et al*., (2007) founded that, the haemolymph trehalose titer decreased remarkably during infection of locusts by *M. anisopliae*. Such results proposed that this fungus may establish superiority over the locust for nutrient utilization.

Conclusion

From our results, we can conclude that reduction in haemolymph protein, carbohydrates and lipid caused by decreasing insect survival and high mortality rate by *B. bassiana* and a drastic effect of this fungus on the population density of *S. gregaria*. also, changes in the ultrastructure of muscles led to decreasing in locust fitness especially flight capability.

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ARABIC SUMMARY

تأثير الفطر الممرض الحشري، بايفاريا باسيانا على عضلات طيران الجراد الصحراوي

محمد خيري الديدامونى، السيد سلطان محمد وسعيد محمد سعيد
قسم بحوث الجراد والنطاط – معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – الجيزة – مصر.

درس تأثير اصابة عضلات طيران الجرد الصحراوي بالممرض الحشري فطر البيوفاريا باسيانا. سبب الممرض الحشري نسب موت 85.56 و81.11% لكلا من حوريات العمر الحوري الخامس والحشرات الناضجة وأيضا كانت قيم الوقت النصفي القاتل 9.17 و10.07يوم.

بينت الدراسات الهستوباثولوجية للعضلات المعاملة ان النواة، الميتوكوندريا، الالياف العضلية والفجوات دمرت بشدة.

تم تقدير الكربوهيدرات، الدهون والبروتين الكلى. اظهرت النتائج ان مكونات هيموليمف الحشرات المعاملة تأثرت بشكل كبير وكانت كل المقاييس التي درست اقل منها فى حشرات المقارنة. زاد النقص فى مكونات الهيموليمف بزيادة الفترة بعد العدوى.

الكلمات الدلالية: عضلات الجراد الصحراوي، الفطريات الممرضة للحشرات، التأثيرات الهستوباثولوجية، الكربوهيدرات والدهون والبروتين الكلى.