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Effect of Two Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* var. *acridum* on the Haemolymph of the Desert Locust *Schistocerca gregaria*

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***Abstract*—**Effect of *Beauveria bassiana* and *Metarhizium anisopliae* var. *acridum* on the 5th instar nymphs of *Schistocerca gregaria* was studied in the laboratory. Infection by these both entomopathogenic fungi caused reduction in the hemolymph total protein. The average amounts of total proteins were 2.3, 2.07, 2.09 µg/100 ml of haemolymph in the control and *M. anisopliae* var. *acridum*, and *B. bassiana* based-treatments, respectively. Three types of haemocytes were recognized and identified as prohaemocytes, plasmatocytes and granulocytes. The treatment caused significant reduction in the total haemocyte count and in each haemocyte type on the 9th day after its application.

Keywords*—Beauveria bassiana*, haemolymph picture, haemolymph protein, *Metarhizium anisopliae* var. *acridum*, *Schistocerca gregaria.*

I. Introduction

LOCUSTS and grasshoppers are major economic pests of crops and grasslands throughout the world's dry zones. Their damages attract much public attention and make headline news [1]. Desert locusts, *Schistocerca gregaria*, consume approximately their own weight (2 g as adults) of fresh vegetation each day. Swarms often contain 50 million individuals per km^2 so that even a moderate swarm measuring 10 km² could consume about 1000 tons of fresh vegetation daily during migration [2]. Interest in using pathogens as biological control agents against locusts and grasshoppers has grown since the last major locust plague of the 1980. Locust control only became efficient after the development of dieldrin [3]. The major advantage of this insecticide was its persistence [4]. Dieldrin and lindane remained the major products used for desert locust control until the early 1980's. However, these compounds are no longer used because of growing concern over their environmental impact.

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The most pesticides recommended by the FAO locust pesticide referee group have a very short persistence in the field in order to avoid their accumulation in the environment. In desert locust control, Ultra Low Volume (ULV) spinning disc spray equipment is mainly used which served for hand application as well as for application by vehicle or aircraft [5]. This technology is the most efficient way of transferring an insecticide to its target [6] and allows low volume application rates. Guidelines for locust control techniques and the organization of control operations have been developed [7], [8]. Different insect growth regulators have been tested for acridid control [9]. These products cause low risk for non-target organisms, except aquatic arthropods. As far as biocontrol agents are concerned, the most extensively studied pathogens are the Deuteromycete fungi such as *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana* [10]. The use of pathogens may offer an environmentally sound method for the management of grasshoppers and locusts, and Hyphomycete fungi are the most promising candidates [11].

The present study aims to examine the effect of two entomopathogenic fungi, *M. anisopliae* var. *acridum* and *B. bassiana*, against haemocytes and protein contents of *S. gregaria* under laboratory conditions.

II. Materials and Methods

A. Tested Insects

Insects used in the laboratory bioassay were 5th instar nymphs of the desert locust, *S. gregaria*. The individuals were taken from stock culture maintained for several generations at the Locust Control Department, National Plant Protection Institute, El-Harrach, Algeria. The insects were reared in group and maintained at 30 ± 5 °C, 45 ± 15% RH under a photoperiod of L12:D12. The individuals of *S. gregaria* were fed on wheat seedlings supplemented with wheat bran.

B. Tested Entomopathogenic Fungi

Spores of *M. anisopliae* var. *acridum* used were from isolate IMI: 330189 provided by Biological Control Products, South Africa (Fig. 1). *B. bassiana* spores were derived from mycosed cadavers of *S. gregaria* collected from Adrar area [12]. The isolate was propagated in locust for several generations.

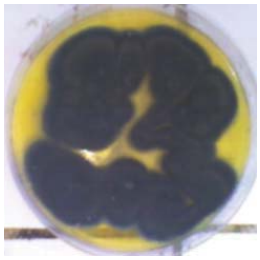

Fig. 1 *Metarhizium anisopliae var acridium* in growth media

C. Effect of Fungal Infection on Protein Content of Schistocerca gregaria Haemolymph

Three treatments were applied: 1) untreated nymphs, 2) nymphs treated with 10^5 spores/nymph of *M. anisopliae* var. acridum and 3) nymphs treated with 10^5 spores/nymph of *B. bassiana*. Fifteen nymphs were used for each treatment. Haemolymph samples were taken after treatment every 3 days till the 9th day. To collect the haemolymph, the arthropodial membrane of a hind leg was pierced with a micro syringe. The haemolymph was collected into a capillary pipette (10 µl).

For haemocyte counting, 3 µl of haemolymph was placed on a glass slide and smeared to a thin film. The smears were first stained with diluted May-Grunwald stain for 3 min, then washed with distilled water and stained for a second time with diluted Giemsa for 10 min then washed again in distilled water [13]. The haemocytes were observed under light microscope with 100 × oil immersion objective and identified according to [14].

To calculate the total haemocyte count, haemolymph was diluted (1: 4) with sterile ice cold anticoagulant buffer then placed in an improved Neubauer haemocytometer.

For estimating the protein contents, a 10µl sample of haemolymph was placed in an Eppendorf tube containing phenyl thiouria. The tubes were centrifuged (3000 rpm for 20 min) and stored at -20 °C. The protein content was determinate based on the method of Bradford [15].

D. Statistical Analysis

To study the effect of *M. anisopliae* var. *acridum* on the haemolymph picture and its protein contents, three treatments were carried, for each one 45 nymphs were used distributed randomly and divided to three repetitions.

Data were subjected to analysis of variance followed by Student-Newman-Keuls post-hoc tests (P=0.05) using the ANOVA procedure of SAS [16]. Mean values are presented with their standard errors (SE).

III. Results

A. Symptoms

After applications of *M. anisopliae* and *B. bassiana* on individuals of *S. gregaria*, they need more feed and their movements become slower and slower before being paralyzed until death. Reddish spots are visible in the chest corresponding to the presence of the mycelium. By moisture mycelium especially pierces the cuticle at the intersegmental membranes and begins to sporulate. Soon after, the corpse is covered with a green powder layer for *M. anisopliae* (Fig. 2 (a)) and a white powder layer for *B. bassiana* (Fig. 2 (b)).

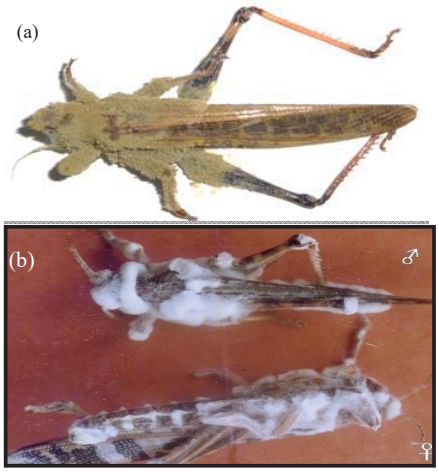

Fig. 2 *M. anisopliae* (A) and *B. bassiana* (B) sporulating on a desert locust

B. Effect of Entomopathogens Tested on Schistocerca gregaria Haemocytes

For this study, three types of haemocytes i.e. prohaemocytes, plasmatocytes and granulocytes were recognized according to [14].

1. Effect on Total Haemocyte Count

Data illustrated in Table I show the effect of *M. anisopliae* var. *acridum* and *B. bassiana* on totalhaemocyte count of the 5th instar nymphs of the desert locust on the 9th day after treatment. It is clear that the crude of haemocyte count was recorded in the infected insect. The treatment with *B. bassiana*showed the lowest reduction in the amount of haemocyte count (P<0.05) while the *M. anisopliae* var. *acridum* based-treatment induced the highest reduction in the total haemocyte amount. The average amounts of the total haemocyte were 30.1, 5.52 and 3.95×10² haemocytes/µl, respectively, in untreated locustsand those treated with *B. bassiana* and *M. anisopliae* var. *acridum*.

TABLE I
Effect of Two Entomopathogenic Fungi on the Total Haemocyte's Count in 3 µL of Haemolymph of 5th Instar Nymphs of *Schistocerca gregaria* Recorded on the 9th Day After Treatment as Compared to the Untreated Control

	Control	*Beauveria bassiana*	*Metarhizium anisopliae var. acridum*
X ± SE (× 10²)	30.1 ± 2.26 a	5.52 ± 0.19 b	3.95 ± 0.15 b

Values (means ± SE) followed by the same letters are significantly similar according to SNK test (at P ≤ 0.05).

Data presented in Table II illustrate the number of the different haemocyte types/3 µl of the 5th instar nymphs of *S. gregaria* in the control, and in *M. anisopliae* var. *acridum* and *B. bassiana* based-treatments recorded on the 9th day post-application. The highest number of haemocyte types was

noted for plasmatocytes followed by prohemocytes and granulocytes. The infection by the tested entomopathegenic fungi induced a significant (P<0.05) decrease of the number of all haemocyte categories: plasmatocytes, prohemocytes and granulocytes.

TABLE II
EFFECT OF TWO ENTOMOPATHOGENIC FUNGI ON HAEMOCYTE'S TYPES COUNT IN 3 µL OF HAEMOLYMPH OF 5TH INSTAR NYMPHS OF *SCHISTOCERCA GREGARIA* RECORDED ON THE 9TH DAY AFTER TREATMENT AS COMPARED TO THE UNTREATED CONTROL

Haemocyte type	*Control* $X \pm SE$ ($\times 10^2$)	*Beauveria bassiana* $X \pm SE$ ($\times 10^2$)	*Metarhizium anisopliae* var. *Acridum* $X \pm SE$ ($\times 10^2$)
Prohemocytes	11.25 ± 1.63 a	1.21 ± 3.75 b	1.21 ± 3.75 b
Plasmatocytes	15.24 ± 1.96 a	1.95 ± 11.75 b	1.95 ± 11.75 b
Granulocytes	5.11 ± 0.73 b	0.68 ± 0.08 b	0.68 ± 0.08 b

For each haemocyte type,values (means ± SE) followed by the same letters are significantly similar according to SNK test (at $P \leq 0.05$).

C. Effect of the Entomopathogens on the Total Haemolymph Protein Content

Table III shows results for total protein content in haemolymph. The total protein content in the infected individuals decreased from the 5th day after treatment until the end of the experiment. The treatment with the fungus *B. bassiana* and *M. anisopliae* var. *acridum* caused significant (P<0.05) reduction in the total protein content.

TABLE III
EFFECT OF TWO ENTOMOPATHOGENIC FUNGION THE TOTAL PROTEIN CONTENT (µG/100 ML) OF HAEMOLYMPH OF 5TH INSTAR NYMPHS OF *SCHISTOCERCAGREGARIA* AS COMPARED TO THE UNTREATED CONTROL

Haemocyte type	*Control* $X \pm SE$ ($\times 10^2$)	*Beauveria bassiana* $X \pm SE$ ($\times 10^2$)	*Metarhizium anisopliae var. acridum* $X \pm SE$ ($\times 10^2$)
1st	2.09 ± 0.01a	2.1 ± 0.02a	2.1 ± 0.02a
3rd	2.24 ± 0.03a	2.14 ± 0.01a	2.16 ± 0.04a
5th	2.38 ± 0.02a	2.08 ± 0.03b	2.11 ± 0.03b
7th	2.39 ± 0.01a	2.04 ± 0.04b	2.06 ± 0.01b
9th	2.39 ± 0.04a	1.97 ± 0.01b	1.99 ± 0.02b

IV. DISCUSSION

In this study, we have established that infection of 5th instar nymphs of the desert locust by two entomopathogenic fungi *B. bassiana* and *M. anisopliae* var. *acridum* had significantly reduced the total haemocyte count and the number of three different haemocyte types. Similarly, [17] noted reductions in the heamocyte's numbers after infection of adult of migratory locust *Locusta migratoria* by *B. bassiana*. Xia et al. [18] have recorded a decline in the total haemocyte count and a marked reduction in the proportion of plasmatocytes and coagulocytes after inoculation of mature adult males of the desert locust infected with *M. anisopliae* var. *acridium*. Gillespie et al. [19] also noted a decrease in the haemolymph protein content of *S. gregaria* treated with *M. anisopliae* var. *acridum*, which is in agreement with our findings.

In this study, we have also noted that the decrease in haemocytes attributed to fungal infection was not caused by the exhaustion of proteins as suggested by [20], indicating that competition of *M. anisopliae* var. *acridum* with the individual *S. gregaria* against haemolymphatic metabolites resulted in depletion of reserves accumulated in the fat body.

The decrease in haemocyte numbers in response to mycosis of *S. gregaria* caused by *B. bassiana* and *M. anisopliae* var. *acridum* may be due to the intervention of these haemocytes in autophagy and humoral defense reactions.

Other studies have shown the increasing of the hemocytopoitic activity as long as conidia dosage. However, the fungus circumvents the host's immune defenses by preferentially destroying prohaemocyte and plasmatocytes, the most common haemocytes types in the termite (*Zootermopsis angusticollis*) [21].

V. CONCLUSION

The infection of 5th instar nymphs of the desert locust by two entomopathogenic fungi *B. bassiana* and *M. anisopliae* var. *acridum* had significantly reduced the total haemocyte count and the number of three different haemocyte types. In this study, we have also noted that the decrease in haemocytes attributed to fungal infection was not caused by the exhaustion of proteins. The decrease in haemocyte numbers in response to mycosis of *S. gregaria* caused by *B. bassiana* and *M. anisopliae* var. *acridum* may be due to the intervention of these haemocytes in autophagy and humoral defense reactions.

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