

## Haemolymph Picture and Its Chemical Components in *Schistocerca gregaria* (Forskål) as Affected by Two Entomopathogenic Fungi

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### ABSTRACT

Effects of *Metarhizium anisopliae* var. *acidum* (Metchnikoff) Soroken, *Beauveria bassiana* (Bals.) Vuill. sole and their combination were studied on the haemolymph picture of the 5<sup>th</sup> nymphal instar of *Schistocerca gregaria* (Forskål), also toxicity effect was evaluated against 3<sup>rd</sup> nymphal instar of *S. gregaria*. The infection of both fungi (sole or in combination) caused reduction in chemical composition of haemolymph in the 5<sup>th</sup> nymphal instar of *S. gregaria*, also reduced the activity of Acid phosphatase (except in case of *B. bassiana* infection), prophenyleoxidase, and phenyleoxidase. Five types of haemocytes were recognized. The treatments also reduced the total haemocytes count and resulted in changes of the percentages of the haemocyte types. The combined infection caused potential effect at all observations when 3<sup>rd</sup> nymphal instar was treated with  $1 \times 10^3$  spores/nymph. While in case of dose  $2 \times 10^3$  spores/nymph of the mixture, there was an additive effect on day 7 and potential effect on days 10, 14, 18 and 21 post infection, the combined infection also accelerated the mortality.

**Key words:** Entomopathogenic fungi, *Metarhizium anisopliae* var. *acidum*, *Beauveria bassiana*, *Schistocerca gregaria*, toxicity effect, haemolymph picture.

### INTRODUCTION

Entomopathogenic fungi have considerable potential for locust and grasshoppers control (Goettel, 1992). *Metarhizium anisopliae* var. *acidum* and *Beauveria bassiana* were efficient against desert locust *Schistocerca gregaria* and clover grasshopper *Eupreocnemis plorans* (Charpentier) nymphs in the laboratory, as well as the common grasshoppers at Sharq Eloinate, Egypt under semi field conditions, while *M. anisopliae* var. *acidum* was a successful biological control agent in the field against *S. gregaria* at Elba Mountains, South east of Egypt, also against common grasshoppers at Baharia oasis, (Abdelatef, 2005 and El-Maghraby *et al.*, 2009). Slow speed act of killing is perceived as a potential drawback to the use of fungi in control operations. It seemed possible that combined infection of *M. anisopliae* var. *acidum* and *B. bassiana* might accelerate the speed act of killing and enhance the efficacy of both fungi against *S. gregaria*.

Insect immune responses are one of the potential factors involved the inability of fungus to infect the non-permissive host insect. The insect immune system is divided into two interactive responses, the first is the ability of the insect haemocytes to identify a non-self entity in the haemolymph and the other response is humoral or noncellular response such as; activation of prophenoloxidase (Gillespie *et al.*, 2000). Acid phosphatase (AP) is one of the major lysosomal enzymes in invertebrates (Anderson, 1981), and considered as non specific body response to integument damage or

fungal toxins (Serebrove *et al.*, 2006).

The objectives of this study were evaluating the effect of combined infection of *M. anisopliae* var. *acidum* and *B. bassiana* against 3<sup>rd</sup> nymphal instar of *S. gregaria*, also on the main haemolymph nutrient resources, acid phosphatase, prophenoloxidase and phenoloxidase activity of the 5<sup>th</sup> nymphal instar haemolymph of *S. gregaria*. The effect of such combined infection on the haemocytes was also studied.

### MATERIALS AND METHODS

#### Insect rearing:

Used insects were the 3<sup>rd</sup> and 5<sup>th</sup> nymphal instars of the desert locust, *S. gregaria*. The insects were obtained from the stock culture maintained for several generations at Locust and Grasshopper Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. The culture is usually fortified with insects brought from the field every year. Insects were reared in the laboratory according to (Robert *et al.*, 2002) in framed cages, both hoppers and adults were fed on branches of Egyptian clover, *Trifolium alexandrinum* and dry wheat bran fortified with 5% yeast powder as a source of vitamin B<sub>1</sub>. The locust's cages were kept at  $30 \pm 2$  °C and 30-50 % R.H.).

#### Entomopathogenic fungi:

Spores of *M. anisopliae* var. *acidum* and *B. bassiana* were mass production as designed method of El-Maghraby *et al.* (2009).