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## Joint Action of Neem and *Metarhizium acridum* on Immature Adults of Desert Locust, *Schistocerca gregaria* (Forsk.)

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### ABSTRACT

The efficacy of Neem, *Metarhizium acridum*, and their mixture on immature adults of *Schistocerca gregaria* were tested. Neem when applied at a tenth of recommended dose mixed with *M. acridum*, caused a significant increase to *M. acridum* efficacy where the mortality reached 96.6 % while mortality of *M. acridum* alone reached 76.6 %, after 14 days post-treatment, mortality of due to Neem alone reached to 26.6%. *M. acridum*, and Neem mixture caused significant reduction of trehalase activity at 2, 4, 6 days post-treatment, while same treatment caused a significant increase in lactate dehydrogenase activity at day 2 post-treatment then this activity was reduced significantly at days 4, and 6 post-treatment.

### INTRODUCTION

Locusts and grasshoppers are considered as the most destroying and a threat to pests agriculture worldwide and responsible for significant loss to the agricultural industry in grassland biomes of the world (Sumit *et al.*, 2020 and wanxi *et al.*, 2020). Threats of locusts and grasshoppers required the application of a huge number of chemical pesticides which rise the risk of environmental pollution, therefore entomopathogenic fungi may be suitable microbial control agents that infect their host through the cuticle with a possibility to mass-produce cheaply and are target specific (Lomer *et al.*, 2000). *M. acridum* proved to be a good solution as an alternative agent for locust and grasshoppers' control in Egypt, (El-maghraby *et al.*, 2009). Plant phytochemicals may also play important role in reducing pesticides programs, Neem tree one of the richest plants with toxic phytochemicals (Jacobson, 1989 and Abdulhadi and Kashere 2020). Azadirachtin (the most active compound of Neem tree) directly interferes with the endocrine system of insects and causes ecdysteroid and juvenile hormone titers imbalance (Subrahmanyam and Rembold., 1989). Trehalase and lactate dehydrogenase are very important enzymes for energy in haemolymph of locust and grasshoppers. Trehalase hydrolyse the trehalose sugar in irreversible action to produce 2 glucose units and energy, (Becker *et al.*, 1996 and Wegener, 1996). While lactate dehydrogenase plays important role in carbohydrate metabolism it is activity indicates active energy metabolism (Diamantino *et al.*, 2001 and Hamadah *et al.*, 2010). The present study aims to investigate the joint action of Neem and fungi *Metarhizium acridum* on mortality

and their effect on two important energy enzymes of the haemolymph of immature adults of desert locust *S. gregaria*.

## MATERIALS AND METHODS

### **Insect Tested:**

Immature adults of the desert locust *S. gregaria* two days after final molting were used in the present experiments and taken from maintained culture for several generations at the Locust and Grasshoppers Research Department, Plant Protection Research Institute (PPRI), Agriculture Research Center (ARC), Dokki, Giza. Desert locust was reared in the laboratory according to Hunter-Jones (1961) and fortified with insects collected from the field whenever possible.

### ***Metarhizium acridum*:**

The entomopathogenic fungus used during the present study was *M. acridum* isolate (IMI330189) from BASF Company, South Africa, under the commercial name Green Muscle®. The conidia of the fungus were suspended in sterilized water, then a trace of Tween (80) was added to be emulsified. The concentration was adjusted to  $5 \times 10^8$  spores/ml., each nymph received 5 $\mu$ l, while the control nymphs were treated with 5 $\mu$ l of sterilized water and traces of Tween (80) (El-Dydamony, 2011).

### **Neem (Azadirachtin):**

Tenth of the recommended dose from commercial product Safe oil was used (1 ml \ 1 liter). Each treated nymph received 5 $\mu$ l.

### **Neem, and *Metarhizium acridum* Mixture:**

Neem Solution was prepared as previously then *M. acridum* spores were suspended in that solution with a trace of Tween 80 and the concentration was adjusted to  $5 \times 10^8$  spores/ml., each nymph received 5 $\mu$ l.

### **Statistical Analyses:**

Mortality was corrected according to Schneider-Orelli's formula (Püntener, 1981).

### **Biochemical Changes of Some Haemolymph Enzymes:**

#### **1- Sample Collection:**

Samples of the haemolymph from the previous treatments and control were taken at 2, 4, and 6 days after treatment. The haemolymph was collected through a fine puncture in the hind leg and from beneath the dorsal pronotal shield membrane and transferred into dry centrifuge tubes (Metaweh *et al.*, 2001).

#### **2- Determination of Lactate Dehydrogenase Enzyme:**

The method described here is derived from the method recommended by the German Society for clinical chemistry (DGCK, 1972).

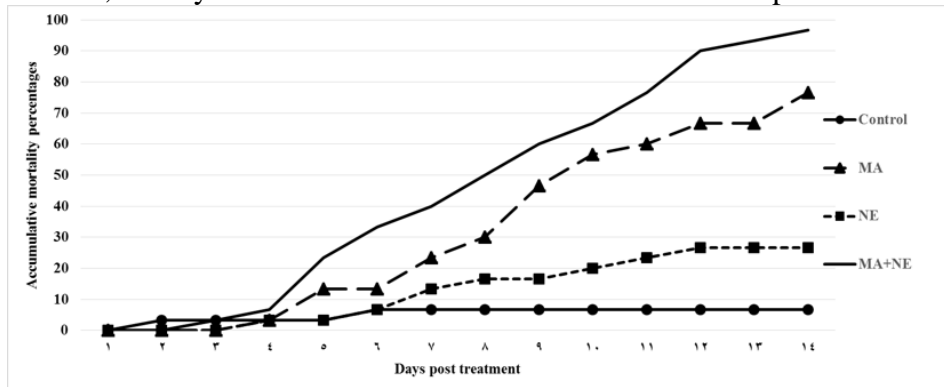
#### **3- Determination of Trehalase Enzyme:**

Trehalase was determined according to the method described by (Ishaaya and Swirski 1976) using trehalose as a substrate for trehalase.

## RESULTS AND DISCUSSION

Figure (1) Show the accumulative mortality caused by *M. acridum* and Neem mixture of them against immature adult *S. gregaria*. *M. acridum* treatment affected mortality after 4 days from inoculation and the mortality reaches 76.6 % after 14 days, while total mortality in case of neem is 26.6 in the same period and the mortality started after 3 days only from treatment, total mortality increased in case of *M. acridum* and Neem mixture to reaches 96.6 % after 14 days.

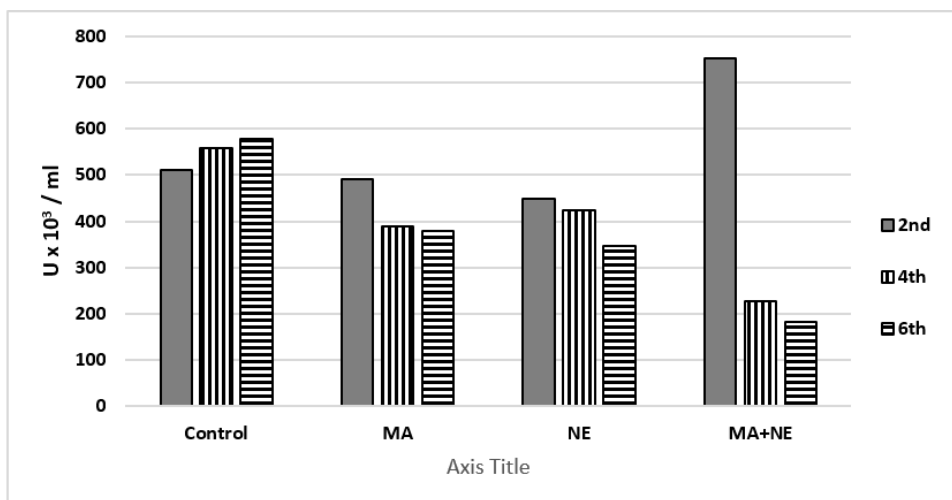
Mortality after *M. acridum* treatment is due to toxins release which causes destruction of locust tissues or reduces nutrition, this results in parallel to (Lednev *et al.*, 2008) who found that the mortality started after four days after treatment of *Locusta migratoria* with *M. anisopliae*. In case of neem the percentage of mortality was lower than *M. acridum* maybe because neems act as antifeedant which affects the amount of food. While mortality in *M. acridum* and Neem mixture may be accelerated due to stimulation of Neem to the ferocity of *M. anisopliae* this result compatible with (El-Dydamone., 2016) who found the mixture from *M. anisopliae* and neem makes the acceleration of mortality if compared with them alone, or maybe due to effect of Neem on the immune response of desert locust.



**Fig 1:** Accumulative mortality due to *M. acridum*, Neem and their mixture against immature adult *S.gregaria*, MA= *M. acridum*, NE= Neem.

**The Activity of Lactate Dehydrogenase Enzyme (LDH):**

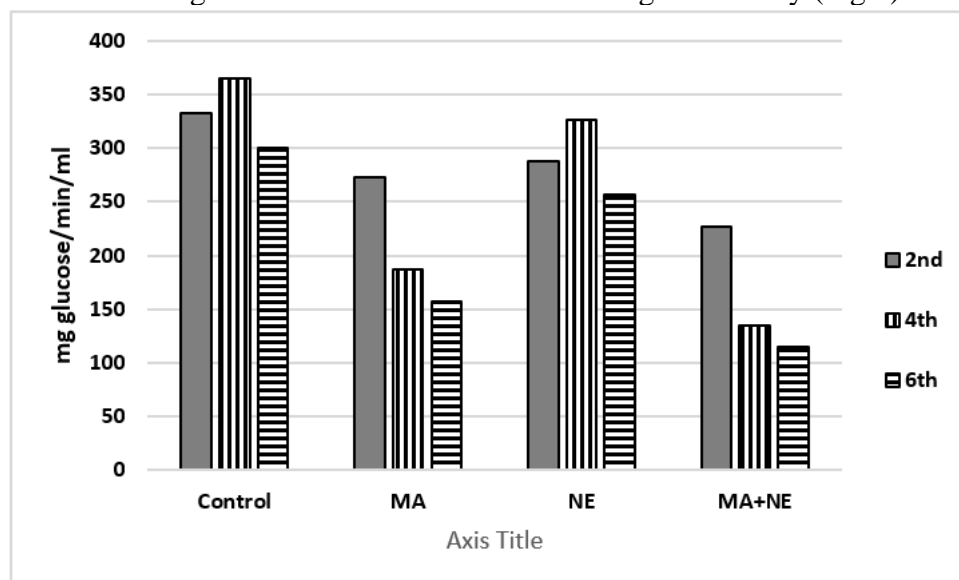
The activity of LDH is decreased than control after 2,4 and 6 days when insect treated with *M. acridum* and neem this result is in parallel to (Soltan, 2009) which found a decrease in the activity of LDH rather than control after 1,2 and 3 days when treated *S. gregaria* with *M. acridum*. Such a decrease in LDH activity may be due to the increase in oxygen uptake. While in case of *M. acridum* and Neem mixture the level of LDH enzyme increase than control after 2 days may be due to the Neem increase efficacy of *M. acridum*, in which the insect wants to keep the cell living in important tissue by active energy metabolism, then the level of enzyme decreases than control after 4 and 6 days due to the fungus distribution in all cells (Fig. 2).



**Fig. 2:** Effect of *M. acridum*, Neem and their mixture on lactate dehydrogenase enzyme activity in haemolymph of *S. gregaria*.

### The Activity of Trehalase Enzyme:

The Activity of trhalase enzyme in *M. acridum* and neem and their mixture on all days of the experiment are lower than control, this decrease due to the toxicity stress of the treatments, (Ishaaya and Swirski, 1976) reported that trehalse could be used as a parameter for assessing the availability of nutrients. The level in the mixture is lower than *M. acridum* and Neem alone, due to the Neem increase the severity of *M. acridum* which effect on the activity of insect to degrade trehalose as foul for all biological activity (Fig.3).



**Fig.3:** Effect of *M. acridum*, Neem and their mixture on trehalase enzyme activity in haemolymph of *Schistocerca gregaria*.

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## ARABIC SUMMARY

الفعل المشترك للنييم و فطر *Metarhizium acridum* على الحشرات الكاملة غير الناضجة جنسيا للجراد الصحراوي *Schistocerca gregaria*

جمال محمد محمود عبداللطيف و محمد خيرى الديدامونى

قسم بحوث الجراد و النطاط – معهد بحوث وقاية النباتات – مركز البحوث الزراعية- مصر

تم اختبار فاعلية النييم و فطر *M. acridum* وكذلك مخلوطهم على الحشرات الكاملة الغير ناضجة جنسيا. ادت المعاملة بالنييم بعشر الجرعه المقرره مخلوطه مع فطر *M. acridum* الى زيادة الفاعلية حيث وصلت نسبة الموت الى 96.6% بينما نسبة الموت فى معاملة الفطر منفردا وصلت الى 76.6% و نسبة الموت فى معاملة النييم منفرد كانت 26.6%. ادت المعاملة بمخلوط النييم و الفطر الى تقليص معنوى فى نشاط انزيم التريهاليز فى اليوم الثانى و الرابع و السادس من المعاملة. بينما ادت نفس المعاملة الى زيادة ملحوظة فى نشاط انزيم اللاكتات ديهيدروجينيز فى اليوم الثانى من المعاملة بينما انخفض نشاط نفس الانزيم معنويا بعد ذلك فى الأيام الرابع و السادس من المعاملة.