THE TOXIC ACTIVITY OF THE ENTOMOPATHOGENIC FUNGUS *Metahizium anisopliae* ON THE HEARTBEATS OF THE DESERT LOCUST *Schistocerca gregaria* (Forskål, 1775)

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Abstract. Locusts are causing significant losses in agricultural production in the countries concerned by the invasion. So far, the control strategy has consisted only of the chemical treatment; this type of treatment has proven harmful to the environment. Therefore, a new biological control method has been discovered which is based mostly on using microorganisms. That's why we have made our contribution by using an entomopathogenic fungus *M. anosopliae* to see its heartbeat effect in the fifth instar locust *Schistocerca gregaria* (Forskål, 1775). Preliminary studies on the pathogenicity of *Metarhizium anisopliae* agent were made in the laboratory on L5 of *S. gregaria*, however we inoculated treatment on the cuticle of L5 of entomopathogenic solution with D1=102 sp. / ml and D2=8.6 x 105. At the same time we tested its effect on the heartbeat of the insects. The inoculum is sprayed directly on the L5 of *S. gregaria*. At the same time witnesses were sprayed with distilled water. The results showed that by using the high dose the heartbeat decreased from 82.5 beats/min in the witnesses to 37 beats/min for D1 and 41.3 beats/min for D2.

Keywords: biological control, *Metahizium anisopliae*, toxicity, desert locust, heartbeat.

Rezumat. Activitatea toxică a ciupercii entomopatogene *Metahizium anisopliae* asupra bătăilor inimii lăcustei de deşert *Schistocerca gregaria* (Forskål, 1775). Lăcustele provoacă pierderi semnificative în producția agricolă în țările vizate de invazie. Până în prezent strategia de control a constat doar în tratarea chimică; acest tip de tratament s-a dovedit dăunător pentru mediu. Prin urmare, a fost descoperită o nouă metodă de control biologic care se bazează în principal pe utilizarea microorganismelor. De aceea, ne-am adus contribuția utilizând o ciupercă entomopatogenă *M. anosopliae* pentru a vedea efectul asupra bătăilor inimii la lăcustele din stadiul al cincilea *Schistocerca gregaria* (Forskål, 1775). Studiile preliminare ale patogenității agentului *Metarhizium anisopliae*, au fost testate în laborator pe L5 de *S. gregaria*; am inoculat tratament pe cuticula lui L5 de soluție entomopatogenă cu D1=102 sp. / ml și D2=8,6 x 105. În același timp am testat efectul acestuia asupra bătăilor inimii insectelor. Inoculul este pulverizat direct pe L5 de *S. gregaria*. În același timp, martorii au fost stropiți cu apă distilată. Rezultatele au arătat că în urma utilizării dozei mari bătăile inimii au scăzut de la 82,5 bătăi/min la martori la 37 bătăi/min pentru D1 și 41,3 bătăi/min pentru D2.

Cuvinte cheie: combatere biologică, *Metahizium anisopliae*, toxicitate, lăcustă, bătăi ale inimii.

INTRODUCTION

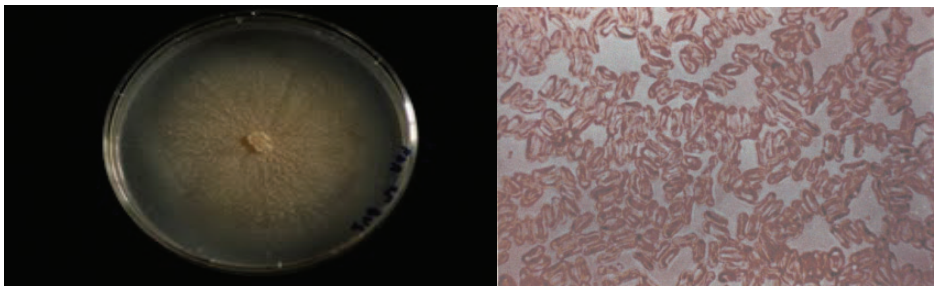
Locusts are causing important agricultural losses in regions covered by their invasion. For a long time, the control strategy consisted of spraying with chemicals only those which are proving to be harmful for the environment. Therefore, a new control method has appeared which is biological control including micro-biological control. This control is based on the use of microorganisms like fungi, bacteria and viruses that are naturally present in the environment (soil, air, water) and infect their host either by ingestion, or through the cuticle. Against this background, we have brought our contribution to studying the effect of the entomopathogenic fungi *Metarhizium anisopliae* (Metch, 1883) (*Ascomycota, Hypocreales*) on the 5th instar larva of the desert locust *Schistocerca gregaria* (Forskål, 1775) (*Orthoptera, Acrididae*).

MATERIAL AND METHODS

Biological material. The individuals of desert locust: *S. gregaria* were kept breeding at 32 ± 1°C temperature and 84 of relative humidity. They were fed grass, lettuce, and cabbage (Fig. 1).

Figure1. 5th instar larva of *S. gragaria* (original).

The Fungal strain: The fungi *M. anisopliae* is grown in a Sabouraud culture at 25 ± 1° C temperature in order to incubate (Fig. 2a; b).



a b

Figure 2. a. cultural aspect of *M. anisopliae*; b. spores of *M. anisopliae* (original).

Treatment process. After 10 days of incubation of the fungi, dilutions were made until obtaining the required concentration (D1= 10^2sp/ml D2= 8,6x 10^5 sp/ml). For this test, locusts received spores of *M. anisopliae* by direct spraying on the body to study the effect of fungi on the *S. gregaria*. Witnesses have been treated with sterilized distilled water. After every 24 hours we calculated the number of heart beats per minute; this count was done by observing a pulsating organ under the wing, after immobilization of the insect and separation of the wings and elytra.

Statistical method. To confirm the effectiveness of the treatment and the doses, we relied on the "Wilcoxon rank test" non-parametric test.

RESULTS

The graphical plot shows that the number of beats per minute has decreased. In fact, a remarkable reduction is seen in the number of beats per minute in the series treated at the low dose compared to the controls; this decrease is stronger in individuals treated with the high dose. We notice that the rate decreases from 57 to 37 beats/min, in the L5 treated by the low dose and from 50.3 to 41.3 beats/min. for the high against 82.5 to 79.5 beats/min for the witness (Table 1; Fig 3).

The statistical test confirms that there is no significant difference for the time factor, so the treatment is indifferent to time. However, the difference is very highly significant for the dose factor, so the dose influences the number of heartbeats per minute in the treated individuales, compared to the witnesses; indeed the difference is significant with $P < 0.05$; in comparison with the control batch, the heartbeats decrease considerably in the batch treated with D1 and even more with D2.

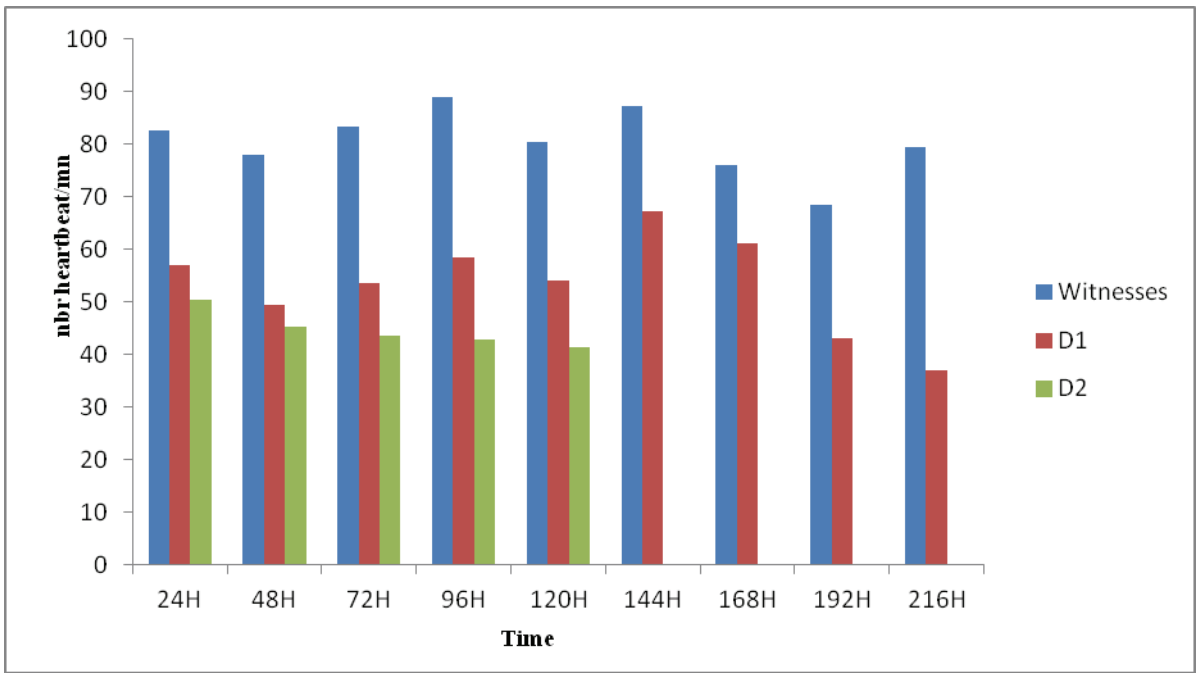


Figure 3. Heartbeat of the 5th instar larva of *S. gregaria* witnesses and treated by *M. anisopliae* (D_1 =10^2sp/ml, D_2= 8,6x 10^5 sp/ml).

Table 1. Heartbeat of the 5th instar larva of *S. gregaria* witnesses and treated by *M. anisopliae*.

Time / Dose	24H	48H	72H	96H	120H	144H	168H	192H	216H
Témoin	82,5	78	83,33	89	80,5	87,13	76	68,5	79,5
D1	57	49,33	53,5	58,5	54	67,25	61,25	43	37
D2	50,3	45,33	43,5	42,8	41,3				

DISCUSSIONS

Similar results have been recorded by several authors, HALOUANE & DOUMANDJI-MITICHE (1996) and by AGRANE 1997. Regarding the effect on heartbeat of *S. gregaria* and *Anacridium aegyptium.* CHAHBAR (1997) and BISSAD (1998) have found results which agree with ours, concerning the imagos of *S. gregaria* treated by *B. bassiana* (BISSAD et al., 1998).

In the same way, HADDADJ (1998; 2021) and HADDADJ et al. (1998) achieved similar results to our own, concerning the effect of *M. anisopliae* against the heartbeats of *S. gregaria*. According to ROUSSEL & CORRIVAULT (1973) there is an evolution in heartbeat with respect to sex, described in the *S. gregaria* adults.

CONCLUSIONS

After inoculation of the fungus, the heartbeats of L5 were altered following treatment. In fact, there is a decrease in the heartbeats of the individuals treated compared to the witnesses.

This decrease is greater at the high dose compared to the low dose. The enthomopathogenic fungus can be selected for biological control in order to minimize chemical treatments, whose consequences are unfortunate for the biosphers and maintain locust populations at a level below the gregarization threshold.

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