THE EFFECT OF THE ENTOMOPATHOGENIC FUNGAL *Metarhizium anisopliae* ON THE DESSERT LOCUST *Schistocerca gregaria*

HADDADJ Fairouz, SAADI Habiba, CHORFI Nassima, REMICHI Hayet, HAMDI Sihem, ZENIA Safia, SMAI Amina, MILLA Amel, DOUMANDJI-MITICHE Bahia

Abstract. The desert locust potentially is the most dangerous for agriculture, because of the ability of swarms to fly rapidly across great distances. The locust control strategy has so far mainly consisted of the application of synthetic insecticides, which can be harmful to the environment. Therefore, research institutes are turning to other methods, including biological control, particularly microbiological control in its various forms, to try to control swarming locusts. Due to its persistence in the soil and its harmlessness to humans and animals and in the context of biological control, we tested an entomopathogenic fungus *Metarhizium anisopliae* on the fifth instar larvae of the locust *Schistocerca gregaria* (Forskål 1775). Preliminary tests have been done in the laboratory on locust larvae. Cryptogam was administered by direct spraying on the treated individuals, three doses were used, namely: D_1 =1,46x10^7 spores/ml, D2= 1,46x10^6 spores/ml, D3= 1,46x10^5 spores/ml. At the same time, the witness was sprayed with sterilized distilled water. We obtained 100% mortality between the 6^{th} and the 8^{th} day of treatment. Therefore, no mortality were observed in the control. In parallel, moulting disruption was observed, preventing the larvae to become adult.

Keywords: *Metarhizium anisopliae*, fifth instar larvae *Schistocerca gregaria*, biological control.

Rezumat. Efectul fungului entomopatogen *Metarhizium anisopliae* asupra lăcustei de deșert *Schistocerca gregaria*. Lăcustele de deșert pot fi cele mai periculoase din agricultură, datorită capacității roiurilor de a zbura rapid pe distanțe mari. Strategia de combatere a lăcustelor a constat până acum în principal în aplicarea insecticidelor sintetice, care pot fi dăunătoare mediului. Prin urmare, institutele de cercetare apelează la alte metode, inclusiv controlul biologic, în special controlul microbiologic în diferitele sale forme, pentru a încerca să controleze lăcustele roitoare. Datorită persistenței sale în sol și inofensivei sale pentru oameni și animale și în contextul controlului biologic, am testat o ciupercă entomopatogenă *Metarhizium anisopliae* pe larvele a cincea etapă a lăcustei *Schistocerca gregaria* (Forskål 1775). Testele preliminare au fost efectuate în laborator pe larvele de salcâm. Criptogamul a fost administrat prin pulverizare directă la persoanele tratate, au fost utilizate trei doze și anume: D1 = 1,46x107 spori/ml, D2 = 1,46x106 spori/ml, D3 = 1,46x105 spori/ml. În același timp, martorul a fost pulverizat cu apă distilată sterilizată. Am obținut 100% mortalitate între a 6-a și a 8-a zi de tratament. Prin urmare, nu s-a observat mortalitate la control. În paralel, a fost observată întreruperea năpârlirii care împiedică larvele să devină adulte.

Cuvinte cheie: *Metarhizium anisopliae*, larvele de stadiul cinci *Schistocerca gregaria*, control biologic.

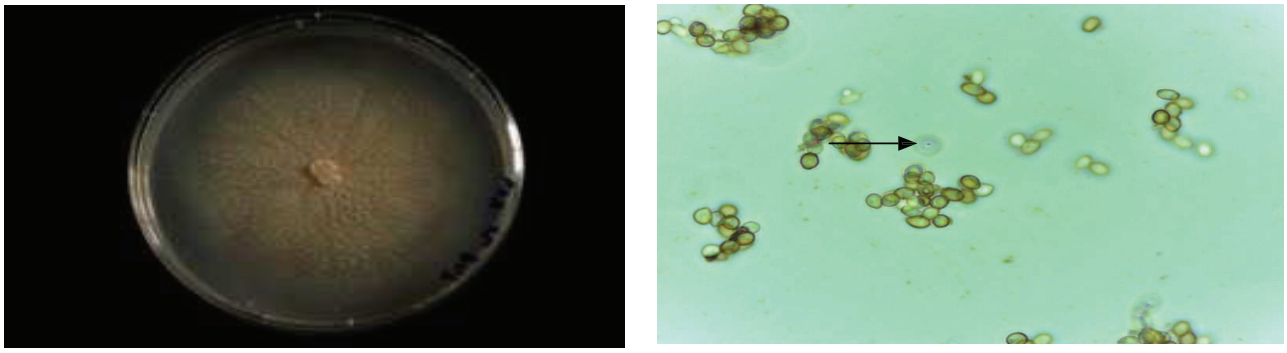
INTRODUCTION

A major danger and enemy of cultures, especially in African and Middle East countries, the Desert Locust *Schistocerca gregaria* (Forskål 1775) has caused significant agronomic damage and economic losses in these countries. In times of invasion, desert locust swarms can invade an area covering more than 29 million km2 or 20% of the land area (DURANTON & LECOQ, 1990). According to COPER (1982) a 10 km2 desert locust swarm contains about 2 billion individuals each consuming the equivalent of its own weight per day, namely 2g, which results in a loss of 4000 tonnes of fresh vegetation per day. It was noticed that hundreds of thousands of litres of pesticides were ordered and delivered to combat locusts because curative control operations required enormous means and high costs by implying several means to provide treatment by air (planes, army) and land (trucks, cars etc.) (LAUNOIS-LUONG et al., 1988).

Pesticides are widely used in agriculture to protect crops and increase productivity but they cause a variety of effects on health like eye and skin irritation; other effects are more serious, such as serious damage in the nervous system and even sterility. A new control method has appeared; it is biological control, in particular microbiological, based on the use of microorganisms such as fungi, bacteria and viruses (LAUNOIS-LUONG et al., 1994). Fungi are entomopathogens present in the humid tropical zone, which sometimes locally eliminate entire populations of locusts. Three fungi are mainly studied, one *Entomophthoral Entomophaga grylli* and two Deuteromycetes: *Metarhizium flavoviride* and *Beauveria bassiana*. According to GREATHED et al. (1994), this can offer the best prospects, especially for those which are formulated and multiplied to be applied as biopesticides.

MATERIAL AND METHODS

Evaluation of the mortality rate of larvae. This test consists of the inoculation of the entomopathogenic fungus *M. anisopliae* on *S. gregaria* larvae 1. The fungal strain: *M. anisopliae var. acridium* strain (coded IMI: 330189) was first identified by Metschnikoff on *Anisoplia anstriaca* and named *Entomophtora anisopliae*, on 1879 Sorokin gave the name of the green muscardine to the genus *Metarhizium*; known under the name of *M. anisopliae* (Metsch) (Zimmerman 1993). Then the fungus is multiplied on a PDA (potato dextrose agar) from the Pastor Institute medium at a temperature between 30-35 ±1°C for incubation (Fig. 1). 2. Desert locusts: The fifth instar larvae *S. gregaria* are raised at a temperature of 30 ± 5°C.



a b

Figure 1. a. cultural aspect of *M. anisopliae*,
b. spores of *M. anisopliae* (original).

Inoculation. Preparation of the entomopathogenic solution (the inoculum): After 7 days of incubation of the fungus, explants are removed and placed in an Erlen-Mayer with sterilized distilled water, which is hermetically sealed to prevent contamination. The Erlen-Mayer is agitated in order to release the maximum of spores, then we dilute till we obtain the desired concentration with the Malassez cell: (D_1 =1,46x10^7 spores/ml, D2= 1,46x10^6 spores/ml, D3= 1,46x10^5). Inoculation mode. For treatment, we used four batches; three were already treated with cryptogam and one batch of control is treated by sterilized distilled water. Each batch contained 30 individuals with two repetitions for each of them. The inoculum is sprayed directly on the insect (by contact) because it is the preferential way of penetration of the fungus (RAPILLY, 1960).

Statistical method. All data were entered into a conventional computer database (Excel 2010). The data verification and statistical processing were carried out on XLSTAT version 7.1 software. The descriptive analysis focused on the determination of mortality rates according to the different doses. We used analysis of variance with the Anova two factor test (Doses and days), showing that the difference is significant for $p<0.05$.

RESULTS

From the results obtained and the graph, we note that the treatment significantly changes with respect to the dose concentration. For the treatment with *M. anisopliae,* the onset of mortalities was recorded in L_5 treated with D1 on the 2nd day to reach 100% on the 8th day. The onset of mortality was delayed until day 4 in L_5 treated with D2, and 100% mortality was reached on day 9 and 10, respectively. Finally, at the low dose D3, mortalities only started on the 4th day and will reach 100% on the 10th day in L_5. At the same time, we did not record any mortality in witnesses during our experiment (Table 1; Fig. 2).

Data analysis. In view of the results concerning the toxicity of the two fungi towards the L5 of *S. gregaria*, we note that their pathogenic activity is highly significant compared to the controls ($P<0.05$). This variation is maintained over time. This mortality rate is more accentuated at high doses: 1.46 x 10^7sp./ml. and 1.46x10^6 sp./ml.

Symptomatology. After inoculation of individuals with *M. anisopliae*, they begin to lose their appetite and their movements become slower and slower until death before becoming paralyzed. We also observe reddish spots on the thorax corresponding to the presence of the mycelium.

The latter are due to the deposition of melanin (CHARNLEY, 1989). In the presence of humidity, the mycelium pierces the cuticle, especially at the inter-segmental membranes and begins to sporulate. Shortly after, the corpse becomes covered with a powdery and greenish layer, characteristic for green muscardine (Fig. 3).

Along with these symptoms we noticed poor mould formation which prevents the insect from moving to the next stage (Fig. 4).

Table 1. Percentage of mortality of *S. gregaria* larvae (L5) controls and treated with *M. anisopliae*.

Days / Doses	1	2	3	4	5	6	7	8	9	10
Témoin	0	0	0	0	0	0	0	0		
D1: 1,46x107 sp/ml.	0	13,33	26,67±5,8	30±0	40±20	63,33±15,3	80±17,3	100±0		
D2:1,46x106sp/ml	0	0	0	30±10	43,33±15,3	60±10	76,67	90±10	100±10	
D3: 1,46x105 sp/ml	0	0	0	13,33±5,8	20±10	20±10	20±10	40±10	90±10	100±0

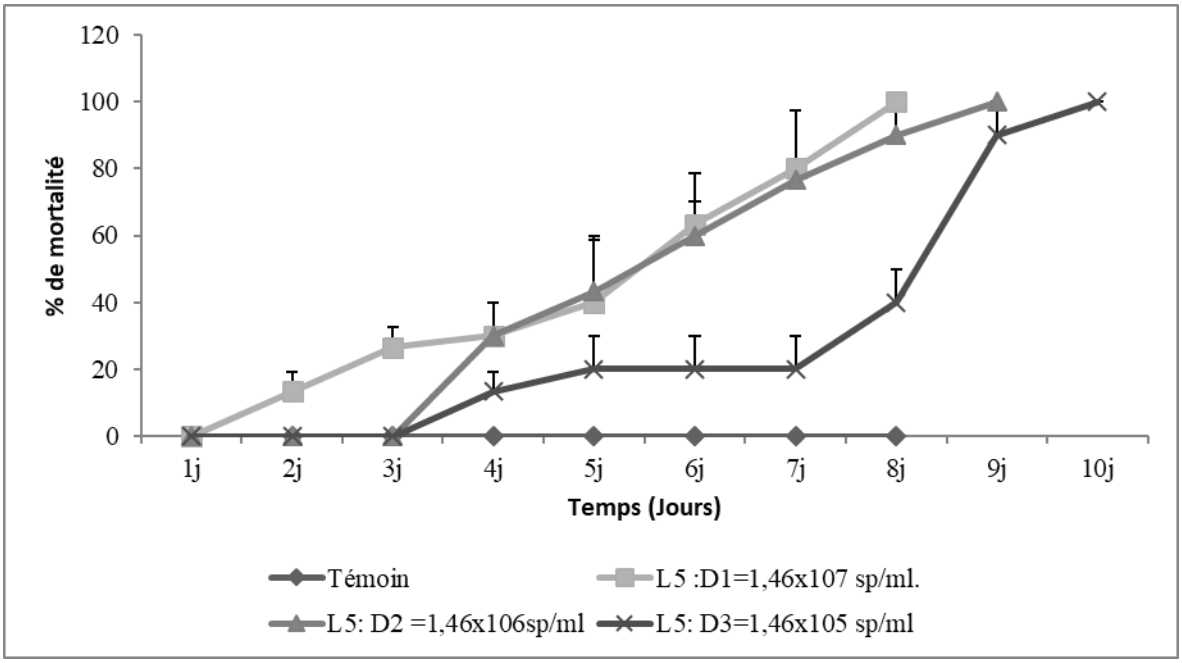


Figure 2. Cumulative daily mortality of *S. gregaria* larvae (L5), treated with *M. anisopliae* per contact at three doses (D1= $1,46x10^7$ sp./ml, D2= $1,46x10^6$ sp./ml, D3= $1,46x10^5$ sp./ml).

Figure 3. *S. gregaria* individual infected with *M. anisopliae* (original).

Figure 4. Molt blockage (original).

DISCUSSIONS

Following the result we obtained, we noted that the cryptogam significantly affected the percentage of mortality. Indeed, we also note from the graphic that the treatment remarkably influenced the treated individuals compared to the witness.

HADDADJ et al. (1998) obtained total mortality in *S. gregaria* after treatment with *Metarhizium anisopliae* (Metsch.).

HADDADJ (2001) recorded 100% mortality between 5 and 7 days after treatment of L5 of *S. gregaria* with *B. bassiana*. In 2014 the same author recoded similar results concerning the same species and the same cryptogamme.

HALOUANE (2008) obtained similar results concerning the effect of *Beauveria bassiana* on L5 and adults of *S. gregaria* after inoculation at the same doses as those we used.

JARONSKI & GOETTEL (1997) working on the effect of *B. bassiana* on adults of *Melanoplus sanginipes* obtained 72% mortalities on the 2nd day at a dose of 1.2.x103 conidia / ml. On this same locust INGLIS et al. (1997) obtained 80% mortality at the start of the 4th day for the 4.3.x103 sp./ml dose.

MILAT-BISSAAD (2011) recorded similar results regarding the effect of *B. bassiana* and *M. anisopliae* against individuals of *S. gregaria*

OUTTAR et al. (2011) recorded results similar to ours regarding the effect of "Green Muscle" on L5 of *Locusta migratoria*.

STEPHAN et al. (1997) report a 90% mortality in adults of *L. migratoria* after two weeks following treatment with *Metarhizium flavoviride* at a dose of 2x107 sp./ml.

SWEARINGEN (1993) obtained a 95% mortality after 7 days of treatment with *B. bassiana*, against the locust *Oedaleus senegalensis* and this for a dose of 2.5x1012 sp./ Ha.

The results of the biological activity of the entomopathogens *M. anisopliae* confirm the satisfactory efficacy of this hyphomycete against *S. gregaria*. However, we note that the higher the dose, the faster the mortalities occur

CONCLUSIONS

Future prospects present themselves in the field of locust control through the use of entomopathogens. Laboratory studies of the efficacy of *M. anisopliae* against *S. gregaria* show that this entomopathogen is potentially effective against this locust. In fact, the observed mortalities and rapidity of action, which increase with the concentrations of the inoculum, suggest good efficacy.

REFERENCES

CHARNLEY A. K. 1989. Mécanisme of fingal pathogenesis in insects in Xhirp S. and Lunsden R. (ed) biothechnologu of fungigal imponing plant grouth. *Insect Biotechnology Journal*. Cambridge University Press. London: 85-127.

COPER A. 1982. The locust and grasshopper agricultural manual for Overseas Pest Research. *I n Van Huis A. eds. Desert locust control with exiting techniques*. Proceeding of the seminar held in Wageningen. London: 11-17.

DURANTON J. F. & LECOQ M. 1990. *Criquet pèlerin au Sahel. Collection Acridologie opérationnelle*. Edit. CIRAD – PRIFAS. Montpellier. **6**. 183 pp.

GREATHEAD D. J., KOOYMAN C., LAUNOIS-LUONG MY. H., POPOV G. B. 1994. *Les ennemis naturels des criquets du Sahel. Collection Acridologie opérationnelle*. Edit. CIRAD – PRIFAS. Montpellier. **8**. 147 pp.

HADDADJ FAIROUZ, DOUMADJI-MITICHE BAHIA, HALOUANE FATMA, DOUMANDJI S. 1998. Efficacité de *Metarhizium anisopliae* sur les œufs, les larves et les imagos de *S. gregaria*. Effet sur la respiration et le rythme cardiaque de cet acridien. *2ème journée d'entomologie*. Institute National Agronomy Press. El-Harrach: 18-25.

HADDADJ F. 2001. *Evaluation de l'activité biologique de l'entomopathogène Beauveria bassiana (Bals) (Hyphomycetes, Deuteromycotina): efficacité sur la cuticule des L5 de Schistocerca gregaria (Forskal, 1775) (Acrididae, Cyrtacantharidinae)*. Thèse Doc. Institute National Agronomy Press. El-Harrach. 96 pp.

HALOUANE F. 2008. *Recherche sur l'acridopathogène B.bassiana (Hyphomycétes, Deuteromyucotina): Bioécologie production et application sur Schistocerca gregaria (Forskal, 1775) et Locusta migratoria (Linné, 1758) (Orthoptera, Acrididae)*. Thèse Doc. Science Agronomy, Ecol. Nat., Sup. Agro. El Harrach. 190 pp.

JARONSKI LAUNOIS-LUONG MY. H., RACHADI T., DEUSE J. 1994. Les biopesticides en lutte antiacridienne par http://www7.inra.fr/opie-insectes/pdf/i92Launois-et-al.pdf (accessed: March 30, 2020).

MILAT-BISSAAD F. Z. 2011. *Etude comparative de l'effet de deux entomopathogènes, Beauveria bassiana (Vuil., 1912) et M.anisopliae var acridium (Metch., 1883) (Hyphomycètes,Deuteromycotina) sur le criquet pèlerin Schistocerca gragaria (Forskål,1775) (Orthoptera, Acrididae)*. Thèse Doc. Agro., Ecol. Nat. Sup. Agro. El Harrach. 205 pp.

OUTTAR F., DOUMANDJI-MITIHE B., MOUHOUHE F., DOUMANDJI S. 2011. *Méthodes alternatives en lutte antiacridienne*. AFPP- 4ème Conférence Internationale sur les méthodes alternatives. London. 45 pp.

RAPILLY F. 1960. *Eléments d'écologie fondamentale*. Edit. Mac. Graw Hill. Paris. 197 pp.

STEPHAN D., WELLINGH H., ZIMMERMAN G. 1997. *Locust control with Metarhizium flavoviride: new approches in the development of biopreparation based on blastospores in new strategies in locust control*. Edit. Krals. Bern. 158 pp.

SWEARINGEN W. D. 1993. Lutte biologique contre les acridiens - Lettre d'information du 19 octobre - *Surveillance des acridiens du sahel CIRAD – PRIFAS*. Montpellier: 17-20.

ZIMMERMAN G. 1993. The Entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol agent. *Pest Science*. Paris. **37**: 375-379.

Haddadj Fairouz, Saadi Habiba, Chorfi Nassima, Remichi Hayet, Zenia Safia, Smai Amina, Milla Amel
National Veterinary High School, of El Alia, Algiers, Algeria.
E-mail: fairouz.haddadj@gmail.com

Hamdi Sihem
University Saad Dahlab of Blida 1; Faculty of Sciences of Nature and Life, Algiers, Algeria.
E-mail: fairouz.haddadj@gmail.com

Doumandji-Mitiche Bahia
National Agronomic High School, of El Harrach, Algiers, Algeria.
E-mail: fairouz.haddadj@gmail.com

Received: April 4, 2021
Accepted: July 23, 2021