THE EFFECT OF THE ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae* ON THE EGGS OF THE DESERT LOCUST *Schistocerca gregaria* (Forskål, 1775)

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

Abstract. The desert locust potentially is the most dangerous for agriculture, because of the swarms' ability to fly rapidly across great distances. So far, the locust control strategy has mainly consisted of the application of synthetic insecticides, which can be harmful to the environment. Therfore 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 *M. anisopliae* on the eggs of the desert locust *S. gregaria*. Preliminary tests have been done in the laboratory on the locust ootheca. The cryptogam was administered by direct spraying on the treated eggs, and two doses were used, namely: $D_1 = 10^2$ spores / ml. and $D_2 = 8.6 \times 10^5$ spores / ml. At the same time, the witness was sprayed with sterilized distilled water. The results obtained showed that 40% of the eggs hatched at the low dose and only 24% at the high dose, therefore the hatching rate in the controls is higher.

Keywords: *Metarhizium anisopliae*, *Schistocerca gregaria*, ootheca, biological control.

Rezumat. Efectul fungului entomopatogenic *Metarhizium anisopliae* pe ouăle lăcustei de deșert *Schistocerca gregaria* (Forskål, 1775). Lăcusta de deșert este potențial cea mai periculoasă din agricultură, din cauza capacității roiurilor de a zbura rapid pe distanțe mari. Strategia de control a lăcustelor a constat până acum în principal în aplicarea insecticidelor sintetice, care pot fi dăunătoare pentru mediu. Institutele de cercetare Therfore apelează la alte metode, inclusiv controlul biologic, în special controlul microbiologic în diferitele sale forme, pentru a încerca să controleze lăcustele pline. Datorită persistenței sale în sol și fiind inofensiv pentru oameni și animale și în contextul controlului biologic, am testat o ciupercă entomopatogenă *M. anisopliae* pe ouăle lăcustei de deșert *S. gregaria*. Testele preliminare au fost efectuate în laborator, pe ootheca lăcustei. Cryptogamul a fost administrat prin pulverizare direct pe ouă tratate, au fost utilizate două doze, și anume: $D_1 = 10^2$ spori / ml. și $D_2 = 8,6 \times 105$ spori / ml. În același timp, martorul a fost pulverizat cu apă distilată sterilizată. Rezultatele obținute au arătat că 40% din ouăle eclozate la doză mică și doar 24% la doză mare, prin urmare, rata de eclozare la controale este mai mare.

Cuvinte cheie: *Metarhizium anisopliae*, *Schistocerca gregaria*, ootheca, 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 of 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.

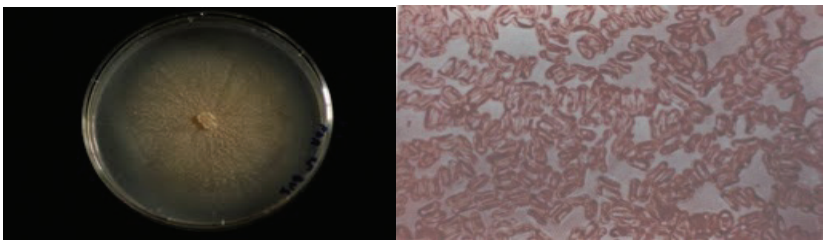
It was noticed that hundreds of thousands of litres of pesticides were ordered and delivered to combat locusts because curative control operations required enormous means and high costs by implying several means to provide treatment by air (planes, army) and land (trucks, cars etc.) (LAUNOIS-LUONG et 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 causing 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 hatching rate of eggs:

This test consists of the inoculation of the entomopathogenic fungus *Metarhizium anisopliae* on *S. gregaria* eggs

1. The fungal strain: *M. anisopliae* is a local strain isolated from a Geotrogus deserticola then the fungus is multiplied on a PDA (potatoes dextros agar) from the Pastor Institute medium at a temperature of 25 ±1°C for incubation (Fig. 1).



a b

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

2. Desert locusts: The eggs of *S. gregaria* are raised in an oven at a temperature of 30 ± 5˚C (Fig. 2).

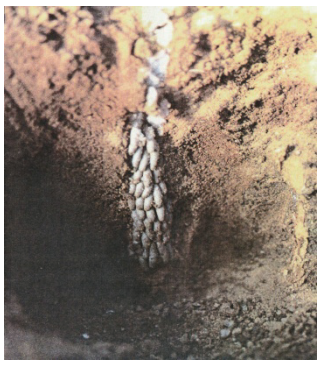


Figure 2. Ootheca of *S. gregaria* (Duranton in POPOV et al., 1991)

Inoculation. Preparation of the entomopathogenic solution (the inoculum):

After 10 days of incubation of the fungus, explants are removed and placed in an Erlen-Mayer with sterilized distilled water; hermetically sealed to prevent contamination. The Erlen-Mayer is agitated in order to release the maximum of spores then it made dilution till we obtain the desired concentration with the Malassez cell: ($D_1 = 10^2$ spores / ml and $D_2 = 8, 6x10^5$ spores / ml).

Inoculation mode. For treatment, we used three batches for eggs; two were already treated with cryptogam and one batch of control is treated by sterilized distilled water. Each batch contained 25 eggs with two repetitions for each of them. The inoculum is sprayed directly on the eggs (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 hatching rates according to the different doses. We used the Kruskal-Wallis test and Wilcoxon test to compare hatching rates recorded at significant level $p<0.05$.

RESULTS

From the results obtained and the graph, we note that the treatment significantly limited the hatching of the treated eggs compared to the control eggs. Indeed, we notice that at the high dose 8.6 x 10^5 sp./ml, 24% of the eggs are hatched and at the low dose 10^2 sp./ml. 40% hatching is noticed versus a rate of 65% for with control; and this after 10 days of treatment. This explains the pathogenic effect of the fungus on the eggs of *S. gregaria* for the two doses, although this percentage is higher at the low dose (Table 1; Fig. 3).

Table 1. Percentage of hatching eggs of *S. gregaria* controls and treated with *M. anisopliae*.

Days / Doses	1	2	3	4	5	6	7	8	9	10
With control	30	30	30	30	40	40	55	65	65	65
D1	0	0	0	12	28	28	28	28	28	40
D2	0	8	8	12	20	20	20	20	20	24

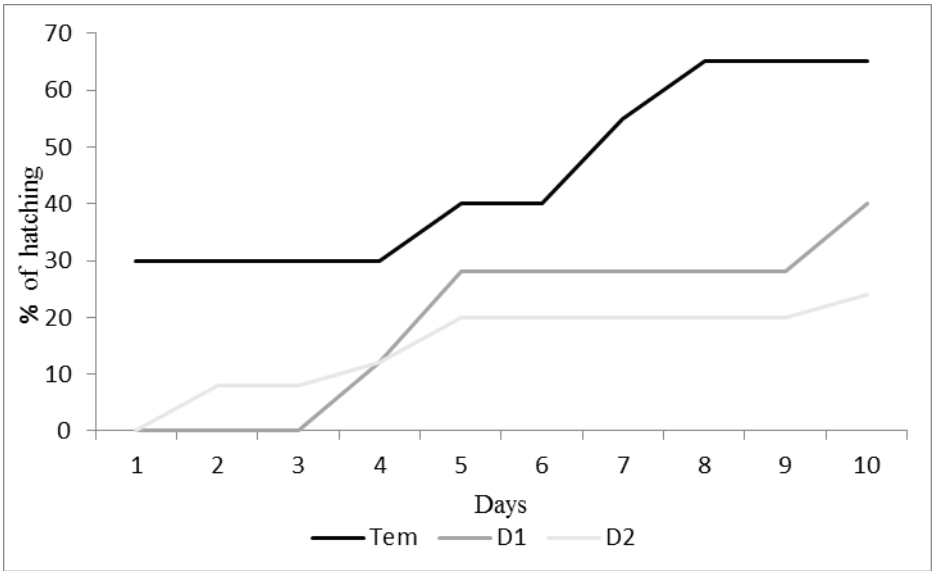


Figure 3. Hatching percentage of the *S. gregaria* eggs using different doses.

Data analysis. Our results are confirmed by the Kruskal Wallis statistical test which demonstrates that the difference between the samples is highly significant. In order to evaluate the effect of the cryptogam on the locust, we have used Kruskal Wallis statistical test (Table 2).

Table 2. Kruskal Wallis statistical test.

H (observed value)	18,547
H (critical value)	5,991
DDL	2
P-value unilateral	< 0,001
Alpha	0,05

Note: H of Kruskal-Wallis was calculated taking into account ex aequo H of Kruskal-Wallis distributed as khi-2.

The study allowed us to record a very high average hatching rate in the control batch with pt (%) = 450.16 and in second position the value recorded in the batch at dose D_1 with pD_1 (%) = 19.20±0,12 and the lowest value observed in the D_2 batch with pD_2 (%) = 15,20±0,11 (Fig. 4).

With the Wilcoxon test, we were able to establish a highly significant difference ($p < 0.005$) for the hatching rates recorded between the control and D_1 batches, as well as between control and D_2, which confirms that the hatching rates are very low for the two lots D1 and D2. However, they did not show any significant difference in the recorded hatching rates. This comparison was illustrated in Figure 3 using different small letters.

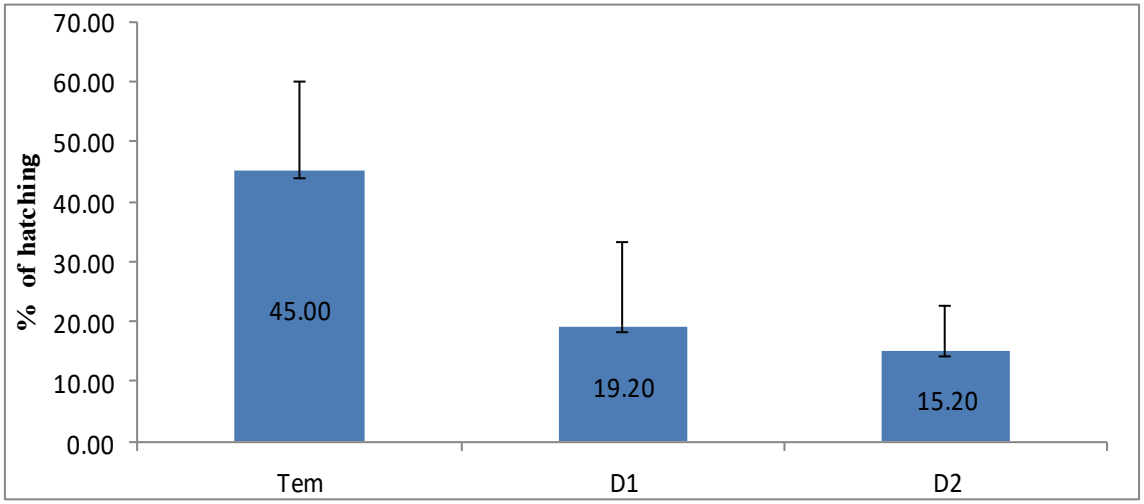


Figure 4. Box in graph concerning the hatching percentage of the *S. gregaria* eggs using different doses.

DISCUSSION

Following the result we obtained, we noted that the cryptogam significantly affected the hatching percentage of eggs; indeed, we also note from the graphic that the treatment remarkably limited the hatching of the treated eggs compared to the witness eggs.

We noticed that at the high dose 8.6 x 10^5 sp./ml. 24% of the eggs are hatched and at the low dose10^2 sp./mL, a 40% hatching rate is obtained versus a rate of 65% among the witness batch; and this after 10 days of treatment. This explains the pathogenic effect of the fungus on the eggs of *S. gregaria* for the two doses, although at a low dose this percentage is higher. Similar results have been reported by HADDADJ et al. (1998) and (2016) concerning the effect of *M. anisopliae* on the hatching rate of S. *gregaria* eggs, in fact the latter significantly reduced this rate, whether at the high dose or at the reliable compared to witness.

MILAT-BISSAAD (2011) mentioned a hatching rate of 31.11% of *S. gregaria* treated by *B. Basiana* and 13.3% treated by *M. anisopliae*, compared to the witness with a 90% hatching rate. In the same direction, OUTTAR (2006) recorded similar results using the same fungus on *Locusta migratoria*. The same author (2009) tested three biopesticides: Henna, triflumiron and *M. anisopliae* on the number of ootheca and the average number of eggs by ootheca for the females of *L. migrotaria*; the treatment significantly decreased the ootheca number and the eggs number by ootheca by contact compared to the average number zero for treatment by ingestion.

AMAR & KALLEL (2016) noted that the treatment by strains of *H. bacteriophora* (HLAB), *H.argentinensis* (CMW) and *H. indicus* (HIW) decreased the hatching eggs rate of *L. migratoria* compare to witness. At the end of the treatment, the hatching of the control eggs exceeds 90%. HIG (*H. indicus*) and CMG (*H. zealandica*) show lower hatching rates compared to other strains (ASI et al., 2013).

The entomopathogenic fungi used in the bioassay were all infective to freshly laid eggs of *S. litura.* Significantly mortalities were caused by the entomopathogenic fungi at each conidial concentration tested. *Metarhizium anisopliae* L6, *I. fumosorosea* 32 and *B. bassiana* 25 resulted in 48.19- 71.56% egg mortality above 1x10^6 conidia ml-^{1}. LAMRI, 2015 has observed the higher average number of eggs by ootheca in female of *L. migratoria* witness 41.89% compare to 38.06% eggs in females treated by *Bacillus thuringiensis* and 27.11% eggs treated by *Bacillus subtilis*. Thereby, these results indicate that *B. subtilis* has a very remarkable effect on this parameter compared to the one shown by MOUSSA (2003), who proved that the treatment with Neem oil resulted in a reduction in the number of egg cells by female and a reduction in the number of eggs by female and even in the eggs number by ootheca in *Locusta migratoria* and *Locusta migratoria migratorioides*.

RODRIGUEZ-RUEDA & FARGUES (1980) found that *I. fumosorosea* was highly virulent against eggs of *Mamestra brassicae* and *Spodoptera littoralis,* LEZAMA-GUTIERREZ et al. (1996) observed that eggs of fall armyworm, *Spodoptera frugiperda*, were highly susceptible to insect pathogenic fungi; *M. anisopliae, I. fumosorosea* and *Isaria javanicus.*

CONCLUSION

In our study, we have evaluated the biological impact of the entomopathogenic fungus *Metarhizium anisoliae* in order to define the efficiency in enlarging its action.

According to the obtained results, we noticed that the cryptogam *M. anisopliae* significantly reduced the hatching rate of treated eggs. In fact, the maximum number of hatched eggs is obtained on the tenth day, compared to with control, where this percentage is staggered over time. This confirms the lethal effect of the fungus.

We can conclude that the biological control by using *M. anisopliae* in agriculture is a promising approach to reduce pesticide use. However, it contributes to promoting organic farming concerned with preserving biodiversity and humanity.

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Haddadj Fairouz, Zenia Safia, Smai Amina, Milla Amel, Chorfi Nassima
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

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