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THE INTERACTION OF L5 LARVAE OF *LOCUSTA MIGRATORIA* (LINNAEUS, 1758) (OEDIPODINAE, ACRIDIDAE) WITH BIOBESTICIDES

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ABSTRACT

Our work is based on the use of three biopesticides belonging to three different categories, a fungus *Metarhizium anisopliae var acridum* a IGRs Triflumuron (TFM) and an excerpt from the henna plant *Lawsonia inermis*, on L5 larvae of *Locusta migratoria* applied with the two modes of penetration, contact and ingestion. For this, we tested their effect on the morphology and mortality. The results show us that the three biopesticides involved morphological deformations and a total mortality of 100% in the L5 larvae.

KEYWORDS: *Locusta migratoria*, *Metarhizium anisopliae var acridum*, Triflumuron, *Lawsonia inermis*, Morphology, Mortality

INTRODUCTION

Grasshoppers and locusts threaten agricultural production since humans have agricultural activities. The migratory locust *Locusta migratoria* locust is that the ecological plasticity and geographic reach of the largest, in fact it rose from the Sahel to the south of Algeria, where it has become more important at times replacing the desert locust *Schistocerca gregaria*. It is likely to cover economic importance by the extent of the damage it can cause to crops in arid, because each individual can eat on a daily basis the value of its own weight. In infested areas, control of grasshoppers and locusts has always required and still requires extensive campaigns chemical causing the use of massive amounts of chemical pesticides. The increasing use of chemical pesticides, the risks of their use for human health and the environment and the high cost of control operations have led some players to ask a number of questions on whether and the effectiveness of current control strategy and its impact on the environment. Within this framework, some donors have focused on finding an alternative control may have little negative impact on the environment while providing a lasting solution to the locust problem. Biological control is an alternative to chemical control with the use of biopesticides, to ensure better protection of human health and the environment.

Our work is designed to use three biopesticides from three different categories, a fungus *Metarhizium anisopliae var acridum*, an endocrine growth Triflumuron (TFM) and an extract of the henna plant *Lawsonia inermis*, in order to test the L5 larvae *Locusta migratoria*, taking into account their effect on the morphology and mortality.

MATERIALS AND METHODS

Biological Material

Our trials were carried out on L5 larvae of *Locusta migratoria* from one of the breeds permanent maintained at the department of Zoology Agriculture and Forestry at the superior National School Agronomic El Harrach, with wooden cages suitable for each stage at a temperature of 30 ± 3 °C and a relative humidity of 50 to 60%.Their food is based on grass and a complement of wheat bran. When we tested for biopesticides, as fungus we used the *Metarhizium anisopliae var acridum*, which comes from the National instlitute of Plant Protection (INPV) in El Harrach, as a biopesticide called Green Muscle "Formulated in a concentrated oil of spores. The Triflumuron (TFM) family of Benzohyles ureas is the growth disrupting, therefore as a plant we used henna *Lawsonia inermis*.

Study of the Effect of Three Biopesticides on the Morphology and the Mortality of L5 Larvae of *L. migratoria*

We chose a dose sublétlale to monitor the effect of these three biopesticides on the parameters studied. The *M. anisopliae* was tested at a dose of 0.22 X 10^8 spores / ml. Counting and determination of spore concentrations were performed using a Neubauer cell under an optical microscope. The Triflumuron and henna extract were tested at a concentration of 0.41 ml / l and 12.5% (S / 8), respectively.

The tests were carried out on larvae from molting L5 stage, these were isolated in blocks of treatment and maintained under the same conditions of temperature and humidity that breeding cages. For each dose, we did 3 replicates and each replicate consists of 10 larvae.

Two types of treatment have been made, the first contact by spraying a volume of 10ml of the three biopesticides directly on the larvae, whereas untreated controls were sprayed with distilled water. The second treatment ingestion, the larvae are fed with treated turf by three biopesticides, while controls have ingested grass sprayed with distilled water.

Each day during the treatment, we record the number of dead larvae for witnesses and the one for the treaties; we also took note of malformations, anomalies and morphological changes observed in the treated insects. The duration of this study was set up to the molting stage, paining formation or death of the witnesses and of the treaties, by the two modes of penetration.

Statistical Analysis

In order to give a statistical significance to the results found through the various parameters studied, data processing is done using the software XL.STAT version 6.0 - ANOVA, which we used to analysis variance at a confidence interval of 95%.

RESULTS

Effect of Three Biopesticides on the Morphology of L5 Larvae of *L. migratoria*

Following the observations made daily during the treatment period, we note that the Triflumuron and henna extract showed the same symptoms at the time of imaginable molting. The larvae are weakened and have difficulties in molting, they suffered to get rid of their exuviae, but they failed, as a result they are trapped and eventually die. On the organized morphology, we observed an increase in size, swelling in the abdomen and pronotum Figure 1. The same observations were noted in henna and in larvae which were able to hardly make their molt, deformations at elytra were observed Figure 2. Larvae treated with *M. anisopliae var acridum* show some morphological changes in coloration: the

larvae become completely red Figure 3 and mummified after their death Figure 4. Symptoms of infection of this fungus on the larvae occur with the existence of spores, which covers the body of the cadaver Figure 5.

Figure 1: L5 Larvae of *L. migratoria* Treated with Triflumuron

Figure 2: L5 Larvae of *L. migratoria* Treated with Henna

Figure 3: L5 Larvae of *L. migratoria* Reddish after Treatment with *M. anisopliae*

Figure 4: Mummification of L5 Larvae of *L. migratoria* Treated with *M. anisopliae*

Figure 5: Female of *L. migratoria* Infected with *M. anisopliae*

Effect of Three Biopesticides on the Mortality of L5 Larvae of *L. migratoria*

The effect of three biopesticides on L5 larvae of *L. migratoria*, manifested with a mortality which differs from one product to another and we can see that the Triflumuron is the most effective with 100% mortality recorded on the 18th day in dose D1, on the19th day in dose D2 and D3, for the treatment by contact Figure 6. The mortality rate was 96.67% on the 20th day in dose D1 and100% in dose D2 on the 18th day as well as in dose D3 on the 20th day for the treatment by ingestion Figure 7. Henna records 100% mortality obtained on the 21th day in dose D1, in dose D2 on the 20th day and in dose D3 on the 16th day for the treatment by contact Figure 8.

The mortality rate was 96.67% on the 22th in doses D1, and 100% in dose D2 and D3 on the 19th and 22th day respectively for the treatment by ingestion Figure 9. *M. anisopliae* showed an effect less important compared to the two previous products, it showed a mortality rate of 79.17% in dose D1, 70.83% in dose D2 and 100% in dose D3 on the 22th day for the treatment by contact Figure 10. The mortality rate reaches 91.67% in dose D1, from 79.17% to 91.67 in dose D2 and D3 on the 22th day for the treatment by ingestion Figure 11. The extract of Henna showed an insecticidal activity similar to that of disrupting growth. Indeed, the larvae treated with Triflumuron and henna die at the stage of molting. We also found a LD50 = 0.32 x 10^{9} spores / ml for *M. anisopliae*, a LD50 = 2.75 ml / l for Triflumuron and LD50 = 5.94% with henna for the treatment by contact. Analysis of variance revealed that there is a highly significant difference between the daily cumulative mortality of witness and of treaties larvae on three products with both type of treatments by contact and ingestion (Probability <0.05).

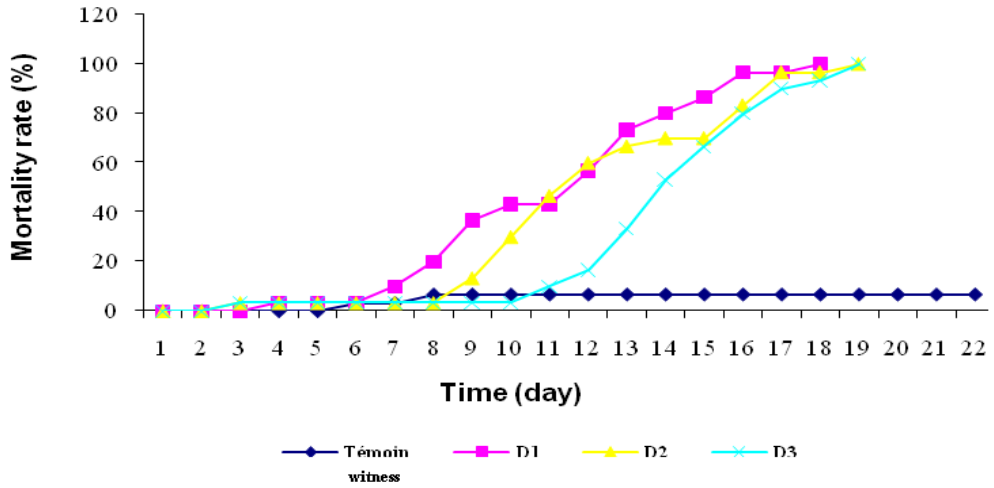

Figure 6: Cumulative Daily Mortality Rate of Larvae L5, Treated with Triflumuron by Contact

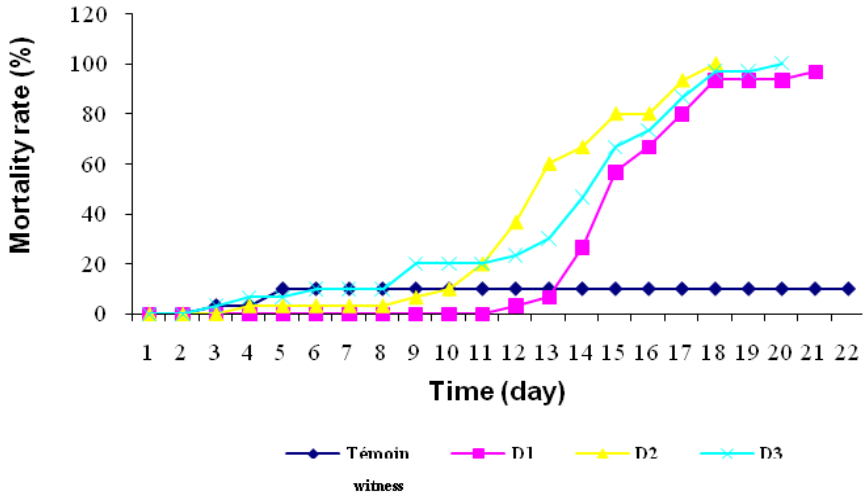

Figure 7: Cumulative Daily Mortality Rate of Larvae L5, Treated with Triflumuron by Ingestion

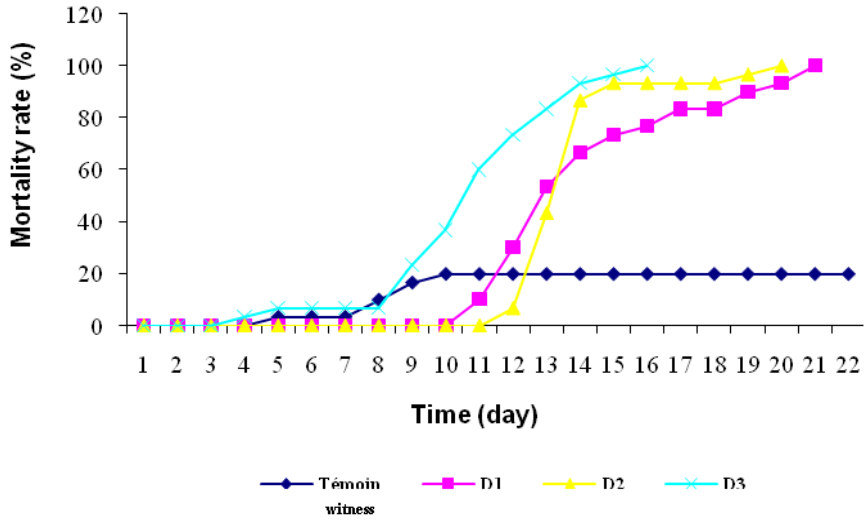

Figure 8: Cumulative Daily Mortality Rate of Larvae L5, Treated with Henna by Contact

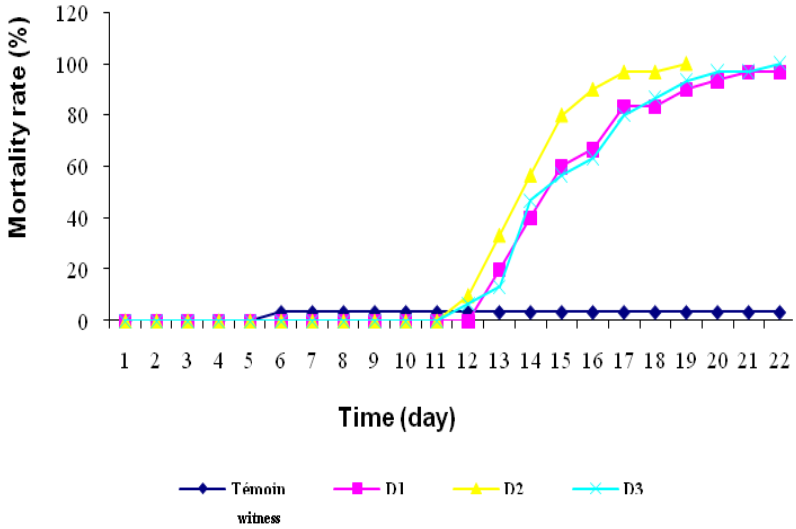

Figure 9: Cumulative Daily Mortality Rate of Larvae L5, Treated with Henna by Ingestion

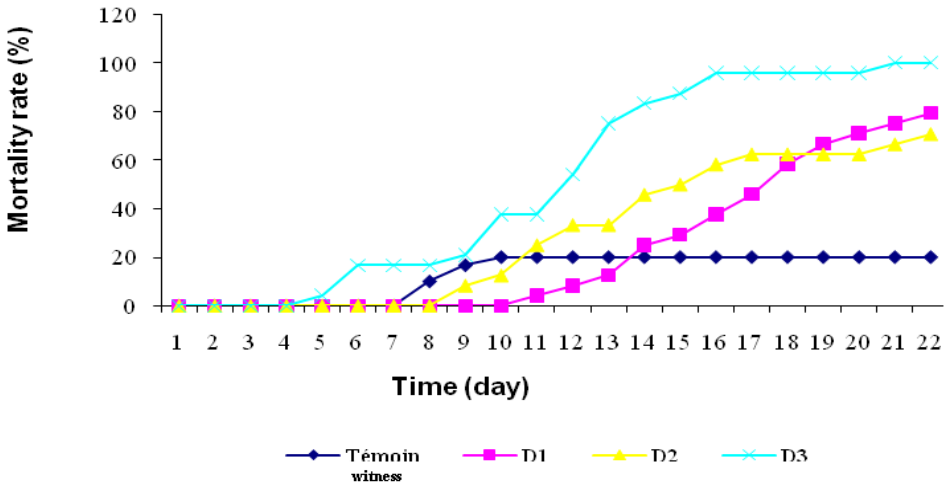

Figure 10: Cumulative Daily Mortality Rate of Larvae L5, Treated with *M. anisopliae* by Contact

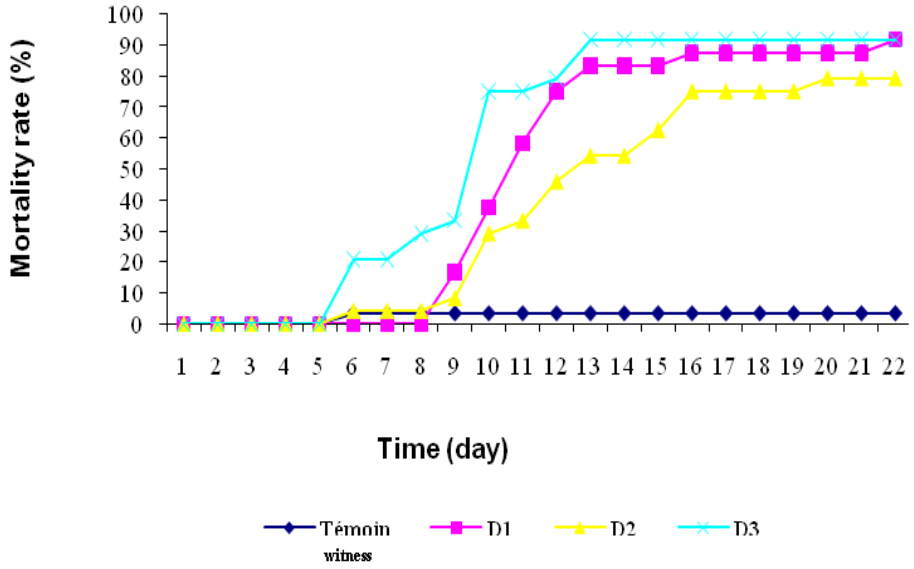

Figure 11: Cumulative Daily Mortality Rate of Larvae L5, Treated with *M. anisopliae* by Ingestion

DISCUSSIONS

Effect of Three Biopesticides on the Morphology of L5 larvae of *L. migratoria*

According to Nasseh and *al*. (1992), treatment by growth inhibitors such as Teflubenzuron and Triflumuron causes disabilities among individuals locust explained by: The insect found unable to molt, reducing the ability to fly due to the deformation of wings, difficulties in food intake due to the malformations or lack of feet and tarsi, barriers to general communication especially in the loss of ability to reach copulation brought by the loss or malformation of extremities and loss of sense of direction following the disappearance of the antennae and eye strain.

Hanrieder and *al*. (1993), added that the larvae of *Locusta migratoria migratorioides* treated with Triflumuron showed an aspect of a molt after their death, no hardening of the cuticle with a break between the membrane segments of the locust is observed.

According Dorn et al. (1997), fenoxycarb was tested on instar larvae of L. migratoria capito in the laboratory and field disturbances caused morphogenic order during metamorphosis, reduced fertility and led the solitarisation gregarious

insects. Changing the phasal condition reported by the color green locusts and the acquisition of typical morphological and behavioral characteristics of solitary locusts.

Rembold (1997) indicated that 80% of malformations in the Desert Locust have been obtained after the treatment with the extract of *Melia volkensii*.

According Soltani et al., (1999), the Alsystin (48% CI), a commercial formulation of Triflumuron was first tested in the laboratory on the fourth-stage larvae of *Culex pipiens pipiens* L. (Diptera: Culicidae) newly exuviées. The treatment causes morphological aberrations and reduced adult emergence.

Kooyman (2007) said that *Metarhizium* infects insects, where the cuticle and green spores are formed 24 or 48 hours after the infection with the fungus.

Effect of Three Biopesticides on the Mortality of L5 Larvae of *L. migratoria*

- Hanrieder and *al*. (1993), announced that the Triflumuron applied to the L2 and L4 *L. migratoria migratorioides* gave mortality rates varying between 60% and 90% after treatment 18 days later.
- Zimmermann and *al*. (1994), found a mortality rate reaching 100% in 8 to 10 days after a treatment of *Metarhizium spp* on L3 of *L. migratoria*.
- Fargues and *al*. (1997), found a mortality rate between 98 and 100% after 8 days of treatment of *Schistocerca gregaria* with *Metarhizium flavoviride* at a temperature between 25 ° C and 30 °C.
- According Welling and Zimmermann (1997), the cuticle of individuals of L. migratoria infected Sorosporella sp. becomes pale and breaks up easily, releasing masses of reddish-brown spores that fill the entire body. These thick-walled spores are globular, with a diameter of 7.5 microns and clumped together in units of tens or hundreds of spores.
- Wilp and Diop (1997), announced that the mortality rate obtained with the growth disrupting: Triflumuron (Alsystin) teflubenzuron (Nomolt) and diflubenzuron (Dimilin) were 40% after 6 days, waiting to reach the maximum of 100% after 10 days of field trials carried out on the desert locust.
- Barbouche and *al*. (2001), confirmed that the toxicity biotests revealed a total mortality of L5 larvae of *Schistocerca gregaria* within 2 to 4 days from a methanol extracted from leaves of *Cestrum parqui*.
- Blanford and Thomas (2001) obtained a higher mortality rate of 90% after 10 days under conditions of constant temperature, 66% after 70 days under optimal thermo treatment with *Metarhizium anisopliae var acridum* applied to adults of *Schistocerca gregaria*.
- Abbassi and *al*. (2004), reported a mortality rate of 100% in larvae of *Schistocerca gregaria* is reached after 15th day of starting treatment with an extract of alkaloids in leaves of *Calotropis procera* in vegetation.
- Tirchi and Mohouche (2008), have made treatment orally with the Triflumuron the five larval stages of locusts, this deregulatory growth has led to a high mortality of up to 100% in all larval stages. The fourth day began mortality in L1 and L3, the 3rd day in L2 and the 6th day in L4 and L5. They also noted that a rate of 100% mortality is achieved by the young stages.

CONCLUSIONS

We can concluded that the study of the effect of contact and ingestion of three biopesticides on the morphology of L5 of *Locusta migratoria*, has allowed us to see an increase in the size of individuals, presence of swelling in the abdomen and pronotum and morphological deformations at the stage of molting in individuals treated with Triflumuron and henna. Larvae treated with henna hardly succeed their imaginable molts and have deformations in the elytra. Larvae treated with *M. anisopliae* show changes in the coloration of their bodies, they become completely red after their death and they are mummified.

Regarding the effect of three biopesticides on mortality, we recorded a total mortality of 100% in L5 treated by the three biopesticides with both modes of penetration.We can also note that the Triflumuron is the most effective followed by henna. And *M. anisopliae* has a smaller effect compared to the two previous products. Even though the extract of henna has an insecticide activity similar to that of growth disrupting, indeed larvae treated with Triflumuron and henna die at the molting stage.

We found a LD50 = 0.32 x 10^9 spores / ml for *M. anisopliae*, LD50 = 2.75 ml / l for Triflumuron and LD50 = 5.94% for the henna with the treatment by contact.

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