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BIOPHYSIOLOGICAL RESPONSE OF THE MIGRATOR LOCUST *LOCUSTA MIGRATORIA* (LINNE, 1758) FIFTH INSTARS SUBJECTED TO HENNA, TRIFLUMURON AND *METARHIZIUM ANISOPLIAE* VAR ACRIDUM BASED TREATMENTS

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Abstract

Description of the subject: The study concerns the evaluation of three products, GREEN MUSCLE, Triflumuron and Henna in the fight against locusts.
Objectives: Our work is based on the use of three products, *Metarhizium anisopliae var acridum*, Metch., Triflumuron (TFM) and henna *Lawsonia inermis* (Lytraceae, Linnaeus, 1753) on L5 larvae of *Locusta migratoria* (Acrididae, Linnaeus , 1758) applied with two modes of penetration, contact and ingestion.
Methods: We tested the effect of these three products on the development and on the hemolymphatic proteins of the L5 larvae, in quantitative and qualitative terms.
Results: The results obtained show us that the three products applied with the two treatment modes do not allow the passage of the L5 stage to the imago stage in the treated insects and induced alterations in the hemolymps proteinemia of the L5 of *Locusta migratoria*.
Conclusion: The results allowed us to demonstrate the effectiveness of these three products on L5 larvae of *Locusta migratoria*.
Keywords: *Locusta migratoria*, *Metarhizium anisopliae var acridum*, Triflumuron, *Lawsonia inermis*, Development, Hemolymphatic Proteins.

RÉPONSE BIOPHYSIOLOGIQUE DES LARVES L5 DU CRIQUET MIGRATEUR *LOCUSTA MIGRATORIA* (LINNE, 1758) SOUMISES AUX TRAITEMENTS À BASE DU HENNE, DU TRIFLUMURON ET DE *METARHIZIUM ANISOPLIAE VAR ACRIDUM*

Résumé

Description du sujet : L'étude porte sur l'évaluation de trois produits, Green muscle, le Triflumuron et l'extrait aqueux du henné appliqués sur des larves L5 de *Locusta migratoria* dans un contexte de lutte anti-acridienne.
Objectifs : Notre travail consiste à l'évaluation des trois produits acridicides sur les larves L5 de *Locusta migratoria* (Acrididae, Linné, 1758) appliqués en deux modes : par contact et par ingestion.
Méthodes : L'effet des trois produits testés a été évalué sur la base de deux paramètres en prenant en considération l'aspect quantitatif et qualitatif à savoir le développement larvaire et les protéines hémolymphatiques des larves L5.
Résultats : Les résultats obtenus ont montré que les trois produits appliqués avec les deux modes de traitement bloquent la mue imaginale chez les individus traités en provoquant des altérations au niveau de la protéinémie de l'hémolymphe des L5 de *Locusta migratoria*.
Conclusion : Les résultats du présent travail nous ont permis de démontrer l'efficacité de ces trois produits sur les larves L5 de *Locusta migratoria*, ce qui soutient l'hypothèse de l'existence d'une possibilité pour les employer dans un programme de lutte antiacridienne
Mots-clés : *Locusta migratoria*, *Metarhizium anisopliae var acridum*, le Triflumuron, *Lawsonia inermis*, développement, proteines hémolymphatiques.

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INTRODUCTION

The devastating ability of the migratory locust is due to its high intake capacity. Each individual can eat as much as its weight daily, as well as the large food range that it can consume. Locusts can form incredibly dense colonies containing from 40 up to 80 million individuals [1]. Ecological plasticity and geographical expansion of the migratory locust *Locusta migratoria* are the most high among all insects [2]. Controling this pest is one of the most important considerations in arid and semi-arid zones where huge volumes of costly chemicals are still being used [3]. Both human health and the environment are threatened. Looking for an alternative to chemical pesticides has led to the use of biopesticides [3]. Many researchers have worked on biopesticides, we can name Blanford et Thomas [4], who assessed *Metarhizium anisopliae var acridum* on adults of *Schistocerca gregaria*. Concerning plant extracts, we give the example of Ould El Hadj et *al*. [5], who studied the toxicity of extracts from *Melia azedarach*, *Azadirachta indica* and *Eucalyptus globules* on L5 instars and adults of *S. gregaria*. Wilps and Diop [6], observed the effect of three growth deregulators: triflumuron (Alsystin), teflubenzuron (Nomolt), and diflubenzuron (Dimilin) on *Schistocerca gregaria* in the field. The aim of our work is to study the effect of two biopesticides: a fungus (*Metarhizium anisopliae va racridum*)and a plant extract (henna *Lawsonia inermis*) along with a growth deregulator (Triflumuron). The development of L5 instars as well as the quantity and quality of hemolymphatic proteins of *Locusta migratoria* were studied.

MATERIALS AND METHODS

1. Biological material

1.1. Insects

Larvae of fifth stage (L5) coming from a permanent mass breeding kept in the acridology laboratory at the department of agricultural and forest zoology at the national high school of agronomy of Algiers, Algeria. The temperature is 30 ± 3°C and the relative humidity ranges from 50 to 60°C. Insects are fed daily with a mixture of grass and wheat bran.

1.2. Biopesticides tested

Three biopesticides were used, the fungus *Metarhizium anisopliae var acridum*, commercially registered as «Green Muscle», is an oily solution of spores that has been provided by the department of acridid control of the national institute of plant protection of Algiers, Algeria. Extract of henna *Lawsonia inermis* is obtained from leaves collected in the region of Adrar (Central Sahara, Algeria). The extraction is performed according to the method proposed by Sasanelli and Divito [7], leaves are pounded after being dried. 25 g of the powder is added to 150 ml of distilled water and the solution is filtered. A suspension of Triflumuron (T.F.M.), a growth deregulator, is certified to control foodstuff pests in Morocco. Its efficiency has previously been assessed against the migratory locust at the department of agricultural and forest zoology at the national high school of agronomy of Algiers, Algeria.

2. Treatments

In order to study the effect of the three pesticides on the biological and physiological chosen parameters, sublethal doses were used. *M. anisopliae* was tested at a concentration of 0.22 X 10^8 spores/ml. The counting of spores and the concentrations were realized thanks to Neubauer's cell under an optical microscope. Henna extract and Triflumuron were tested at concentrations of 0.41 ml/l and 12.5% (S8) respectively. Eight groups of L5 instars larvae were separated in boxes keeping the temperature and the humidity at the same levels. A volume of 10 ml from one pesticide was sprayed on three groups, while a forth group was sprayed with distilled water. Other three groups were fed with grass mixed with one pesticide whereas the last group was made to feed on grass sprayed with distilled water.

2.1. Effect of the three pesticides on the development duration of the locust L5 instars

A total amount of 40 recently molted L5 instars was split into 8 groups of 5 individuals each. Thus, 4 groups were observed for the contact mode and 4 other groups for the ingestion mode. Each individual is considered as a repetition.

Larvae were closely monitored to molt into imagoes, for those having been able to, so as to determine the development duration from L5 to imago.

2.2. Effect of the three pesticides on the hemolymphatic proteins of the locust L5 instars

We measured the quantity an analyzed the quality of the hemolymphatic proteins taken from 24 recently moulted L5 instars. The larvae were splited into 8 groups of 3 individuals. The three pesticides and also distilled water were used either by contact and ingestion. Hemolymph was extracted from each larva, at the fourth and eighth day after treatment. A volume of 10 µl of hemolymph was taken from the insect's thorax backside part, then preserved at -20°C. The quantity of hemolymphatic proteins was determined according to Bradford method [8]. The hemolymphatic proteins concentration is calculated by extrapolating the optical density from a standard graph drawn in the same conditions from a 1% Bovine Albumin Serum (BAS) mother-solution. A spectrophotometer at 595 nm was used to read the absorbance of BSA and that of the other different samples. The quality of hemolymphatic proteins was tested using the electrophoretic analysis according to the technic of Leammli [9]. Hemolymphatic proteins migration was observed on the SDS-PAGE electrophoresis gel.

3. Statistical analysis

To be able to show the effect of the three treatment modalities (TFM, Ma, Henna) on the duration of development and the concentration of hemolymphatic proteins in L5 larvae of *Locusta migratoria*, we performed an analysis of variance (ANOVA) at the 5% threshold, followed by the Post-Hoc test (Dunnett, Tukey) using XL STAT software version 6.0.

RESULTS

1. Effect of the three pesticides on the development duration from instar L5 to imago

Control larvae were able to complete their metamorphosis normally without any malformation. Nonetheless, treatments with all three pesticides have led to a toxic effect on L5 instars. None of the treated larvae could develop into imago. The duration of development was prolonged compared to that of the control larvae due to the fact that moulting has not occurred, (Fig. 1, 2 and 3).

Figure 1. L5 of *L. migratoria* treated with Triflumuron

Figure 2. L5 of *L. migratoria* treated with *Lawsonia inermis*

Figure 3. Female of *L. migratoria* infected with *M. anisopliae*

The development duration was 14.5±0.91 days and 18.75±2.36 days respectively for both larvae sprayed with distilled water and those fed with grass sprayed with distilled water (Fig. 4). Difference of development duration from L5 to imago was highly significant between control and treated larvae for each type of treatment (p<0.0001, ANOVA Fisher test). The comparison of treatment methods with distilled water was subjected to a Dunnett test and revealed a significant difference between the three produced compared to the control.

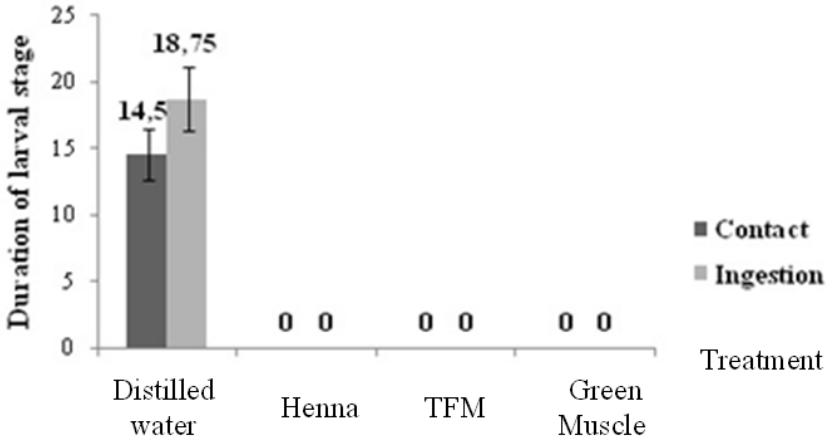

Figure 4: Development duration in days of *L. migratoria* fifth instar under the effect of the different treatments by contact and ingestion (Average ± standard deviation).

2. Effect of the studied pesticides on the L5 hemolymphatic proteins

Effect of contact application has shown that protein contents decreased with Henna and Triflumuron but increased with *M. anisopliae* infection compared with the control. We have recorded amounts of 22±0.35µg/ml, 38.38±0.18µg/ml and 55.5±0.35µg/ml at day 4 and 39.63±0.18µg/ml, 46.25±0.35µg/ml and 83.13±0.18µg/ml at day 8 in larvae treated by Henna, Triflumuron and *M. anisopliae* respectively against an amount of 43±0.35µg/ml at day 4 and 64.88±0.18µg/ml at day 8 for the control. As for the effect of the three substances introduced by ingestion, the hemolymphatic proteins amount decreases with henna while it increases with Triflumuron and *M. anisopliae* compared to the distilled water treated larvae. Amounts of 42.88±0.18µg/ml, 68,38 ± 0.18µg/ml and 68.5±0.35µg/ml at day 4 and 58.5±0.35µg/ml, 73.63±0.53µg/ml and 84.75±0.35µg/ml at day 8 respectively in larvae treated by henna, Triflumuron and *M.anisopliae* against an amount of 50.63±0.18µg/ml at day 4 and 66±0.35µg/ml at day 8 for the samples (Fig. 5 & 6).

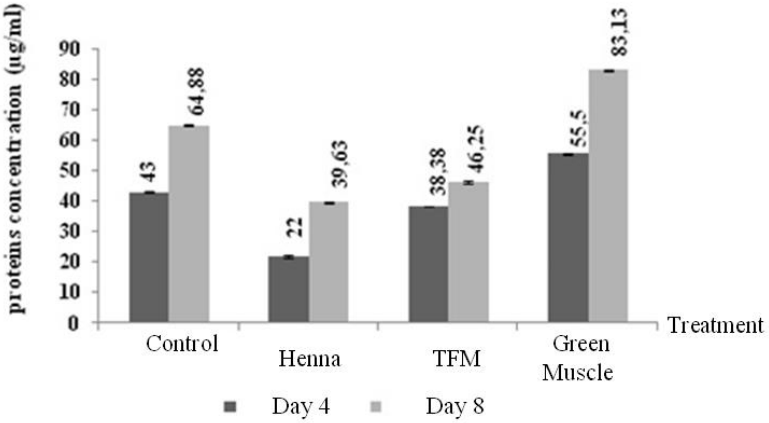

Figure 5. Protein concentrations in Hemolymph of treated *L. migratoria* L5 larvae at day 4 and day 8 after contact application, (Average ± standard deviation).

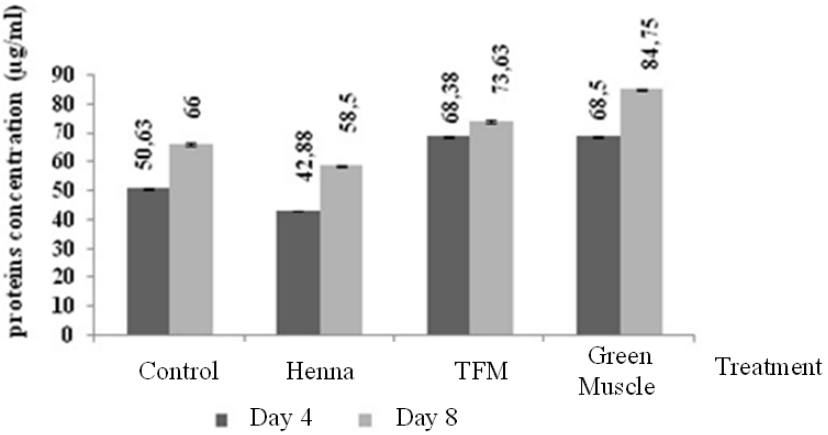

Figure 6: Protein concentrations in Hemolymph of treated *L. migratoria* L5 larvae at day 4 and day 8 after ingestion application, (Average ± standard deviation).

Protein concentrations in haemolymph of the treated larvae and the controls were significantly different regardless to the application mode of treatment (p<0.0001). The Tukey test groups the treatment mode in interaction with the product (mode × product) into three homogeneous groups. Thus, we find *Metarhizium anisopliae* in ingestion mode and contact with Triflumuron in ingestion mode on the one hand and Henna in ingestion mode and contact with Triflumuron in contact mode on the other hand, the third group contains Triflumuron and Henna in mode ingestion with *Metarhizium anisopliae* in contact mode (Table 1).

Table 4: Tukey test

Category	LS means	Groups		
ING×Ma	76.625	A		
ING×TFM	71	A	B	
CONT×Ma	69.312	A	B	
ING×Henna	50.687		B	C
CONT×TFM	42.312			C
CONT×Henna	30.812			C

The electrophoretic analysis of the larval hemolymphe of *L. migratoria* sprayed with *M. anisopliae* solution shows an increase in the number of bands; after 4 days (13 bands) and 8 days (14 bands). Whereas a diminution of the number of bands at day 4 (12 bands) and a total alteration of the bands at day 8 for larvae sprayed with henna solution.

The use of Triflumuron solution has revealed the same number of bands noticed for the samples at day 4 (10 bands) and an increase of this number at day 8 (17 bands) compared to the sample's (13 bands). Number of bands for the hemolymphatic proteins in larvae having ingested *M. anisopliae*is was the same as the control one's at day 4 (10 bands) but less at day 8 (10 and 13 bands respectively). For the Triflumuron effect, we report an increase in the number of bands at day 4 (11 bands) and day 8 (15 bands).However, we highlighted a decrease in the number of bands at day 4 and day 8 (9 bands) for henna. The bands quality does not seem to be influenced by the treatment by contact of *M. anisopliae* and we notice almost the same intensity at day 4 and day 8 for both the treated larvae and the samples. For the individuals treated by contact with henna, the bands have been altered and the intensity is weak compared to the sample's at day 4 and no band is noticed at day 8. As for the larvae treated by contact with Triflumuron, the bands intensity is weak either in day 4 or day 8 compared to the samples. For the treatment by ingestion, the intensity of the bands is as strong as the sample's for the individuals treated by *M. anisopliae*, this is not the case for the larvae treated with Triflumuron neither in day 4 nor in day 8. The bands'intensity remains weaker, compared to the sample's for the individuals treated with henna in day 4 and day 8 (Fig. 7).

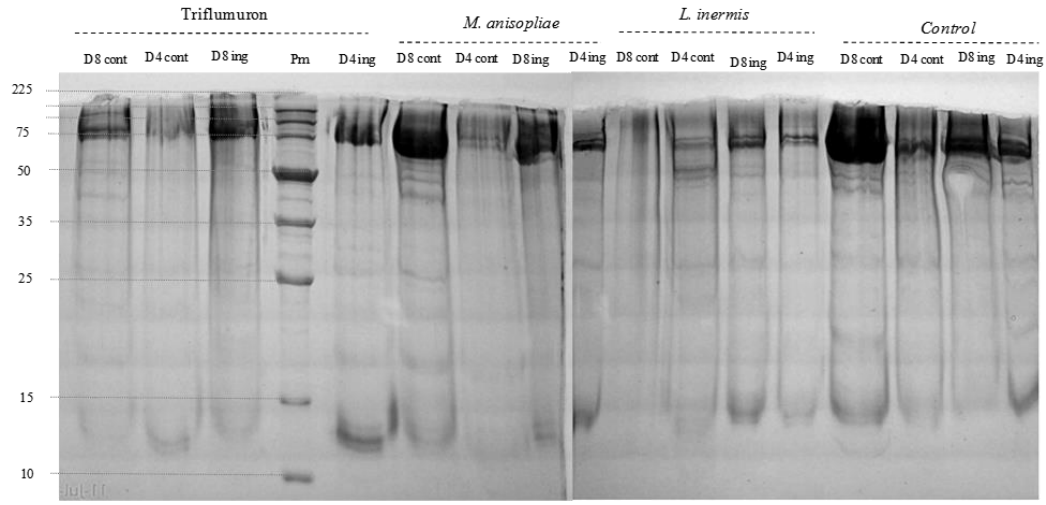

Figure 7: Results of electrophoretic profiles of haemolymph proteins L5 larvae of*L. migratoria* treated with three biopesticides by the two types of treatment in the 4th and 8th day
D: day; Cont: conact; Ing: ingestion; Pm : Protein marker.

DISCUSSION

1. Effect of the three pesticides on the development duration from instar L5 to imago

Our results have revealed the positive effect of all three tested pesticides on the duration from L5 stage to imago of *L. migratoria* under controled conditions. This parameter has been studied by many authors, like Tirchi and Mouhouche [10], who report that the application by ingestion of the Triflumuron on larvae of the migratory locust inhibits the molt which can be due to an abnormally prolonged larval stage duration. Belhadi [11], has shown in this context that of *Rosmarinus officinalis* and *Nerium oleander* leaves have affected the development of *Schistocerca gregaria* L5 instar, where the first plant species caused the larvae mortality before even starting to molt, whereas the second plant species prolonged the larval stage and inhibited the molt. Ould El Hadj et *al.* [5], reported that L5 larvae of the same locust species fed with cabbage treated with neem solution have been unable to molt. According to these authors, only 20% of individuals fed in the presence of *Eucalyptus* have reached their final moult. Soltani et *al.* [12], have confirmed that the use of Alsystine (48% EC), which is a commercial formulation of triflumuron, at doses ranging from16 to 79 ng/l on newly exuviated larvae from the fourth stage of *Culex pipiens pipiens* L. (Diptera: Culicidae), provokes the lengthening of their development duration.

Nasseh et *al.* [13], pointed out that in the case of *Melia*, the delays in larval development of *Schistocerca gregaria* and even the absence of the molt in some cases, were very clear. After 18 days, for the laboratory larvae and 20 days for captured larvae, all of the individuals both treated and samples, developed into imagoes. By comparison, the use of *Melia* extracts caused a two-week delay in average in the larval stage for the laboratory larvae and a three-week delay for the captured larvae. While all captured larvae have developed into imagoes after approximately 20 days, some of laboratory individuals stayed in complete stagnation never overcoming the larval stage. Boubekka [14], has shown that the use of plantextracts aqueous *Datura innoxia* on the fifth instars larvae of migratory locust: *Locusta migratoria* caused an extension of the duration of the larval stages. Abdelaoui et *al.* [15], have noticed that the development had been inhibited which slowed down the molt process causing the larval-stage's duration to extend after the application of the gibberellic acid on larvae of *Locusta migratoria migratoria*.

2. Effect of the studied pesticides on the L5 hemolymphatic proteins

Tripathi et *al.* [16], have shown that *Lawsonia inermis* affects the proteins synthesis in the nematode *Helminthosporium oryzae*.

The electrophoretic analysis of the hemolymphe of the males and females of *Schistocerca gregaria* reveals a very low proteins concentration due to the consumption of *Rosmarinuus officinalis* et *Nerium oleander,* they seem to have a negative effect on the larva appetite [11]. Acheuk [17], demonstrated that the activity of teflubenzuron (TFB benzoylphenylurea) on the structural parameters of the cuticle caused a significant reduction in its chitin content and an increase in protein cuticles in the treated series. Gillespie et *al*. [18], have found that the use of *M. anisopliae var acridum* on adult males of *S. gregaria* leads to a decrease in the concentration of the hemolymphatic proteins. According to St Leger et *al.* [19], the entomopathogenic fungus *M. anisopliae* produces during the infection process protease 1 and protease 2 which ensure the destruction of host proteins. Seymoun et *al*. [20], indicate that competition from *M. anisopliae var. acridum* with the individual of *S. gregaria* with respect to its haemolymphatic metabolites leads to the depletion of the reserves accumulated in the fat body. Hua et *al.* [21], claim that topical application of *Metarhizium anisopliae var. acridum* on *Locusta migratoria manilensis* caused decreases in trehalose and glucose in the hemolymph of treated individuals. Mahdjoubi et *al*. [22], notes that it is crucial to establish the link between the requirements of this locust species and its physiological behavior in order to be able to improve locust control strategies.

CONCLUSION

In conclusion, this study allows us to say that L5 of *L. migratoria* do not develop after being treated with the three pesticides, regardless to the way of application. Moreover, all the larvae died before reaching the adult stage. We notice that the three pesticides have a negative effect on the larval hemolymphatic proteins. Triflumuron leads to their diminution when used by contact while it provokes an increase in their concentration when the individuals ingest it. The bands are as intense as the samples' with *M. anisopliae*, less important with Triflumuron and weak at day 4 and day 8 ; the hemolymphatic proteins have been altered. Results can be of value in the domain of pest management and control.

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