Biological activity of *Calotropis procera* Ait on mortality and haemogram of *Schistocerca gregaria* (Forskal, 1775) and *Locusta migratoria* (Linné, 1758).

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**ABSTRACT**

The treatments based on acetone extracts of *Calotropis procera* against imagos of *Schistocerca gregaria* and *Locusta migratoria* have been very convincing due to the speed and efficiency of the action of said plant, on mortality and on haemogram of treated locusts. The process consists, after having carried out a mass breeding of locusts, in placing the latter in containers containing food soaked in the acetone extract of *C.* obtained by cold-molding. The first contribution allowed us to see that *C. procera* generates 100% mortality in the space of a few days, eight (08) days after treatment with a mortality recorded beginning from the 2nd day. As we observed among imago’s of *S. gregaria* and *L. migratoria*, who had ingested lettuce and grass soaked in acetone extract of *C. procera*, instant decrease in food intake and mobility accompanied by strong tremors of the legs and an almost total paralysis of the insect after only 3 hours of treatment. We found, as well, rotting and dismemberment corpses, 24 hours after the death of each individual with a bluish coloration of the oral appliance of dead locusts. A second intake, confirms, therefore, the toxicity of the plant used against both species of locust treated, especially on haemogram of the species. We have thereby seen strong cytological disturbances in the hemolymph, and this, whether for the qualitative aspect as for that quantity. These disturbances are, therefore, translated into structural alterations marked and significant drop in the number of haemocytes, ranging from 271-123 haem / 3μl in *S. gregaria* and from 321.33 to 65.66 haem / 3μl in *L. migratoria*. Also note that some cells become empty of their contents gradually and others have been disaggregated, leaving only debris.

**KEYWORDS:** Schistocerca gregaria, Locusta migratoria, Calotropis procera, Mortality, Haemogram, Toxicity.

**INTRODUCTION**

Considered as fearsome plagues in agriculture, desert Locust *Schistocerca gregaria* and the migratory locust *Locusta migratoria* are known since antiquity and its invasions constitute a major phenomenon that is perceived by Saizonou [25], as apocalyptic, a phenomenon, which is not new. The swarms of desert locusts, fearsome scourge of agriculture - particularly in Africa - in invasion period, are able to invade an area covering over 29 million km² representing 20% of the land [7].

However, to combat these invasions, chemical control has greatly contributed to avoiding the worst by using an arsenal of insecticides that prove to be harmful to the environment by causing problems of biodegradability and ecological toxicity by accumulation of residues.

Awareness of these problems leads us to turn to other methods for biological control in its various forms, to try to contain the teeming locusts. In addition to fungi and bacteria
Pathogens of insects, the use of toxic plants is a very promising alternative to ensure more effective phytosanitary protection. It is with this in mind that we wanted to make our contribution by conducting a study of the toxic effect of Calotropis procera, with respect to the mortality and hemogram of S. gregaria and L. migratoria.

**MATERIALS AND METHODS**

**Biological material:**

**Animal material:**

The imagos of S. gregaria and L. migratoria come from a permanent breeding held at Crop Protection laboratory of the National Institute of Agronomic Research in Algeria (experimental station Mehdi Boualem, Baraki, Algiers, Algeria).

Breeding is conducted in rectangular cages with fine mesh on both sides for ventilation. These cages are equipped with two sliding glass doors, to enable cleaning and renewal of the food and the verification of nests. An average temperature of 32+ 4 °C and an area humidity of the order of 75+ 3%, are maintained constant. Photoperiod of 12 hours of light on 12 hours of darkness is ensured. The fresh feed is composed of grass, lettuce and a wheat bran balance

**Materiel vegetal:**

Fresh leaves of Calotropis procera, are collected at of the wadis Abadel (23 ° 22 'N 04° 29' E, 23 ° 25 'N 04 ° 30') and Amded (22 ° 51 'N 04 ° 29' E) at the Tamanrasset region. The method of preparing the acetone extracts of C. procera consists to rinse fresh leaves thoroughly and then drying them in an oven controlled at 30 ± 2 ° C for 5 days [20], and then grinding them. One hundred grams of leaf powder are macerated in 200 ml of acetone for 24 hours. Filtration is then carried out in vacuo using a vacuum flask and a funnel. The dry residue is discarded while the recovered filtrate is subjected to vacuum evaporation in the rotary evaporator to remove acetone. The resulting product is an extract, which is added 20 ml of acetone. We obtain subsequently a mixture, which will serve as treatment product [3].

**Biological tests:**

The imagos are placed in rectangular wooden cages whose faces are screened. After fasting for 24 hours, allowing them to empty the contents of their digestive tract and starve them, S. gregaria individuals and L. migratoria were fueled by fresh leaf lettuce Lactuca sativa and Lolium perene grass, soaked for a few seconds in the acetone extract of C. procera and left for 15 to 20 minutes in the open air to evaporate the acetone.

For control subjects, were fed by lettuce leaves soaked in acetone and left still for 15 to 20 minutes in the open air to evaporate the acetone. Experimentation is followed until the total death of all individuals treated lots.

**Parameters studied:**

- **Effect on mortality of S. gregaria and L. migratoria:**
  
  The experimental device for this study is comprised of 04 batches of 20 individuals for each species of cricket being a lot intended to witnesses and 3 lots for rehearsals. The test was monitored daily for 14 days for both treatments.

- **Effect on the haemogram of S. gregaria and L. migratoria:**
  
  For this study, we used 40 individuals for each species of cricket, with 10 individuals for witnesses and 30 individuals (03 repetitions) for treated. The blood smear is made 24 hours after treatment, according to the method of Guzo and Stoltz (1987) cited by Habes [8] (Figure 1)

- **Statistical tests:**
  
  The results were compared by analysis of variance (ANOVA) using the STATISTICA software (2008), in order to approve the effectiveness of treatment and to test the statistical significance of differences between the factors studied.
RESULTS AND DISCUSSION

Effect of Calotropis procera on mortality of S. gregaria and L. migratoria imagos:

The results of the cumulative daily mortality rate recorded at imagos of S. gregaria and L. migratoria, witnesses and treated with acetone extract of C. procera are carried respectively in Figures 2 and 3.

Among imagos witness of S. gregaria and L. migratoria, we recorded a mortality rate of 3.33% between the 9th and 11th day and a 10% rate from the 12th day to the last day observation. As for imagos treated by C. procera, 50% of mortality was reached from the 4th day of treatment for both species of locust studied. A 100% mortality was recorded in the 8th and 9th day, respectively, in imagos treated of S. gregaria and L. migratoria.

Fig. 1: Realization of imagos blood smear of S. gregaria and L. migratoria according to the method of Guzo et Stoltz (1987) cited by Habes [8]

Fig. 2: Cumulative daily mortality rate of S. gregaria imagos treated by C. procera
Fig. 3: Cumulative daily mortality rate of \textit{L. migratoria} imagos treated by \textit{C. procea}

The analysis of variance with two classification criteria, the time factor and the factor by \textit{C. procera} treatment, reveals a highly significant difference (F = 17.85, df = 2 and p <0.005) and (F = 2.48; df = 12 and p <0.005) between the mortality percentages recorded for the treated and the witnesses at different levels of time.

Also worth noting that the ingestion of lettuce and grass soaked in the acetone extract of \textit{C. procera} by imagos of \textit{S. gregaria} and \textit{L. migratoria} caused an instant decrease in food intake and mobility accompanied by strong tremors of the lugs, followed by an almost total paralysis of the insect after only 3 hours of treatment. As, we have found rotting and dismembered corpses, 24 hours after the death of each individual, accompanied by a bluish oral appliance of dead locusts.

Foliar acetone extract of \textit{C. procea} seems to have a highly significant effect on the food intake by adult individuals of \textit{S. gregaria} and \textit{L. migratoria}. The choice of a plant to give to the insect as a food depends on the relative amounts of stimulating agents or inhibiting the absorption of food present in the plant, according to Descoins [7] cited by Kemassi and al. [13]. The latter adds that generally locusts explore the surface of the sheet with their palps before biting. The rejection of the plant is usually done after the bite. However, among \textit{Locusta migratoria} and \textit{Schistocerca gregaria}, there may be an unusual rejection of the plant just after the step of palpation and without bite. This behavior results from a kind of learning insect associating stimuli registered by their palps with rejection following the first bites [16].

Indeed, the continuity individuals to feeding lots lettuce leaves soaked in acetone extract of \textit{C. procera} can be explained by the adaptation to the presence of lettuce leaves and grass, as their daily food during their larval stages, to adulthood.

According to Mamadou et al. [19], \textit{C. procera} leads to a decrease food intake, decreased weight and a water loss of larvae and imagos of \textit{Schistocerca gregaria}.

Abassi and al. [1] recorded a 100% mortality rate among the larvae, after 15 days from the start of treatment with \textit{C. procea}. These same authors add, also, that the extract of alkaloids of young leaves of the said plant is an anti-palatable, toxic and anti-fertilizer for \textit{S. gregaria}.

The same authors report that these extracts cause to imagos treated, ovarian blocking development in previtellogenesis among females and lack of sexual maturity among male with a motor reduction among imagos of both sex. They conclude that these physiological disturbances are due to the action of the plant alkaloids of which the precursors are histamines highlighted in the latex by Saha and Kasinathan [24]. These histamines are manifested by balance disorders and convulsive movements. These manifestations underlie alterations in the nervous system of individuals. Thiaw and Sembene [26] note that by treating the eggs of the bruche \textit{Caryedon serratus} with extracts of \textit{Calotropis procera}, survivors see the sex ratio of their offspring undergo an imbalance. Thiaw et al., [26], who treated eggs and adult \textit{C. serratus} by \textit{Senna occidentalis}, mention that the extracts of the latter causing an imbalance in the sex ratio towards males and limited fertility.

As a reminder, several studies highlight the biocidal effect of some plant extracts on the desert locust. We quote those made by Barbouche and al. [5] who studied the toxic effect of the plant Solanaceae \textit{Cestrum parqui}; the works of Linton et al. [18] showed the insecticidal effect of \textit{Melia azedarach} but also those of Abassi et al. [2] and Idrissi-Hassani and Hermas [11] concerning the toxic effect of \textit{Peganum harmala}, always against the desert locust.
**Effect of C. Procera on haemogram of S. gregaria and L. migratoria imagoes:**

Further to comparisons with the works of references relating to the hemolymph of orthopterans, namely the works of Wigglesworth [29], Arnold [4], Raccaud-Shoeller [22] and Woodring cited by Klowden [14] we have managed to identify four types of cells among *S. gregaria* and *L. migratoria*, namely prohaemocytes, plasmatocytes, coagulocytes and granulocytes (fig. 4 and 5). However, the counting of cell categories involved only the prohaemocytes and plasmatocytes because the last two categories mentioned above were observed infrequently.

Indeed, Lopesme [17] distinguishes among adults of *S. gregaria* two Haemocyte categories: Proleucocytes and Phagocytes. He added that the examination of blood smears *L. migratoria*, *Anacridium aegyptium* and *Pamphagus elephas*, showed a deep similarity to the locust. Halouane [9], Halouane et al. [10] and Kaidi [12] have been identified among *S. gregaria* and *L. migratoria*, two types of cells namely prohaemocytes and plasmatocytes.

After the examination of blood smears made from the hemolymph individuals of *S. gregaria* and *L. migratoria* treated with *C. procera*, we observed an important reduction in the number of cells, some are emptied of their contents gradually and other disaggregated, leaving only debris.

![Fig. 4](image1.png)

**Fig. 4:** Different categories of haemocytes identified in the hemolymph of *S. gregaria*

![Fig. 5](image2.png)

**Fig. 5:** Different categories of haemocytes identified in the hemolymph of *L. migratoria*
The total number of haemocytes accounted in 3μl of hemolymph levied from imago *S. gregaria* and *L. migratoria*, 24 hours after ingestion of these lettuce and grass soaked in acetone extract of *C. procera* are illustrated in Figure 6.

We have, indeed, a remarkable decrease in both haemocyte locust species treated, a fall of 271.66 to 123 hem / 3μl among *S. gregaria* and 321.33 in 65.66 hem / 3μl at imagos of *L. migratoria*. The other, was disaggregated, leaving only debris (Fig. 7 and 8).

**Fig. 6:** Number of haemocytes of *S. gregaria* and *L. migratoria* imagos witness and treated by *C. procera*

**Fig. 7:** Hemolymph of *S. gregaria* imagos treated with *C. procera*

**Fig. 8:** Hemolymph of *L. migratoria* imagos treated with *C. procera*
The rate of pro-haemocyte has also declined by 37.91% to 23.66% and 29.56% to 15.75%, respectively among imagos treated *S. gregaria* and *L. migratoria* (Fig. 9).

As for the plasmatocytes, they had undergone the same fall that is 40.98% to 9.75% among *S. gregaria* and 33.71% to 15.22% among *L. migratoria* (Fig. 10).

This remarkable decrease can be explained by their intervention in the immune process and the depletion of these cells after phagocytosis of toxins from *C. procera*. Therefore, the said toxin plants certainly escape the phagocytic barrier of the immune systems of individuals, causing 24 hours after treatment an almost complete cell lysis.

According Chauvin [6] and Arnold [4], the introduction of foreign particles can disrupt insects. Paillot [21], mentions that this perturbation can be translated either by the increase of the number of cells or by its diminution.

McNamara cited by Lahoues et al. [15] mentions that certain sterols including tetra cyclic triterpenes present in the plant *C. procera* are endowed with necrotizing and cytotoxic properties among rodents.
Upadhyay [28] also mentioned the cytotoxic activity of *Calotropis procera* against larvae of *Anopheles stefensi, Culex quinquefasciatus, Aedes aegypti* and *Musca domestica*, at a very low topical application of latex.

In parallel, the same reduction in the number of haemocytes and the rate of prohemocytes and plasmatocytes was observed among individuals of desert locusts treated with the entomopathogenic fungus, *M. anisopliae* var. *acidum* [30,10,12].

**Conclusion:**

Treatment with the acetone extract of the leaves of *Calotropis procera* of the imagos *Schistocerca gregaria* and *Locusta migratoria*, have been proved very convincing due to the speed and efficiency of their action, following behavioral disturbance and the first recorded deaths respectively only 3 hours and 24 hours after treatment. Toxins in the leaves of *C. procera* have acts on the two species of locusts radically. A fact reflected in a decrease in food intake in the treated individuals followed by total paralysis of the body and finally death locusts.

Hence, these natural toxic compounds could then be a promising base for the synthesis of molecules acridicides and insecticides that will be in better harmony with the environment, the use of which I would be beneficial for locust control especially because the *Calotropis procera* is widely widespread in the natural biotopes of the two species of studied locusts, that to say in southern Algeria.

**REFERENCES**


