Effectiveness of *Calotropis procera* Ait. latex against late nymphal instars of *Locusta migratoria* L. (Orthoptera: Acrididae)

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**Abstract**

*Calotropis procera* latex treatments against *Locusta migratoria* were very persuasive due to mortality efficiency and haemolymph contents of treated nymphs. This study showed the impact of five Usher latex doses 10, 20, 30, 40, and 50 µl by topical applications on 3rd, 4th and 5th. The Ld₅₀ values were 10.3, 10.9 and 12.2 µl respectively. The effect of latex usher concentration LD₅₀ (12.2 µl) on haemolymph contents, total carbohydrates, total lipids, total protein and cholesterol of 5th nymphal instar were carried out and showed that the haemolymph content of the treated insects was highly affected and all the studied parameters were lower than the control.

**Key words:** *Locusta migratoria*, *Calotropis procera*, Mortality, Haemolymph contents.

INTRODUCTION

Insect chemical interactions have appeared in recent years to have the potential to use secondary plant metabolites, or allelochemicals as control agents for insects. That interest in botany compounds arising from the need for alternatives to the traditional pesticides in integrated pest management (IPM) programs which have a high negative effect on agro-ecological systems (Whitten, 1992).

Usher, *Calotropis procera* is growing widely in the African and Asian tropics and they have plentifully grown in arid and semi-arid regions without irrigation, fertilization and pesticides or other types of agronomy. The contents of those plant's green parts seem to be a defense plant method against virus, fungi and insects (Qari, 2008). It is a tiny 2-4 m. tree exuding milky, active sap when broken or cut; leaves opposite, grey-green, up to 15 cm long and 10 cm
high, with a spiky tip (Kleinschmidt & Johnson, 1977). It possesses potential anti-inflammatory, anti diarrhoeal, analgesic and antipyretic (Kumar et al., 2001).

The migratory locust, Locusta migratoria L., is widely distributed in the old world (Uvarov, 1977), and consume large amount of grass and sometimes endangers farm crops (Pener & Simpson, 2009). L. migratoria from the late 1990s up to the present day and its scale is growing progressively, especially infesting and breeding areas in the southwest and west of the country. During the years from 2015 to 2019 many consecutive outbreaks occurred in Sharq El-Owinat and Toshka southwest Egypt so recently, L. migratoria became a major economic pest after the expansion of land reclamation projects undergoing continuous irrigation (Moustafa, 2019).

This study aims to develop insecticide alternatives using purified Usher latex safe for environment, particularly for agriculture, non-target organisms and human beings. Through evaluating the values of LD50 and some haemolymph contents.

MATERIALS AND METHODS

1.1. Experimental Insect:
In this study Nymphs of L. Migratoria insects had been reared in wooden formed cages measuring: 60 cm length x 60 cm Width x 70 cm heights. The front side of the cage had small door to facilitate regular routine work and insects care. The bottom was filled with a15 cm deep sandy layer and 10-15% humidity suitable for laying egg. A 100 watt electric bulb was adjusted to hold a continuous 12 Light: 12 Dark photoperiod in each cage and 32±2 °C ambient temperature.

The insects were reared and handled under the crowded conditions outlined by Hunter–Jones (1961). Before introduction of the fresh food, the faeces, dead locusts and food remains were collected daily. Fresh Alexandranium trifolium were used as an insect's food.

1.2. Crude Usher latex:
Latex of Usher, Calotropis procera Ait. (Gentianales: Apocynaceae) was collected during August 2017 from plants that are naturally grown in the region of Baharia oasis, western Egyptian desert. Crude of Usher's latex was gathered from about two meter height plants by partially broken tip of stem. The latex was stored in conical flasks which were surrounded by crushed ice. Latex was partially purified by centrifugation before topical application In order to remove inert coagulum. (Alawi, 2004).

1.3. Nymphal treatments:
The doses of Usher latex, 10, 20, 30, 40, and 50 µl were used on the newly moulted abdomen 3rd, 4th and 5th nymphal instars of L. migratoria. Six groups were used in each group three replicates (ten nymph/replicate), where each group was used for each concentration and sixth group was used as a control. All mortality of treated and control insects, were recorded for 14days post-treatment. Mortality data have been summarized as estimates of the Median Lethal Doses. LD50 values and regression lines slope have been determined using (Lpd line) software for calculating and drawing the mortality curve according to Finney Method (1971).
1.4. Samples collection and preparation:
Sixty nymphs for each group, treatment and control (three replicates/group) under lab condition were treated with LD$_{50}$ value and distilled water. They were kept in cages (25 x 25 x 60cm). Haemolymph samples were collected at different periods, 2, 4, 6 and 8 days post-treatment. The haemolymph was obtained by fine puncture in the hind leg membrane and moved into clean, dry centrifuge tubes. A known volume centrifuged on 13000 rpm to 15 min. to remove blood cells and pigments. Then the supernatant collected for analyses (El Gawhary, 1997).

1.5. Determination of total carbohydrates:
Total carbohydrates were determined according to the method described by (Dubois et al., 1956).

1.6. Determination of total lipids:
Total lipids were determined according to the method described by (Kinght et al., 1972).

1.7. Determination of total proteins:
Total proteins were determined according to the method described by (Bradford, 1976).

1.8. Determination of total cholesterol:
Total cholesterol was determined according to the method described by (Richmond, 1973).

1.9. Statistical analysis
The mortality percentages were corrected according to Abbott's formula (Abbott, 1925). The values LD$_{25}$, LD$_{50}$, LD$_{90}$ and regression lines slope were determined using (Lpd line) software for drawing toxicity lines according to Finney (1971). Other Data have been subjected to analyze of variance (ANOVA). Means were compared using LSD according to SAS 6.12 (SAS Institute, 1996).

RESULTS

1.10. Effect of C. procera on mortality of L. migratoria:
The results of the cumulative daily mortality percentages were recorded at L. migratoria, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ nymphal instars treated with 10, 20, 30, 40 and 50µl of C. procera latex are held in Table (1) and Figure (1) showing the association between the mortality percentages and days after treatment to determinate LD values, Slope, and the potent different concentrations in mortality for Five latex usher concentrations.

Fig. (1) display the LD$_{50}$ value of latex usher on 3$^{rd}$, 4$^{th}$ and 5$^{th}$ nymphal instars. The LD$_{50}$ for concentrations (10.3, 10.9 and 12.2µl) respectively.

Results in Table (1) show the effect of five concentrations of usher latex were: 10, 20, 30, 40, and 50 µl topical applications on 3$^{rd}$, 4$^{th}$ and 5$^{th}$ nymphal instar of L. migratoria.
Table (1): Mortality percentage in the nymphal instars of *L. migratoria* treated with different usher latex concentrations.

| instar | Doses | Observed response % | Linear response % | Linear probit | LD | Dose (µl/nymph) | Slope |
|--------|-------|---------------------|-------------------|--------------|----|----------------|-------|
| third  | 10    | 52                  | 48.0231           | 4.9504       | 10 | 4.0032         | 3.1+/0.34 |
|        | 20    | 75                  | 81.1376           | 5.8831       | 25 | 6.2854         |       |
|        | 30    | 97                  | 92.3342           | 6.4287       | 50 | 10.3756        |       |
|        | 40    | 98                  | 96.5023           | 6.8161       | 75 | 17.1273        |       |
|        | 50    | 99.5                | 98.2296           | 7.1163       | 90 | 26.8916        |       |
| fourth | 10    | 49                  | 46.3079           | 4.9073       | 10 | 3.2929         | 2.46+/0.27 |
|        | 20    | 68                  | 74.1875           | 5.6492       | 25 | 5.8065         |       |
|        | 30    | 87                  | 86.0618           | 6.0832       | 50 | 10.9044        |       |
|        | 40    | 96                  | 91.7839           | 6.3912       | 75 | 20.4779        |       |
|        | 50    | 99.9                | 94.8349           | 6.63         | 90 | 36.1095        |       |
| fifth  | 10    | 47                  | 40.9829           | 4.7721       | 10 | 3.9157         | 2.59+/0.26 |
|        | 20    | 62                  | 70.9155           | 5.551        | 25 | 6.7208         |       |
|        | 30    | 81                  | 84.2897           | 6.0066       | 50 | 12.2485        |       |
|        | 40    | 94                  | 90.8192           | 6.3301       | 75 | 22.3226        |       |
|        | 50    | 98                  | 94.289            | 6.5808       | 90 | 38.314         |       |
1.11. Characterization of the fifth nymphal instar haemolymph of *L. migratoria* after treatment with Usher latex:

Usher Latex LD<sub>50</sub> (12.2 µl) was applied topically on 5<sup>th</sup> nymphal instar of *L. migratoria* and the haemolymph chemical analysis was performed in days 2, 4, 6 and 8 after treatment the results on control nymphs were compared.

1.11.1 Effect on total carbohydrate:

The effect of LD<sub>50</sub> (12.2 µl) Usher latex on total carbohydrate content at fifth nymphal instar summarized in Table (2).

It is clear that total carbohydrates level decreased markedly in the treated 5<sup>th</sup> nymphal instar than that of the untreated at all periods of application (LSD=14.68)

Table (2): Estimated total carbohydrates, total lipids, total protein and total cholesterol (mg/dl Haemolymph) of the 5<sup>th</sup> nymphal instar of *L. migratoria* after treated with usher latex.

| Days after treatment | Total carbohydrates | Total lipids | Total protein | Total cholesterol |
|----------------------|---------------------|--------------|---------------|------------------|
|                      | Control±SE<sup>a</sup> | treatment±SE<sup>b</sup> | Control±SE<sup>a</sup> | Treatment±SE<sup>b</sup> | Control±SE<sup>a</sup> | Treatment±SE<sup>b</sup> | Control±SE<sup>a</sup> | Treatment±SE<sup>b</sup> |
| 2<sup>nd</sup>       | 316.00±2.42<sup>a</sup> | 295.23±2.25<sup>b</sup> | 221.17±2.61<sup>a</sup> | 218.67±1.2<sup>a</sup> | 5256.67±137.76<sup>a</sup> | 5230.00±119.3<sup>a</sup> | 21.67±1.2<sup>a</sup> | 18.33±0.35<sup>b</sup> |
| 4<sup>th</sup>       | 392.53±5.90<sup>a</sup> | 326.67±8.82<sup>b</sup> | 195.33±2.58<sup>a</sup> | 153.34±2.72<sup>b</sup> | 6486.67±70.75<sup>a</sup> | 4650.00±180.28<sup>b</sup> | 22.43±0.86<sup>a</sup> | 15.67±0.43<sup>b</sup> |
| 6<sup>th</sup>       | 482.00±6.10<sup>a</sup> | 291.00±3.21<sup>b</sup> | 182.27±0.99<sup>a</sup> | 104.47±0.95<sup>b</sup> | 5002.00±57.77<sup>a</sup> | 3906.67±23.33<sup>b</sup> | 18.00±0.58<sup>a</sup> | 10.30±0.26<sup>b</sup> |
| 8<sup>th</sup>       | 368.73±4.56<sup>a</sup> | 210.23±0.44<sup>b</sup> | 210.43±1.1<sup>a</sup> | 86.57±2.66<sup>b</sup> | 4650.00±28.87<sup>a</sup> | 3499.67±5.49<sup>b</sup> | 17.23±1.2<sup>a</sup> | 6.34±0.69<sup>b</sup> |

<sup>a</sup> means in the same row with same letter are not significantly difference
1.12. **Effect on total lipids level:**

The effect of latex usher concentration LD$_{50}$ (12.2 µl) on Fifth nymphal instar total lipids content summarized in Table (2). There were no significant differences between treated nymphs after 2$^{nd}$ days compared with control but on 4$^{th}$, 6$^{th}$ and 8$^{th}$ day, significant differences were noticed (LSD=14.68)

1.12.1. Effect on total protein level:

The effect of latex usher concentration LD$_{50}$ (12.2 µl) on total protein level on Fifth nymphal instar are summarized in Table (2). It was no significant differences between treated nymphs after 2$^{nd}$ day compared with control but on 4$^{th}$, 6$^{th}$ and 8$^{th}$ day, significant differences were noticed (LSD=291.17).

1.12.2. Effect on total cholesterol level:

The effect of latex usher concentration LD$_{50}$ (12.2 µl) on total cholesterol content on fifth nymphal instar are summarized in Table (2). It was found that total cholesterol level decreased markedly in the treated 5$^{th}$ nymphal instar than that of the untreated at all periods of application (F= 48.43)

**DISCUSSION**

1.13. **Effect of *C. procera* on mortality of *L. migratoria***:

The African migratory locust is considered one of Egypt's most important recent pests, especially in the last two decades, since it began to represent a large burden on the authorities responsible for the control operations, as well as on the investors in the breeding area of the African migratory locust, which are represented in two main areas, Shark Al-Owinat and Toshka. Due to the presence of vast areas of newly reclaimed agricultural land, the irrigation method used, as well as the type of soil, temperatures, humidity, and cultivated crops, all of these factors led to the availability of an optimal environment for the rapid, strong and effective reproduction of the African migratory locust, which led to a steady and rapid increase in numbers. This enormous increase in the census must be accompanied by a similar increase in the use of control methods, especially machinery and pesticides, which results in an increase in costs, as well as massive damage caused by those pesticides to the environment, crops, non-target organisms, soil, and human, which are the main source of irrigation operations all of that. It prompts us to search for modern alternatives and methods for control operations, alternatives that are safer, less expensive, and harmful to the system that are considered modern and a less polluted environment than the old cultivation areas such as the delta or even the most recent places, Ismailia, Salehia and Nubaria. In this study we discussed the use of the Ushar latex to know the extent of its effect on the various nymph ages of the African migratory locust from several sides, the first of which is its ability to reduce the numbers of the pest, as it used five concentrations of the substance from 10 to 50µl on the third, fourth and fifth ages and the results showed a marked decrease in the census after treatment 14 days for all ages used. This death rate may be due to the toxic effect of this substance and its effect on the vital components of the blood of the nymphs, which resulted in death, or it is the result of preventing the nymphs from feeding and consequently the death occurs after a while. All these speculations and more can explain the causes of death nymphal. Many authors have
explained many reasons, and most important of them. such studies have also identified extracts from different plant parts of C. procera and Azadirachta indica have insecticidal effect. Abbassi et al. (2003) the alkaloid has been extracted from C. procera leaves of was able to induce a substantial mortality of S. gregaria. Abassi et al. (2004) reported a nymphal mortality rate 100% of S. gregaria after 15 days of treatment with C. procera. The same authors reported that these extracts cause to nymphs treated, ovarian blocking development in previtellogenesis among females and lack of sexual maturity among male with reduction in motor skill among nymphs of both sex. Jahan et al (1991) had shown the toxicity of leaf powder of C. procera against larvae of Tribolium confusum. Singhi et al., (2004) mentioned that C. procera latex solution showed a remarkable effect as a larvicide against Aedes aegypti and highly important observations on the ovipositing behavior. Kaidi et al. (2017) clarified that locusts in general explore the layer surface of the sheet C. procera with their palps before biting. The rejection of the plant is usually done after the bite. However, among L. migratoria and S. gregaria, there may be an unusual rejection of the plant just after the step of palpation and without bite. This behavior is the results of a kind of learning insect associating stimuli registered by their palps with rejection following the first bites.

1.14. Characterization of the haemolymph of the fifth nymphal instar of L. migratoria after treated with the usher latex:

An insect's activities day-to-day demand a continuous energy supply. The adults insect need intake of food to assistance their activities (dispersal, reproduction). Especially flight is a very energy-intensive activity, requiring rapid energy sources mobilization, transport, and transformation of food energy into ATP. Those metabolic reactions are directly involved in mobilizing stored energy reserves and in releasing that energy for flight (Chapman, 1971).

Insects require nitrogen resources for ovaries and eggs maturation. Those are protein, and essential amino acids privation may appear itself in the fail to excrete Juvenile hormone (JH) which is required for development of ovary and egg in adult females. If JH or an analog as methoprene is given to protein-starved insects, they do not provide the usual supplement for eggs simply because they do not have enough stores for protein in the body. Some insects make use of Proline as a fuel for flight (Nation, 2002).

Immature stages of some groups of insects need polyunsaturated fatty acids for normal development. Some species (Lepidoptera, Orthoptera, and some others) use lipids from burning (fatty acids) as flight fuels which release large amounts of energy per unit weight of the metabolized substratum. Some insects that metabolize lipids can fly for hours continuously and undertake long distance migration (Chapman, 1971). Various forms of lipid molecules such as phospholipids and sphingolipids are considered to be essential structural components in cell membrane whereas other forms are considered used as reservoirs of energy. Other forms of lipid molecules act as vitamins, chemical signals, or pigments. Finally, some lipid molecules that exist in various organisms outer coatings functions have protective or waterproofing functions. Other lipid molecules also function as hormones, antioxidants, essential factors of growth (Nation, 2002).

Carbohydrates are not only an essential source of fast development energy production and growth living cells, but also acting as structural cell building blocks and components
numerous intermediates in metabolism (Wang et al., 2007). Flight muscles in insect contain glycogen in small amounts are sufficient for only a few minutes flying time (Nation, 2002).

Insects are unable to synthesize sterols and thus immature insects require sterols as precursor be converted into the moulting hormone with sterol structure. Eggs contain sterols, as well as the first instar may be molten without a dietary source but subsequent moults may not be possible if sterol is not present in the diet. Some adult insects need sterol to produce the normal number and/or eggs hatching. In our results the Ushar latex occurred a great decrease in haemolymph contents such as, total protein, total lipids, total carbohydrates and total cholesterol this decrease increased as the treatment period increased. These findings were in line with Said (2009) who showed the impact of Metarhizium anisopliae var. acridum in total protein; total carbohydrate, total lipids and cholesterol content of the desert locust haemolymph were all lower than control. Abdellaoui et al. (2018) demonstrated that biochemical analyzes of extracts from olive leaf caused decrease dramatically to metabolites of the haemolymph (proteins, lipids, and carbohydrates). Das et al., (2007) mentioned that plant-derived phytochemicals may act as larvicide, insect growth regulators, ovipositor attractant and repellent. Mordue (Luntz) & Nisbet (2000) & Martinez & Emden (2001), appear that the neurosecretory brain system influenced by extracts of C. procera and A. indica which caused morphogenetic peptide hormones and allatostatins blockage. These control the function of the prothoracic glands and corpora allata respectively. It regulates the development of new cuticles and ecdysis, while the corpora allata's juvenile hormone controls the formation of juvenile stages at each moult. In adults there can be both hormones implicated in regulating deposition of yolk in the eggs. Any disruption in these cascade events by plant extracts results in the many various but well-defined effects as seen as moult disruption, moult defects and sterility effects.

Therefore, latex usher which gave a highest mortality and some biochemical changes could be used as insecticidal agent in an integrated L.migratoria pest control program. The use of the plant materials in the pest control could become important supplements to imported synthetic pesticides, especially in developing countries.
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فعالية المادة اللبنية لنبات العشار على الاعمار الحورية للجراد الأفريقي

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أدت معالمة الجراد الأفريقي بالمادة اللبنية لنبات العشار إلى نتائج مقنعة جدا بسبب فاعليتها على نسب الموت ومحتويات الهيموليمف في الحوريات المعالمة. تبين هذه الدراسة تأثير خمس تركيزات من المادة اللبنية لنبات العشار 10, 20, 30, 40 و50 ميكرو ليتر بالتطبيق الموضعي على حوريات العمر الثالث والرابع والخامس للجراد الأفريقي مهاجر. كانت قيم التركيز النصف مميت للأعمار الثلاثة 10.9, 10.3, 10.1 ميكرو ليتر، و12.2 ميكرو ليتر بالترتيب. استخدمتركيز النصف مميت للعمر الخامس (12.2 ميكرو ليتر) لمعرفة تأثيره على مكونات الهيموليمف العمر الحوري الخامس الكربوهيدرات الكليه, الدهون الكلية, البرتين الكلي و الكوليسترول الكلي. أوضحت النتائج أن محتويات الهيموليمف الحشرات المعالمة تأثرت بشكل كبير بكل مقاييس الدراسة بها وانخفاضت عن المقارنة.