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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 Ld_{50} values were 10.3, 10.9 and 12.2µl respectively. The effect of latex usher concentration LD_{50} (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.

The treatments *Calotropis procera* latex 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 Ld_{50} values were 10.3, 10.9 and 12.2µl respectively. The effect of latex usher concentration LD_{50} (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.

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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 LD_{50} 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 ºC 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. LD_{50} 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*, 3rd, 4th and 5th 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 3rd, 4th and 5th 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 application**s** on 3rd, 4th and 5th 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	

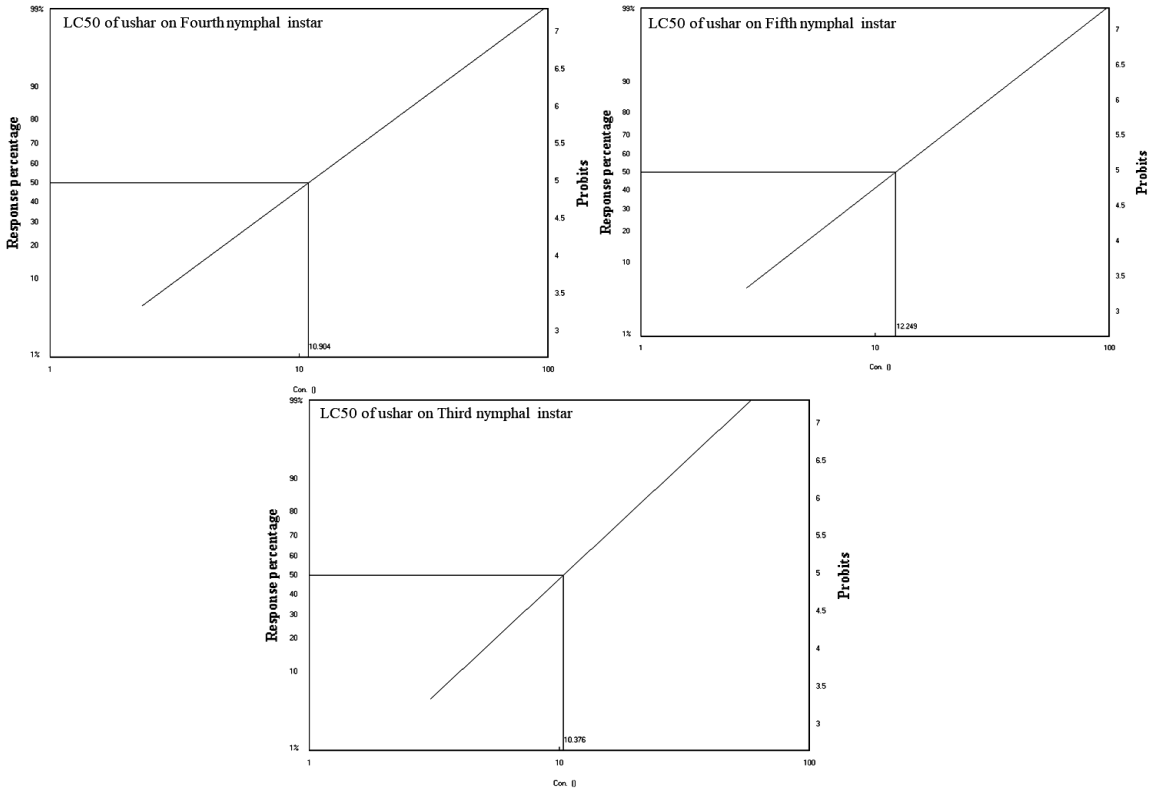

Figure (1): The usher latex LD_{50} values on the nymphal instars of *L. migratoria.*

1.11. Characterization of the fifth nymphal instar haemolymph of *L. migratoria* after treatment with Usher latex:

Usher Latex LD_{50} (12.2 µl) was applied topically on 5th 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_{50} (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 5th 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 5th nymphal instar of *L. migratoria* after treated with usher latex.

Days after treatment	Total carbohydrates		Total lipids		Total protein		Total cholesterol	
	Control±SE[a]	treatment±SE[a]	Control±SE[a]	Treatments±SE[a]	Control±SE[a]	Treatments±SE[a]	Control±SE[a]	Treatments±SE[a]
2nd	316.00±2.42a	295.23±2.25b	221.17±2.61a	218.67±1.2a	5256.67±137.76a	5230.00±119.3a	21.67±1.20a	18.33±0.35b
4th	392.53±5.90a	326.67±8.82b	195.33±2.58a	153.34±2.72b	6486.67±70.75a	4650.00±180.28b	22.43±0.86a	15.67±0.43b
6th	482.00±6.10a	291.00±3.21b	182.27±0.99a	104.47±0.95b	5002.00±57.77a	3906.67±23.33b	18.00±0.58a	10.30±0.26b
8th	368.73±4.56a	210.23±0.44b	210.43±1.1a	86.57±2.66b	4650.00±28.87a	3499.67±5.49b	17.23±1.26a	6.34±0.69b

a= 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 2nd days compared with control but on 4th, 6th and 8th 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 2nd day compared with control but on 4th, 6th and 8th 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 5th 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 breading 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 w 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, moulting 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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الملخص العربي

فاعلية المادة اللبنية لنبات العشار على الاعمار الحورية للجراد الأفريقي

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أدت معاملة الجراد الأفريقي بالمادة اللبنية لنبات العشار إلى نتائج مقنعة جدا بسبب فاعليتها على نسب الموت ومحتويات الهيموليمف في الحوريات المعاملة. 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 لمهاجر. كانت قيم التركيز النصف مميت للأعمار الثلاثة 10.3, 10.9 و12.2 ميكرو ليتر بالترتيب. استخدم تركيز النصف مميت للعمر الخامس (12.2 ميكرو ليتر) لمعرفة تأثيره على مكونات الهيموليمف العمر الحوري الخامس الكربوهيدرات الكلية, الدهون الكلية, البرتين الكلى و الكوليسترول الكلى. اوضحت النتائج ان محتويات الهيموليمف الحشرات المعاملة تأثرت بشكل كبير بكل مقاييس الدراسة بها وانخفضت عن المقارنة.