Acta Entomologica Serbica, 2021, 26(2): 1-17

UDC: 665.345/.347:632.95.021.2
632.95.021.2:595.727
DOI: 10.5281/zenodo.5541184

LARVICIDAL EFFECT OF THE SEED OILS OF TWO INDIGENOUS PLANTS FROM THE ALGERIAN SAHARA ON THE DESERT LOCUST

AHMED AIT AOUDIA[1], ABDELLAH KEMASSI[1,2*], AMEL HEROUINI[1], ZAKARIA TAIBAOUI[1], RIMA DEROUICHE[1], NAWAL BOUZIANE[2] and DIDI MOHAMED OULD EL HADJ[2]

1 Mathematics and Applied Sciences Laboratory, Faculty of Natural, Life and Earth Sciences
University of Ghardaia, POB 455 Ghardaia, Algeria

2 Laboratory of Ecosystem Protection in Arid and Semi-arid Areas, Faculty of Natural and Life Sciences
Kasdi Merbah University, Ouargla POB 511 Ouargla, Algeria

*E-mail: akemassi@yahoo.fr (corresponding author)

Abstract

The lethal and sublethal effects of the seed oils of two indigenous plants of the Algerian Sahara, *Peganum harmala* L. (Zygophyllaceae) and *Datura stramonium* L. (Solanaceae), were investigated. Administration of 60 µl of oil by forced oral injection using a micropipette to the L_5 larvae of *Schistocerca gregaria* Forsk. (Orthoptera: Acrididae) revealed the deterrent effect of these oils on treated larvae. The treatment resulted in various toxicological symptoms, such as intense defecation, diarrhea, weight loss, reduction in motor activity, delay and difficulty in molting and, in the most extreme cases, the death of treated individuals. During the treatment of L_5 larvae of *S. gregaria* with *P. harmala* seed oil, various toxicological symptoms appeared: 81.81% of individuals presented with diarrhea; 68.18% of individuals lost weight; 72.72% exhibited reduced motor activity, and 100% of surviving individuals experienced a delay in their molt. On the other hand, in L_5 larvae treated with *D. stramonium* seed oil, 77.27% of individuals had diarrhea, 100% showed weight loss and 100% of individuals reduced their motor activity. *D. stramonium* seed oil has been shown to be more toxic than *P. harmala* seed oil. The oral administration of 60 µl of *D. stramonium* seed oil caused the blocking of the phenomenon of exuviation in 100% of the treated L_5 larvae, resulting in death after 16 days. While *P. harmala* seed oils caused 50% mortality after 12 days, the 50% surviving individuals were able to complete their imaginal molt with difficulties, which resulted in malformations. The estimated lethal time 50 (LT_{50}) in larvae (L_5) treated with *D. stramonium* seed oil was 3.67 days. It was more toxic than the LT_{50} obtained in larvae (L_5) treated with the oil of *P. harmala* seeds, which was 12 days.
The food intake in L_5 larvae of *S. gregaria* treated with *D. stramonium* seed oil was 0.28 ± 0.18 g/day, it was lower than the average daily consumption recorded in the L_5 larvae treated with *P. harmala* seed oil, which was 0.67 ± 0.36 g/day, *D. stramonium* seed oil appears to be more toxic, and profoundly affects food intake. The values of the apparent digestive utilization coefficient (DUC a) reported for L_5 larvae treated with seed oil of *P. harmala* and *D. stramonium* were 39.32 ± 13.07% and 34.23 ± 29,07%, respectively. These values were significantly lower compared to the control group value, which was 70.63 ± 19.56%. Likewise, the digestive conversion coefficients (CCD) recorded in the L_5 of *S. gregaria* treated with the seed oils of *P. harmala* and *D. stramonium* were -75.07 ± 54.45% and -3.08 ± 1.18, respectively. However, in the control group of L_5 larvae, the noted CCD was 1.004 ± 0.073. Values of the consumption index (CI)

reported for L_5 larvae treated with the seed oils of *P. harmala* and *D. stramonium* were low, 6.74 ± 4.45 and 3.82 ± 2.45, respectively, while for the L_5 larvae of the control group, it was 15.74 ± 3.51.

KEY WORDS: larvicide, oil, *Schistocerca gregaria*, *Datura stramonium, Peganum harmala*, forced oral injection

Introduction

In several regions of the world, apart from climatic factors, food security relies primarily on crop protection. Locusts are a particularly alarming group of insects. During periods of invasions, juveniles and adults invade fields in their billions, devastating food resources and crops in many countries. The desert locust *Schistocerca gregaria* is the most feared locust species for crops. More than 60 African and Asian countries are affected by intermittent invasions and upsurges (Lecoq, 2005; 2014). Faced with this scourge, for years chemical pesticides were the most used and effective means to stop the progression of hopper bands and swarms. In Africa more than 13 million liters of insecticides were sprayed during the last invasion of 2003-2005 (Brader *et al*., 2006). Although they are effective, the collateral effects on contaminated environments are severely damaging through the pollution of natural ecosystems, water, soil, etc.

In order to minimize the large-scale spreading of chemical pesticides, the scientific community is interested in the research and development of other effective control methods that are less toxic to the environment. Several studies have highlighted the potential of biocide extracts of many plant species including the work of (Ould El Hadj *et al*., 2006; Kemassi *et al*., 2010, 2012a, 2012b, 2013, 2014, 2015, 2018, 2019, Cherif *et al*., 2016; Bouziane *et al*., 2018), and some Saharan plant species have been characterized by certain pharmacological properties that partly emanate from the various adaptation methods that allow them to develop in extreme and harsh environments; such phenomena make the flora of the Sahara the object of study and biological conservation. In the Sahara, there are more than 500 taxa, the majority of which have medicinal uses (Ozenda, 1991). *Peganum harmala* L. (Zygophyllaceae) and *Datura stramonium* L. (Solanaceae) are two Saharan species known for their various medicinal uses and strong toxicities because of their richness in secondary metabolites, particularly in alkaloids.

The present study sought to evaluate the lethal and sublethal effects of the seed oils of two Saharan plant species, *P. harmala* L. (Zygophyllaceae) and *D. stramonium* L. (Solanaceae), on fifth-stage juveniles of the desert locust *Schistocerca gregaria*.

Materials and methods

Biological material

The biological material used consisted of the ripe seeds of two plant species, *D. stramonium* and *P. harmala*, and 5th stage larvae of the desert locust *Schistocerca gregaria* (Forskal, 1775).

Datura stramonium is an annual plant of the Solanaceae family (Fig. 1) Its height varies from 30 cm to 2 m. It is characterized by large oval leaves and white flowers. The fruits are in the form of a thorny capsule filled with seeds (Bellakhder, 1997). It can be found in various environments; it grows wild in all hot and temperate regions of the world and in some regions of Europe (Reynaud-Maurupt, 2006). Its flowering period is between July and September. *D. stramonium* is rich in secondary metabolites, in particular alkaloids (Goulle *et al*., 2004).

Peganum harmala is a perennial herbaceous plant with a height of 30 to 90 cm and an ordinary and sparsely branched stem, densely leafed. The leaves are elongated and irregular with a size of 5x5 cm (Fig. 2). It is characterized by white flowers. The fruits are small, spherical capsules depressed at the top containing small black seeds (Ozenda, 1991). Given its richness in alkaloids, *P. harmala* is known for its high toxicity for animals and humans (El Bahri & Chemli, 1991; Bruneton, 1993; Bellakhder, 1997).

Photos of the plants and locusts were taken by the first author of this paper (Ahmed Ait Aoudia 2020).

Figure 1. *Datura stramonium* at the vegetation stage (Oued Metlili, Ghardaia Region - Northern Sahara of Algeria).

Figure 2. *Peganum harmala* at the vegetation stage (Oued Metlili, Ghardaia Region - Northern Sahara of Algeria).

Desert locust *Schistocerca gregaria*

The desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) is the most destructive locust species for crops in the arid and semi-arid zones of North Africa up to the Indian/Pakistani border, where it threatens the food resources of populations in more than 50 African and Asian countries (Roffey, 1982; Lecoq, 2004). It is a locust exhibiting a phenomenon of phase polymorphism, that is to say the possibility of developing various and reversible aspects, depending on the density of the populations. These different aspects are referred to as phases. We speak of solitary phase for low density populations and gregarious phase for high density populations. During periods of invasions, gregarious larvae and imagos invade fields over a geographic area of over 12.5 million km2 in Africa and Asia (Symmons & Cressman, 2001). In addition, the desert locust is polyphagous and has an exceptional capacity for dispersal and adaptation to different climatic conditions (Lecoq, 2004).

Extraction of oils

The ripe seeds of the two species come from plants sampled in Oued Metlili (Ghardaia region of the Algerian Sahara). After harvesting the seeds and cleaning them of any debris or dust, grinding was carried out using an M20 Universal type knife mill, IKA®- Germany. The powder was then stored in hermetically sealed and labeled jars. We used 100 g of *P. harmala* and *D. stramonium* seed powder, which produced 3.9 mL and 3 mL of plant oil, respectively. The extraction of the seed oil was carried out by hexane in a 250 ml capacity Soxhlet device. Extraction from the seed powder took about 6 h at a temperature of 50°C. To purify the obtained product, it was passed through a vapor rotor until the complete elimination of hexane.

Desert locust mass rearing

Individuals from a single population captured in pivot-irrigated cereal perimeters in the Hassi Lefhal Ghardaïa area (Algerian Sahara) were kept in a parallelepiped cage with a wooden frame measuring 120 cm x 70 cm x 60 cm. The cage was covered with a fine wire mesh. A small sliding door on the front face of the cage allowed access. The bottom of the cage had circular openings where nest boxes filled with sand from dunes were placed and moistened regularly. Individuals were maintained at a temperature of 35 ± 1°C, with continuous lighting and a relative humidity of 55 ± 3%. The food consisted mainly of Poaceae, including durum wheat (*Triticum durum* L.), barley (*Hordeum vulgare* L.), sod (*Stenotaphrum americanum* L.) and cabbage leaves (*Brassica oleracea* L.) (Brassicaceae), with wheat bran as a supplement. Provision of food, cage cleaning, humidification and checking the nest boxes were carried out daily.

For laboratory convenience and to demonstrate the action of these vegetable oils on the molt, 5^{th} stage (L_5) larvae were selected for experimentation.

Application of tests

L_5 larvae were placed individually in 500-mL jars, fitted with supports to allow the larvae to roost during the molt. L_5 larvae were fasted for 24 h to empty their digestive tract and to starve them. The test consisted of injecting into the esophagus of the insect a volume of 60 µl of vegetable oil or a control liquid (distilled water) by forced injection. Given their exceptional nutritional value and their palatability to this locust, after injection, the treated larvae were returned to their boxes and fed with specified surfaces and weight of the fresh leaves of cabbage *Brassica oleracea* fragments After 24 h, the jars were cleaned. Not ingested fragments of cabbage leaves by the larvae were collected in order to take their imprints on graph paper. These will be used to calculate the leaf area consumed which will be used to estimate the quantity of cabbage leaves consumed. To study the effect of the extracts on the digestion of L_5 larvae, the feces were collected. The weight changes of individuals, the number of deaths, the weight of feces and the weight and area of non-ingested

cabbage fragments were recorded daily. The experiment was pursued until the total mortality of the individuals of the treated batches or in the case of the passage of the entire treated population to the imago instar.
Three batches of 22 larvae (11 males and 11 females) were used, making a total of 66 L_5 larvae, with two batches for treatment and one for the control.

Studied parameters

In the present study, the lethal and sublethal effects of the seed oils of two Saharan plants on some biological parameters of L_5 larvae of *S. gregaria* were studied, namely mortality, molting, consumption, weight growth, digestion, motor skills, etc.

Effect on mortality

In toxicology, mortality is the most important parameter for evaluating the effectiveness of a natural or synthetic product. It is calculated by different formulas including an assessment of cumulative morality, corrected mortality and lethal time 50, which were used for this study.

Cumulative mortality corresponded to the number of dead individuals relative to the total number of individuals within the treated population. This was calculated using the following formula:
Mc (%) = (No. of dead individuals / No. of individuals treated) x 100 (Lazar, 1968).

Corrected mortality was estimated by applying Schneider's formula, as follows:
Corrected mortality (%) = Mc = [M2 - M1/100 - M1] x 100 (Lazar, 1968).
Mc:% corrected mortality
M2:% of mortality in the treated population
M1:% mortality in the control population

Lethal time LT_{50} is the time required for 50% of individuals in the treated population to die from exposure to a given product. It was calculated from the probit regression line corresponding to the percentages of mortalities corrected for the logarithms of treatment duration (Ramade, 2007).

Effect on digestion

The effects of the tested extracts on the digestion of desert locust L_5 larvae were studied by estimating the apparent digestive utilization coefficient (DUC a), the consumption index (CI) and the efficiency of conversion of ingested food (ECI).

Apparent digestive utilization coefficient (DUC a) is the percentage of nutrients ingested note excreted in feces. It represents the results of the interaction between the digestive tract and the composition of the plant consumed. The DUC a was determined according to the equation of Walbauer (1968).
DUC = ((Weight of food ingested - weight of feces) / Quantity ingested) × 100

Consumption index (CI) was evaluated by calculating the ratio between the amount of food consumed by an animal during a specified period and its gain in live weight during the same period (Boccard, 1963), using the following formula:
CI = Weight of food ingested / gain in live weight

Efficiency of conversion of ingested food was the ratio of the increase in an animal's weight over 24 h to the amount of food ingested during the same period Walbauer, 1968). It was estimated by the following formula:
ECI (%) = [(Bodyweight gain) / (Weight of food ingested)] × 100

Statistical analyses

The experiment results were statistically analyzed by the mean of one-way analysis of variance ANOVA and when results were significant at p =0.5. Tukey (HSD) and Kruskal-Wallis tests was used. XLSTAT Version 2012 software was used to interpret the experimental results of the various tests.

Results

Effect on mortality

An insect is considered to be sensitive to a given product if the latter is lethal at low doses. The mortality rates observed in control L_5 larvae treated with the seed oils of the two Saharan species are shown in Table I. It was noted that the seed oils of the two species cause appreciable rates of mortality.

Table I. Cumulative mortality rate recorded in L_5 larvae in control and larvae treated with *Peganum harmala* and *Datura stramonium* seed oils.

Time (days)	**Experimental batches (Mortality rates %)**		
	Control	***P. harmala***	***D. stramonium***
1	0 (A)	0(A)	0(A)
2	0 (A)	18.18(B)	13.63(B)
3	0 (A)	22.72(B)	40.90(C)
4	0 (A)	36.36(B)	54.54(C)
5	0 (A)	36.36(B)	59.09(C)
6	0 (A)	36.36(B)	68.18(C)
7	0 (A)	36.36(B)	81.81(C)
8	0 (A)	36.36(B)	90.90(C)
9	0 (A)	40.90(B)	90.90(C)
10	Imago	40.90(A)	90.90(B)
11	Imago	40.90(A)	90.90(B)
12	Imago	50(A)	90.90(B)
13	Imago	Imago	90.90(A)
14	Imago	Imago	95.45(A)
15	Imago	Imago	95.45(A)
16	Imago	Imago	100(A)

The cumulative mortality rate recorded in L_5 treated with the *P. harmala* and *D. stramonium* seed oils was higher than those recorded in larvae of the control group where no mortality was recorded (Table I). The 100% mortality rate was reached after 16 days in L_5 larvae treated with *D. stramonium* seed oil (Fig. 3). On the other hand, the L_5 seem less sensitive to the action of *P. harmala* seed oil, where a mortality rate of 50% was reported in the L_5 larvae after 16 days, and the surviving L_5 larvae were able to complete their imaginal molt (Fig. 3). No mortality was observed in the control L_5 (Fig. 3) and they were able to complete their imaginal molt after 8 ± 0.5 days. It was important to note that the L_5 larvae treated with the two oils presented symptoms before their death, including intoxication manifested by a decrease in locomotor activity, liquefied feces, reduced ability to perch. In some L_5 larvae treated with *D. stramonium* seed oil, there was a blackening of the ventral surface a few hours after their death (Fig. 4) Difficulties in molting were observed in L_5 larvae treated with seed *P. harmala* oil (Fig. 5), leading either to the death of the individuals treated following a blockage of the exuviation phenomenon (50%), or to deformation of wings and legs (36.36%) (Fig. 6) Only a few larvae (13.64%) of the treated population completed their imaginal molt without morphological abnormalities. For L_5 larvae treated with *D. stramonium* seed oil, no larvae succeeded in the imaginal molt.

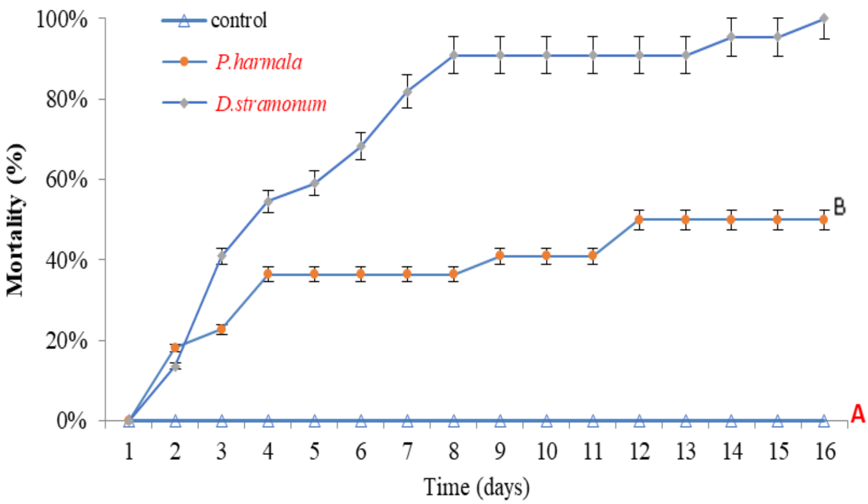

Figure 3. Variations in the mortality rates observed in control L_5 larvae and those treated with seed oils of *Peganum harmala* and *Datura stramonium*.

Lethal time 50 (LT_{50}) evaluation

The evaluation of the LT_{50} makes it possible to assess the speed of action of the studied oils. To evaluate the LT_{50} of the seed oils of *P. harmala* and *D. stramonium* on L_5 larvae of *S. gregaria*, the probit regression lines of the mortality rate as a function of the logarithms of the treatment times were drawn (Fig. 7). Estimation of the LT_{50} showed that the seed oil of *D. stramonium* was more toxic compared to that obtained from the seeds of *P. harmala*, being in the order of 3.67 days, while it was 12 days for *D. stramonium* and *P. harmala* seed oil respectively (Fig. 7).

Effect on food intake

The average daily quantities in grams ingested by the L_5 larvae of *S. gregaria* are shown in Table II. In the control larvae, the average daily consumption of fresh cabbage leaves was 1.55 ± 0.87g/day. A difference in consumption between sexes was reported. For males, it was 1.29 ± 0.81 g/day and for females 1.8 ± 0.95 g/day. For L_5 larvae treated with *P. harmala* seed oil, the average daily consumption was lower than the control; it was 0.67 ± 0.36g/day. Male L_5 larvae appear to be more sensitive to the deterrent effects of *P. harmala* seed oil, the average consumption noted was 0.24±0.22 g/day, while it was 1.03 ± 0,49 g/day in females. In addition, *D. stramonium* seed oil appears to be the more toxic, and profoundly affects food intake in *S. gregaria* L_5 larvae; the average recorded consumption was 0.28 ± 0.18 g/day. At the same time, male L_5 larvae were more sensitive than females; the mean consumption reported was 0.18 ± 0.14 g / day and 0.34 ± 0.24 g/day in male and female L_5 larvae, respectively.

Figure 4. Blackening of the ventral surface observed in L_5 larvae of *Schistocerca gregaria* treated with seed oils of *Datura stramonium.*

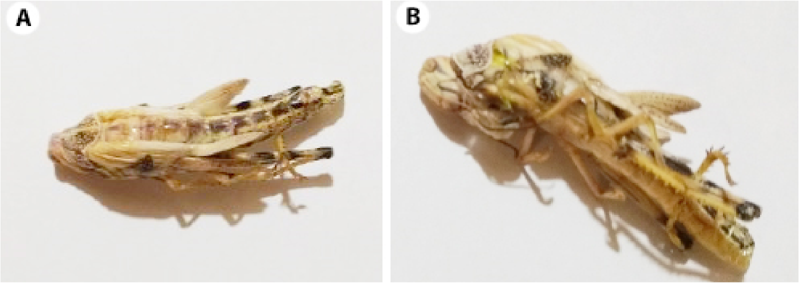

Figure 5 (A, B). Dead larvae as a result of the inability to molt observed in L_5 of *Schistocerca gregaria* treated with *Peganum harmala* seed oil.

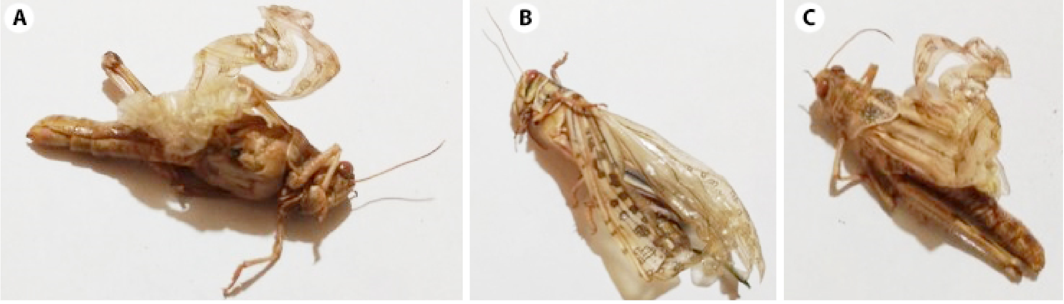

Figure 6 (A, B, C). Morphological abnormalities observed in L_5 larvae of *Schistocerca gregaria* treated with *Peganum harmala* seed oil.

A B

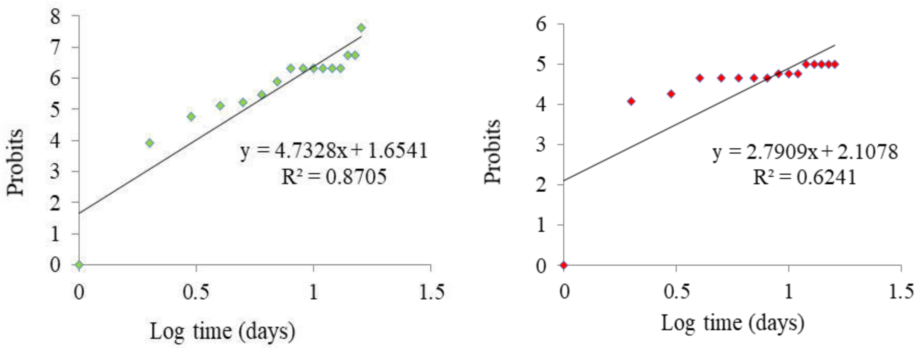

Figure 7 (A, B). Regression lines of mortality rates recorded in L_5 larvae treated with *Peganum harmala* and *Datura stramonium* seed oils as a function of time. A – Variation over time in the mortality rate recorded in L5 larvae of *Schistocerca gregaria* treated with *Datura stramonium* seed oil. B – Variation over time in the mortality rate recorded in L5 larvae of *Schistocerca gregaria* treated with *Peganum harmala* seed oil.

Table II. Variation over time of the average consumption of cabbage leaves recorded in control L_5 larvae of *Schistocerca gregaria* and those treated with the seed oils of *Datura stramonium* and *Peganum harmala.*

Time (days)	Experimental batches (Consumption in grams)		
	Control	*P. harmala*	*D. stramonium*
1	1.95±0.79(A)	0.68±0.44(B)	0.42±0.14(B)
2	2.24±0.64(A)	0.76±0.29(B)	0.44±0.26(B)
3	2.68±0.95(A)	1.09±0.79(B)	0.47±0.29(B)
4	2.70±0.78(A)	1.12±0.76(B)	0.5±0.09(B)
5	1.67±0.96(A)	1.21±0.79(B)	0.29±0.35 (B)
6	0.57±0.72(A)	0.64±0.39(A)	0.31±0.03(A)
7	0.17±0.31(A)	0.21±0.04(A)	0.45±0.18(A)
8	0.79±0.76(A)	0.17±0.15 (B)	0.35±0.13(B)
9	1.18±0.73(A)	0.31±0.11 (B)	0.31±0.13 (B)
10	Imago	0.3±0.004(A)	0.22±0.22(A)
11	Imago	0.75±0.48(A)	0.10±0.10(A)
12	Imago	0.88±1.24(A)	0.00±0.00(A)
13	Imago	Imago	-
14	Imago	Imago	-
15	Imago	Imago	-
16	Imago	Imago	-

From the obtained results, the L_5 larvae of the desert locust appear to be sensitive to the seed oils of *D. stramonium* and *P. harmala*. These oils significantly affect the intake of food, indicating that their anti-appetite power is very strong. In the light of these results, it appears that the applied treatments generate a significant reduction in the consumption of cabbage leaves by the L_5 larvae of desert locust particularly during the first five days of treatment, where it appeared a particular difference in the consumption of cabbage leaves between the larvae L_5 of the control lot and those of the lots treated with the seed oils of *P. harmala* and *D. Stramonium*. By extending the experimental follow-up period either from the sixth day, no significant difference is recorded in the quantities of cabbage leaves consumed by the larvae of different lots. At the tenth day of experimental follow-up, all L_5 larvae were able to complete their imaginal moult, on the other hand at the batch treated by the seed oil of *P. harmala* and *D. Stramonium*, the larvae L_5 of the desert locust showed signs of intoxication including death, unusual water losses in the form of diarrhea, difficulties and the inability to moult followed by the death of the individual. For L_5 larvae of the batch treated with seed oil of *P. harmala* having succeeded their imaginal moult, they undergo death a few days (delayed mortality).

Effect on growth and development

The deterrent effects of *P. harmala* and *D. stramonium* seed oils on food intake are limited weight growth or a drop in body weight of the treated larvae. Variations in the average daily weight of the L_5 larvae of *S. gregaria* for the control group and those treated with the seed oils of *P. harmala* and *D. stramonium* are given in Table 3. The obtained results show that a low weight gain was reported in L_5 larvae treated with *P. harmala* seed oil compared to the control group (Table 3, Fig. 8), where a weight gain of 19.65% and 11.63% was noted for male and female larvae, respectively. This weight gain was markedly higher in the control L_5 larvae; it was around 53.38% for males and 53.33% for females. The forced administration of the seed oil of *D. stramonium* to *S. gregaria* L_5 larvae caused a significant drop in weight of 28.03% and 7.07% in male and female L_5 larvae, respectively (Fig. 8).

Table III. Weight variation recorded in control *Schistocerca gregaria* L_5 larvae and those treated with *Peganum harmala* and *Datura stramonium* seed oils.

Time (Days)	**Experimental batches (Weight variation in grams)**		
	Control (g)	***P. harmala***	***D. stramonium***
1	0.75±0.13(A)	0.74±0.1(A)	0.59±0.1(B)
2	1.21±0.22(A)	0.78±0.21(B)	0.60±0.19(C)
3	1.40±0.22(A)	1.02±0.35(B)	0.65±0.27(C)
4	1.58±0.22(A)	1.16±0.4(B)	0.77±0.29(C)
5	1.74±0.23(A)	1.48±0.29(B)	0.87±0.27(C)
6	1.81±0.22(A)	1.73±0.29(A)	0.9±0.33(B)
7	1.77±0.26(A)	1.75±0.3(A)	0.98±0.31(B)
8	1.67±0.27(A)	1.71±0.31(A)	1.30±0.35(B)
9	1.67±0.20(A)	1.59±0.31(B)	1.58±0.17(B)
10	Imago	1.53±0.3(A)	1.64±0.14(B)
11	Imago	1.55±0.41(A)	1.65±0.04(B)
12	Imago	1.49±0.63(A)	1.63±0.009(B)
13	Imago	Imago	1.57±0.003(A)
14	Imago	Imago	1.52±00(A)
15	Imago	Imago	1.43±00(A)
16	Imago	Imago	1.29±00(A)

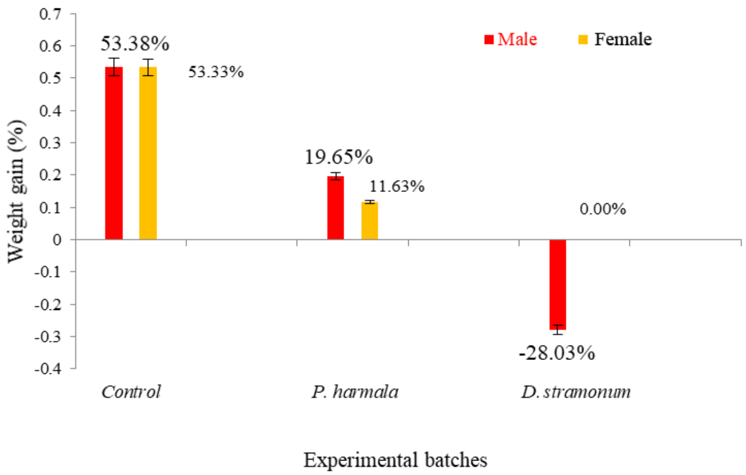

Figure 8. Weight change from initial weight of *Schistocerca gregaria* L_5 larvae in the control group and those treated with *Peganum harmala* and *Datura stramonium.*

Effect on digestion

The effects of the seed oils of two Saharan plant species on digestion was examined by estimating the apparent digestive utilization coefficient (DUC a), consumption index (CI) and the efficiency of conversion of ingested food (ECI).
The average values of the DUC a, CI and ECI estimated for *S. gregaria* L_5 larvae revealed the harmful effects of the applied treatments (Table IV). The tested seed oils caused digestive disturbances in the insects, translated into a loss in the ability to convert ingested food into weight, intense defecation, and liquefied feces.

Table IV. Digestive parameters estimated for the L_5 larvae of control *Schistocerca gregaria* and those treated with the seed oils of *Peganum harmala* and *Datura stramonium*.

	Parameters	**Apparent Digestive Utilization Coefficient (DUC a) (%)**	**Consumption Index (CI)**	**Efficiency of Conversion of Ingested food (ECI%)**
Experimental batches	Controls	70.63±19.56(B)	15.74±3.51(A)	1.004±0.073(A)
	P. harmala	39.32±13.07(B)	6.74±4.45(B)	-5.07±54.45(A)
	D. stramonium	34.23±29.07(B)	3.82±2.45(B)	-3.08±1.18(A)

The values of the DUC a reported for L_5 larvae treated with seed oil of *P. harmala* and *D. stramonium* were 39.32 ± 13.07% and 34.23 ± 29.07%, respectively. These values were significantly lower than that of the control group which was 70.63 ± 19.56%.

Likewise, the ECI recorded in L_5 of *S. gregaria* treated with the seed oils of *P. harmala* and *D. stramonium* was -75.07 ± 54.45% and -3.08 ± 1.18, respectively. However, in the control L_5 larvae, the noted ECI was 1.004 ± 0.073 (Table IV). The negative ECI values were from the weight loss recorded in L_5 larvae treated with the seed oils of the two plant species. The digestive capacity and the altered conversion of food into weight resulted in appreciable losses in weight; the CI confirms the obtained results. The values of the CI reported for L_5 treated with seed oil of *P. harmala* and *D. stramonium* were low, they are 6.74 ± 4.45 and 3.82 ± 2.45, respectively. For L_5 of the control batch, the CI was15.74 ± 3.51 (Table IV). Table IV shows that for the results of the DUC a, group A contains the control individuals and group B contains *P. harmala* and *D. stramonium*, indicating that the average of the controls, which was 70.63 ± 19.56%, was much higher than the averages of *P. harmala* and *D. stramonium* (39.32 ± 13.07 and 34.23 ± 29.07, respectively). For the CI, group A contains the controls and group B contains *P. harmala* and *D. stramonium*, showing that the mean of the control group of 15.74 ± 3.51 was higher compared to the means of *P. harmala* and *D. stramonium* (6.74 ± 4.45 and 3.82 ± 2.45, respectively). Moreover, in the results of the digestive conversion coefficient (ECI), group A contains the controls and *P. harmala* and *D. stramonium*; this result is explained by the absence of a significant difference between the means of the controls and those of *P. harmala* and *D. stramonium*.

Discussion

Effects on mortality

An insect is sensitive to a given insecticide, so when applied at a low dose it causes death. L_5 larvae of *S. gregaria* treated with *P. harmala* seed oil showed a mortality rate of 50% after 12 days, and a mortality rate of 100% after 16 days for those treated with *D. stramonium* seed oil. Some individuals died from the inability to molt, while the survivors were able to complete their imaginal molt, but morphological abnormalities were observed, including wing deformities. However, no mortality was observed in control L_5 larvae. The study on toxicity by contact and by ingestion of the ethanolic extract of the seeds of *P. harmala* by the L_5 larvae of *S. gregaria* shows their sensitivities; a mortality rate of 100% was obtained after 5 days for the dose D1 = S (100% of the stock solution) and after 6 days for the dose D2 = S / 2 (50% of the stock solution), and on the 8th day for the dose D3 = S / 4 (25% of the stock solution). Similarly, this extract, applied by ingestion, caused a mortality rate of 100% after 15 days for the dose D1 = S (100% of the stock solution) and after 16 days for the dose D2 = S / 2 (50% of the stock solution) and a mortality rate of 77.78% after 16 days for the dose D3 = S / 4 (25% of the stock solution) (Aissaoui, 2014). This toxicity of the ethanolic extract of *P. harmala* seeds is probably due to the effects of secondary substances, including indole alkaloids (harmine and harmaline), which are predominant in the seeds of this species (Aissaoui, 2014). Kemassi *et al*., (2013) reported on the contact toxicity of essential foliar oils of *P. harmala*; a 100% mortality rate was reached after 8 min and 30 s in L_5 larvae of *S. gregaria.* In addition, ingestion of lettuce leaves treated with the ethanolic extract of *P. harmala* leaves caused a mortality rate of 75% after 14 days in L_5 larvae of *S. gregaria* (Abbassi *et al*., 2005). Indole alkaloids including harmine and harmaline identified in leaf and seed extracts of *P. harmala* or those isolated from seeds of *D. stramonium* including hyoscyamine and scopolamine, are the main cause of the lethal effects of these extracts on the insects, they act on the central nervous system of insects and generates the intestine distension by a lack of nervous tone. These metabolites have an action on the digestive system by generating a deficit of assimilation, the lesions of the intestinal mucosa and disturb the water metabolism (Fuentes & Longo, 1970; Baran, 2000).

Evaluation of lethal time 50 (LT_{50})

LT_{50} values in 5 min and 43 s are recorded for the essential oils of *P. harmala* applied by contact to *S. gregaria* L_5 larvae (Kemassi *et al*., 2013). L_5 larvae showed sensitivity to two ethanolic extracts of *P. harmala*. Ingestion application of ethanolic extracts of *P. harmala* to *S. gregaria* L_5 larvae gave LT_{50} values of 2.2 days, 2.29 days and 2.57 days for doses D1 = S and D2 = S / 2, D3 = S / 4 respectively. Likewise, the application of the same extracts by contact obtained LT_{50} values of 2.61 days, 0.75 days and 1.06 days for doses D1, D2 and D3, respectively (Aissaoui, 2014). Kemassi *et al*. (2018) in their study on the toxicity of the total alkaloids of *Cleome arabica* L. (Capparidaceae) on *S. gregaria* reported lethal times of 8.77 days and 11.19 days, estimated for L_5 male and female larvae, respectively, and 13.42 days noted for female imagoes and 17.58 days for male imagoes. The alkaloid extract of *Euphorbia guyoniana* Boiss. & Reut. (Euphorbiaceae) seems to be toxic for the desert locust. The estimated lethal times were around 7.05 days for male L_5 larvae, 7.31 days for female L_5 larvae, and 5.99 days and 9.06 days for male and female imagoes, respectively (Kemassi, 2014).

Effect on food intake

The desert locust is characterized by its great voracity and polyphagia (Duranton and Lecoq, 1990). The low consumption of cabbage noted in L_5 larvae treated with *P. harmala* and *D. stramonium* seed oils was probably linked to the presence of substances with an anti-appetizing effect; a notable decrease in food intake was reported in the treated larvae. It is commonly accepted that the seeds of *P. harmala* and *D. stramonium* are very toxic, they contain between 3% and 4% of alkaloid substances (Baran, 2000; Abbassi *et al*., 2003). The observed decrease in food intake probably arises from the dissuasive action of these toxic compounds on the taste organs of the insect including the maxillary palps, which play an important role during food prospecting (Chapmane, 1974; Idrissi-Hassani *et al*., 2002).

Effect on growth and development

L_5 larvae treated with *P. harmala* and *D. stramonium* seed oils showed a drop in weight, especially in those treated with *D. stramonium* seed oil. However, in the control L_5 larvae, a change in weight was also observed. It is commonly accepted that digestion in locusts is sequential; the food ingested is mainly assimilated at the level of the mesenteron of the digestive tract. Weight gain is linked to the ability of insects to convert ingested food into growth tissue (Nicole, 2002). Therefore, the drop in weight observed in L_5 larvae treated with seed oil of *P. harmala* and *D. stramonium* was likely the result of the action of these oils on the digestive tract. In addition, Idrissi-Hassani and Hermas (2008) noted that ingestion of the aerial parts of *P. harmala* causes lesions in the digestive tract of *S. gregaria* imagoes, in particular on the epithelial cells of the mesenteron. Similar works have shown that the L_5 larvae of *S. gregaria* treated by different modes with *P. harmala* seed extracts lose weight following the annihilation or limitation of food intake or by the effects of digestion and water metabolism, externalized through intense defecation or liquid feces (Abbassi *et al*., 2003; Kemassi *et al*., 2012a). Abbassi *et al*., (2003) reported that alkaloids have an inhibitory action on the reabsorption of water by the rectum, hence the increase in urine in the form of hydrated feces through the disruption of the regulatory function of the Malpighi tube in *S. gregaria*. Deltamethrin, an insecticide widely used in locust control, induces in the imagoes of *Locusta migratoria* L. (Orthoptera: Acrididae) a harmful action on the neuro-endocrine system responsible for the regulation of water balance (Proux *et al*., 1993; Alaoui, 1994).

Effect on digestion

Legal (1989) noted that the apparent digestive utilization factor represents the interaction between the digestive tract and the composition of the plants consumed. Therefore, it should be noted that the low values of the DUC a, ECI and CI recorded for L_5 larvae of *S. gregaria* treated with *P. harmala* and *D. stramonium* seed oils compared to the values of the various indices reported for the control group of larvae L_5 arise from the action of these oils on the digestive tract by affecting the processes of nutrient assimilation and the reabsorption of the water contained in the food consumed, phenomena which were externalized by intense defecations and by unusual water losses in the form of diarrhea. Proux (1991) noted that water metabolism in locusts was controlled by the neuroendocrine system, which acts on the subesophageal ganglion that acts on the Malpighi tube for the secretion of primary urine, and which also controls the reabsorption of water contained in food. Hence, any disturbance of these mechanisms places the insect in a situation of imbalance.

Conclusion

The study of the larvicidal activity of seed oils from two indigenous plants collected in the Algerian Sahara highlights the lethal and sublethal effects of the oils on L_5 larvae of *S. gregaria*. Administration of *P. harmala* and *D. stramonium* seed oils markedly affected food intake, digestion, molting, digestive conversion and weight growth in L_5 larvae. These harmful effects resulted in the death of treated larvae with differences in toxicity; *D. stramonium* seed oil appears to be more toxic than *P. harmala* seed oil in terms of time.

References

Abbassi, K., Mergaoui, L., Atay-Kadiri, Z., Ghaout, S., & Stambouli, A. (2005). Biological activities of *Peganum harmala* (Zygophylacea) leaves at floral stage on the mortality and reproductive activity of the desert locust. *Zoologica baetica*, *16*, 31-46.

Abbassi, K., Mergaoui, L., Atay- Kadiri, Z., Stamboli, A., & Ghaout, S. (2003). Activités biologiques de l'extrait des graines de *Peganum harmala* sur le criquet pèlerin (*Schistocerca gregaria* Forskal, 1775). *Journal of orthoptera research*, *12*(2), 71-79. [In French].

Aissaoui, H. (2014). *Activité biologique des extraits de Peganum harmala, Nerium oleander et d'un insecticide sur le criquet pèlerin Schistocerca gregaria* (Forskal, 1775) *(Orthoptera- Acrididae)*. Thèse Magister. Zoologie agricole et forestière. Institut national. Agronomique (INA), El Harache, Algérie, 110pp. [In French].

Alaoui, A. (1994). *Mode d'action d'un insecticide de synthèse la deltamithrine, sur le métabolisme glucidique et hydrominéral, chez le criquet migrateur mâle adulte*. Thèse de Doctorat es-sciences. Faculté des Sciences, Rabat, Maroc, 152 pp. [In French].

Baran, J. M. (2000). *Datura plantes magiques, Halluc, inogenes et medcinales. l'ile L'ile de la réunion et dans le monde.* Thèse docteur en médecines. Université HENRIPOIN Nancy, France, 254 pp. [In French].

Bellakhder, J. (1997). *La pharmacopée marocaine traditionnelle médecine arabe ancienne et savoirs populaires*. Ed le Fennec, Casablanca *Rabat, Ibis, Presse*, Maroc, 1370 pp. [In French].

Boccard, R. (1963). Etude de la production de la viande chez les ovins. *Annales de zootechnie, 12*(3), 227-230. [In French].

Bouziane, N., Kemassi, A., & Oud El Hadj, M. D. (2018). Étude de la toxicité des extraits foliaires de *Peganum harmala* L. (Zygophyllaceae) sur *Schistocerca gregaria* (Forskål, 1775) (Orthoptera-Acrididea). *Ciencia e tecnica jornal, 33*(8), 2-19. [In French].

Brader, L. H., Djibo, H., Faye, F. G., Ghaout, S., Lazar, M., Luzietoso, P. N., & Ould-Babah, M. A. (2006). *Evaluation multilatérale de la compagne 2003- 2005 contre le Criquet pèlerin*. Ed. FAOUN, Rome, 101pp. [In French].

Bruneton, J. (1993). *Pharmacognosie, phytochimie: Plantes médicinales (2e édition)*. Ed. Lavoisier, Paris, 915 pp.

Cherif, R., Kemassi, A., Boual, Z., Bouziane, N., Benbrahim, F., Hadjseyd, A., Gharib, T., Ould El Hadj-Khelil, A., Sakeur, M. L., & Ould El Hadj, M. D. (2016). Activités biologiques des extraits aqueux de *Pergularia tomentosa* (Asclepiadaceae). *Lebanese science journal*, *17*(1), 25-35. [In French].

Duranton, J. F., & Lecoq, M. (1990). *Le criquet pèlerin au Sahel. Collection Acridologie Opérationnelle N°6*. Ed., CIRAD/PRIFAS, Montpellier, 178 pp. [In French].

El- Bahri, L., & Chemli, R. (1991). *Peganum harmala* L. a poisonous plant of North Africa. *Veterinary and human toxicology*, *33*(3), 276-277.

Funtes, J. A., & Longo, V. G. (1970). An investigation on the system nervous central effects of harmine, Harmaline, and related B-carbolines, *Neuropharmacologigy*, *10*, 15-23.

Goulle, J. P., Pepin, G., Lacroix, C. (2004). Botanique, chimie et toxicologie des Solanacées hallucinogènes: Belladone, Datura, Jusquiame, Mandragore. *Annales de toxicologie analytique*, *16*(1), 22-35. [In French].

Gry, J., Coquard, J., & Coquard, G. (1966). Appéciation en laboratoire de l'activité des insecticides à l'égard du criquet migrateur. *L'agronomie tropicale*, Série 3, Agronomie Générale. Etudes Scientifiques, *21*(7), 837-855. [In French].

Idrissi-Hassani, L., M., & Hermas, J. (2008). Effet de l'alimentation en *Peganum harmala* L. (Zygophyllaceae) sur le tube digestif du Criquet pèlerin *Schistocerca gregaria* Forsk. (Orthoptera, Acrididae*). Zoologica baetica*, *19*, 71-84. [In French].

Idrissi-Hassani, L., M., Ouled Ahmedou, M. L., Maya, E. H., and Bouichi, A. (2002). Pouvoir insecticide de *Peganum harmala* sur *Schistocerca gregaria*. Effets de l'huile et des extraits de feuilles. *Biologie et santé*. *2*(2), 122-133. [In French].

Kemassi, A. (2014). *Toxicité comparée des extraits d'Euphorbia guyoniana (Stapf.) (Euphorbiaceae), Cleome arabica L. (Capparidaceae) et de Capparis spinosa L. (Capparidaceae) récoltés de la région de Ghardaïa (Sahara septentrional) sur les larves du cinquième stade et les adultes de Schistocerca gregaria (Forskål, 1775) (Orthoptera-Cyrtacanthacridinae)*. Thèse de doctorat en Écologie Saharienne et Environnement. Université de Kasdi Merbah-Ouargla, Algérie, 264 pp. [In French].

Kemassi, A., Boual, Z., Bouziane, N., Ould El Hadj-Khelil, A., & Ould El Hadj, M. D. (2013). Biological activity of essential oils leaves from one Sahara plant*: Peganum harmala* L. (Zygophyllaceae) on the desert locust. International *Journal of current microbiology and applied sciences*, *2*(8), 389-395.

Kemassi, A., Boual, Z., Hadjseyd, A., Bouziane, N., Herouini, A., Mensouri, K., Bouras, N., Ould El Hadj-Kelil, A., & Ould El Hadj, M. D. (2018). Effets bitoxiques des extraits de *Cleome arabica* L. (Cappardicaceae) sur le criquet pèlerin *Schistocerca gregaria* (Forskål, 1775) (Orthoptera, Acrididae). *Algerian journal of arid environment*, *8*(2), 79-98. [In French].

Kemassi, A., Boual, Z., Lebbouz, I., Daddi Bouhoun, M., Saker, M. L., Ould El Hadj-Khelil, A., & Ould El Hadj, M. D. (2012b). Étude de l'activité biologique des extraits foliaires de *Cleome arabica* (Capparidaceae). *Lebanese science journal, 13*(2), 81-97. [In French].

Kemassi, A., Boual, Z., Ould El Hadj-Khelil, A., Dadi Bouhoun, M., & Ould El Hadj, M. D. (2010). Activité biologique de l'extrait *d'Euphorbia guyoniana* (Boiss. & Reut.) (Euphorbiaceae) chez le Criquet pèlerin *Schistocerca gregaria* (Forskål, 1775) (Orthoptera-Acrididae). *Annales de sciences et technologie*, *2*(1), 60-71. [In French].

Kemassi, A., Boukhari, K., Cherif, R., Ghada, K., Bendaken, N., Bouziane, N., Boual, Z., Bouras, N., Ould Elhadj-Khelil, A., & Ould El Hadj, M. D. (2015). Evaluation de l'effet larvicide de l'extrait aqueux d'*Euphorbia guyoniana* (Boiss. & Reut.) (Euphorbiaceae). *Revue El-Wahat pour les recherches et les etudes, 8*(1), 44–61. [In French].

Kemassi, A., Bouziane, N., Boual, Z., & Ould El Hadj M. D. (2014). Activité biologique des huiles essentielles *de Peganum harmala* L. (Zygophyllaceae) et de *Cleome arabica* L. (Capparidaceae) sur *Schistocerca gregaria* (Forskål, 1775). *Journal de phytothérapie de la recherche à la pratique*, *12*, 348–353. [In French].

Kemassi, A., Herouini, A., Hadj, S. A., Cherif, R., & Ould El Hadj, M. D. (2019). Effet insecticide des extraits aqueux d'*Euphorbia guyoniana* (Euphorbiaceae) récoltée dans Oued Sebseb (Sahara Algerien) sur le *Tribolium castaneum*. *Lebanese science journal*, *20*(1), 55-70. [In French].

Kemassi, A., Ould El Hadj-Khelil, A., Boual, Z., Hamid- Oudjana, A., & Ould El Hadj, M., D. (2012a). Activités biologiques des huiles essentielles brutes foliaires de *Peganum harmala* L. (Zygophyllaceae) sur les larves du cinquième stade et sur les adultes de *Schistocerca gregaria* (Forskål, 1775) (Orthoptera- Cyrtacanthacridinae). *PhytoChem & BioSub Journal*, *6*(2), 71-77. [In French].

Lazar, P. (1968). Les essais biologiques. *Revue de statistique appliquée, 16*(3), 5-35. [In French].

Lecoq, M. (2004). Vers une solution durable au problème du Criquet pèlerin. *Sécheresse, 15*(3), 217-224. [In French].

Lecoq, M. (2005). *Enseignement de la récente invasion du Criquet pèlerin en Afrique*. Ed. CIRAD, Montpellier, 170 pp. [In French].

Lecoq, M. (2014). *Les criquets: une menace pour l'agriculture, une plaie toujours d'actualité. Note de recherche*. Ed. CIRAD. Montpelier, 97 pp. [In French].

Legal, P. (1989). Le choix des plantes nourricières et la spécialisation trophique chez les Acridoidea (Orthoptéres). Bulletin d'écologie, *20*(3), 245-261. [In French].

Nicole, M. C. (2002). Les relations des insectes phytophages avec leurs plantes hôtes. *Antennae*, *9*(1), 1-7. [In French].

Ould El Hadj, M. D., Tankari-Danbadjo, A., Halouane, F., & Doumandji, S. (2006). Toxicité comparée des extraits de trois plantes acridifuges sur les larves du cinquième stade et sur les adultes de *Schistocerca gregaria* (Forskål, 1775) (Orthoptera-Cyrtacanthacridinae). *Sécheresse, 17*(3), 407-414. [In French].

Ozenda, P. (1991). *Flore et végétation du Sahara. Ed. CNRS, (3ème édition augmentée)*, Paris, P 662 pp. [In French].

Proux, J. (1991). La régulation hormonale du métabolisme hydrique chez les criquets grégarisables. Ed. *AUPELF-UREF, John Libbcy Eurotext*, Paris, 129-134. [In French].

Proux, J., Alaoui, A., Moreteau, B., & Baskali, A. (1993). Deltamithrine induced deregulation of the water balance in the migratory locust. *Comparative. Biochemistry and physiology*, *106*(C), 351-357.

Ramade, F. (2007). Introduction à l'écotoxicologie: Fondements et applications. *Ed. Lavoisier Tec & Doc*. 618 pp. [In French].

Reynaud-Maurupt, C. (2006). Usages contemporains de plantes et champignons Hallucinogènes. Une enquête qualitative exploratoire conduite en France. *Ed. Observatoire français des drogues et des toxicomanies (OFDT)*, 106 pp. [In French].

Roffey, J. (1982). The desert locust upsurge and its termination 1977 – 79 In: Field Research stations. Technical series. FAO Report no AGP/DL/TS/23.111– 1V, 1-74.

Symmons, P. M., & Cressman K. (2001). Desert locust guidelines 1: *Biology and behaviour. Rome, FAOUN* (2e éditione), 43 pp.

Walbauer, G. P. (1968). The utilization and consumption of food by insects. *Journal of insect physiology*, *5*, 229-288.

УТИЦАЈ УЉА СЕМЕНА ДВЕЈУ АУТОХТОНИХ БИЉАКА ИЗ АЛЖИРСКЕ САХАРЕ НА ЛАРВЕ ПУСТИЊСКОГ СКАКАВЦА

Ахмед Аит Аудиа, Абделах Кемаси, Амел Херуини, Закариа Тебауи, Рима Деруише, Навал Бузиане и Диди Мохамед Улд Ел Хадј

Извод

Истражени су летални и сублетални ефекти уља семена двеју аутохтоних биљака из алжирске Сахаре, *Peganum harmala* L. (Zygophyllaceae) и *Datura stramonium* L. (Solanaceae). Примена 60 μл уља принудном оралном ињекцијом помоћу микропипете на ларве Л5 *Schistocerca gregaria* Forsk. (Orthoptera: Acrididae) открили су одвраћајући ефекат ових уља на третиране ларве. Третман је резултирао различитим токсиколошким симптомима, као што су интензивна дефекација, пролив, губитак тежине, смањење моторичке активности, кашњење и потешкоће у пресвлачењу и, у најекстремнијим случајевима, смрт третираних јединки. Током третмана ларви Л5 *S. gregaria* уљем семена *P. harmala*, појавили су се различити токсиколошки симптоми: 81,81% јединки са дијарејом; 68,18% јединки са губитком тежине; 72,72% је показало смањену моторичку активност, а 100% преживелих јединки је каснило са пресвлачењем. С друге стране, код ларви Л5 третираних уљем семена *D. stramonium*, 77,27% јединки је имало дијареју, 100% је показало губитак тежине и 100% јединки је имало смањену моторну активност. Показало се да је уље семена *D. stramonium* токсичније од уља семена *P. harmala*. Орална примена 60 μл уља семена *D. stramonium* изазвала је блокирање феномена егзувијације у 100% третираних ларви Л5, што је довело до смрти након 16 дана. Док су уља семена *P. harmala* проузроковала 50% смртности након 12 дана, 50% преживелих јединки је успело да заврши своје пресвлачење у адулте са потешкоћама, што је резултирало малформацијама. Процењено смртоносно време 50 (ЛТ50) код ларви (Л5) третираних уљем семена *D. stramonium* било је 3,67 дана. Био је токсичнији од ЛТ50 у ларвама (Л5) третираним уљем семена *P. harmala*, што је трајало 12 дана.
Унос хране код ларви Л5 *S. gregaria* третираних уљем семена *D. stramonium* износио је 0,28 ± 0,18 г/дан, што је било мање од просечне дневне потрошње забележене код ларви Л5 третираних уљем семена *P. harmala*, која је била 0,67 ± 0,36 г/дан, уље семена *D. stramonium* је отровније и веома утиче на унос хране. Вредности привидног коефицијента искоришћења дигестије (DUC) забележене за ларве Л5 третиране уљем семена *P. harmala* и *D. stramonium* биле су 39,32 ± 13,07% и 34,23 ± 29,07%, респективно. Ове вредности су биле значајно ниже у односу на вредност контролне групе која је износила 70,63 ± 19,56%. Слично, дигестивни коефицијенти конверзије (CCD) забележени у Л5 *S. gregaria* третираних семенским уљима *P. harmala* и *D. stramonium* били су -75,07 ± 54,45% и -3,08 ± 1,18, респективно. Међутим, у контролној групи ларви Л5 забележена CCD је била 1.004 ± 0.073. Вредности индекса потрошње (CI) за ларве Л5 третиране уљима из семена *P. harmala* и *D. stramonium* биле су ниске, 6,74 ± 4,45 и 3,82 ± 2,45, респективно, док је за ларве Л5 контролне групе то било 15,74 ± 3,51.

Received: November 3rd, 2020
Accepted: August 25th, 2021