Antifeedant and Antigonadotropic Effects of *Ruta chalepensis* Methanolic Extract against *Locusta migratoria*

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ABSTRACT

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The migratory locust *Locusta migratoria* is one of the most important pests due to its extensive and serious damage to crops in large parts of Africa and Asia. To identify novel new environment friendly products for the management of the migratory locust, experiments were conducted to assess the effect of a methanolic extract of *Ruta chalepensis* (*ME-Rc*) on feeding activity and different reproductive phases of *L. migratoria*. The results showed that *ME-Rc* caused a significant decline in food intake and insect digestibility. The treatment applied to adult females caused a significant lengthening of the preoviposition period and a significant reduction in both fecundity and fertility. *ME-Rc* also affected growth and development of oocytes as evidenced by measurements of ovarian weight, length and volume of terminal oocytes and ovarian index. In addition, *ME-Rc* based-treatments led to disturbances in the incorporation of haemolymph metabolites (proteins and carbohydrates) in oocytes resulting in a significant decrease in their concentrations in ovaries.

Keywords: Antifeedant, *Locusta migratoria*, ovarian metabolites, reproduction, *Ruta chalepensis*

Locusts are characterized by the pronounced ability to exhibit a continuum of forms between the extreme solitarious and gregarious phases (32). In the gregarious phase, migratory locusts cause substantial damage to crops and grazing and are responsible for enormous losses in agriculture in many regions of the world (1, 10). Therefore, it is necessary to search and develop some effective control strategies for suppressing population

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density aiming to prevent the outbreak of mobile swarms. Control has traditionally relied on conventional insecticides. Three representative insecticides, including deltamethrin (pyrethroid), carbaryl (carbamate) and chlorpyrifos (organophosphate) have been used in locust control (35). However, chemical control of grasshoppers and locusts has been associated with dangers like environmental pollution and adverse effects on non target organisms (26). In addition, frequent applications of insecticides have led to the occurrence of resistance in some field locust populations (17, 23, 34). Therefore, searching for new anti-locust substances allowing more effective control and reducing risks for operators, inhabitants, livestock and environment is increasingly needed. Many scientists believe that alternatives to conventional pesticides can be developed using plant-derived active ingredients (2, 4, 15).

Botanicals are an important source of insecticides. Nicotine from *Nicotiana tabacum*, piretrins from *Tanacetum cinerariifolium* and rotenone from *Derris* and *Lonchocarpus* were recognized as effective agents in controlling insect pests. Recently, there has been a growing interest in researching for possible use of plant extracts as alternatives to synthetic insecticides. Allelochemicals produced by secondary metabolism of plants have been considered as potential sources of natural compounds for insect control (24). Research in this area has led to the isolation of new compounds that can affect the general metabolism of target insects including development, behavior and reproduction (20). The botanical derived insecticides are generally pest-specific and are relatively harmless to non-target organisms such as pollinators and natural enemies of locusts. They are also biodegradable and with no adverse effects on environment (19). Therefore, molecules of botanical origin may offer a safe source of compounds for pest management, being environmentally friendly and an excellent alternative to persistent synthetic insecticides.

Among the plant families, naturally occurring within the Tunisia flora, the Rutaceae, *Ruta chalepensis* has attracted a lot of attention due to the wide range of biological activities exhibited by their secondary metabolites. In an effort to identify both environmentally acceptable and effective locust-control products, we assessed in the present study the effects of *R. chalepensis* methanolic extract on *L. migratoria*. For this purpose, we studied (i) the feeding activity, (ii) the reproductive potential (fecundity and fertility); (iii) the morphometric parameters of ovaries; and (iv) the ovaries biochemical composition.

MATERIALS AND METHODS

Insect rearing.

Insects used for testing came from a gregarious stock, which had been reared in breeding cages measuring 50 cm^3 and containing a few hundred specimens. The temperature was kept at 30 ± 1°C and a light/dark cycle of 12/12 h was used. *L. migratoria* were fed on fresh sorghum leaves supplemented with wheat bran. The substratum used for oviposition was composed of 2/3 peat and 1/3 sand.

Plant material and treatments.

R. chalepensis was collected from Kairouan region (Center of Tunisia) during spring seasons (March-April 2013). Plant extract was prepared based on the procedures described by Gökçe et al. (16). Ten gram samples of dried plants were placed into 100 ml Erlenmeyer flask and supplemented with 100 ml of methanol. Flask was covered with aluminum foil and subjected to

continuous shaking at 100 rpm during 24 h in the dark at 25°C. The obtained suspension was filtered and the obtained extract was transferred into a 250 ml evaporating flask and dried at 40°C in a rotary evaporator in order to evaporate the excess of methanol. The resulting dry residues were weighed and mixed with ethanol to yield a 20% (w/w) plant organic extract.

Crude methanolic extract from *R. chalepensis* (*ME-Rc*) was sprayed on sorghum leaves which were subsequently offered as a mono-specific diet for larvae and adults of the migratory locust. The effects of *ME-Rc* on *L. migratoria* feeding activity and reproduction were investigated by exposing the young (0-1 day-old) larvae and adults to three concentrations 0.5, 1 and 2%, denoted respectively as *ME-Rc-*C_1, *ME-Rc-*C_2 and *ME-Rc-*C_3. Control locusts were fed on untreated sorghum leaves.

Feeding activity.

Antifeedant activity was assayed by exposing freshly emerged 5^{th} instar larvae to fresh sorghum leaves treated with *ME-Rc* in no-choice format. Recently molted nymphs were starved for 8 h to ensure that their guts were empty, weighed and randomly assigned to feed on one of tested treatments. The bioassays were conducted in individual 2 liter plastic boxes containing food and placed under the same conditions described above for mass rearing.

Cut leaves were weighed and placed in experimental boxes. After 24 h, larvae were fed on new sample of freshly cut leaves removed from uncut plants reared in the same chamber and used to feed them originally. Some sorghum leaves of the same weights were kept in a similar container under the same conditions to estimate natural loss of moisture for correcting the weight of consumed leaves. Fecal pellets and uneaten food were collected daily and weighed.

Reproductive potential and ovarian development.

Treated males and females (15 couples per each concentration) were immediately paired in individual 2 liter plastic boxes containing food and placed under the same conditions described above for mass rearing. The time of first oviposition (TFOp) and the number of eggs per ootheca (NE/Ot) were recorded for each pair. Fecundity (number of eggs deposited by the female during its lifespan) and fertility rate (number of hatched eggs per ootheca/number of deposited eggs per ootheca × 100) were also noted in control and treated insects. The corrected sterility rate was calculated by applying the following formula (21):

(% fertility in control - % fertility in treatment)/(% fertility in control) ×100.

To follow the effects of *ME-Rc* on ovarian morphometric parameters, periodic dissections (at 2, 4, 8 and 12 days of imaginal life) were carried out in a physiological liquid (Ringer's solution). Five control and treated females were weighted and dissected. For each dissected female, the ovarian fresh weight, the length and volume of terminal oocytes (five oocytes per female) were measured. The volume was calculated using the formula of Lambreas et al. (22): $V= 4\pi/3\ (L/2)\ (l/2)^2$, where V: volume ($mm^3$), L: length (mm), and l: width (mm). An ovarian index was also determined in order to compare oocyte growth in ovaries of treated and control females. This index was expressed as the ratio between the length of oocytes in treated and control individuals (11).

Quantification of ovarian metabolites.

Proteins and carbohydrates were extracted from the same ovary sample following the procedure of Shibko et al. (31). At appropriate times (2, 4, 6, 8 and 12 days after emergence), females (n = 5) were sampled from control and treated individuals and their ovaries were removed and weighed. Each sample of gonad was individually homogenized in 1 ml of trichloroacetic acid (20%) and then centrifuged (5,000 g for 10 min). The supernatant was used for determination of carbohydrates as described by Duchateau and Florkin (13) using anthrone as reagent and glucose as standard. Protein concentration was determined using the Bradford (9) assay with Coomassie Brilliant Blue (G 250, Merck) as reagent and bovine serum albumin (Sigma) as standard.

Statistical analysis.

The assimilation of ingested food (digestibility) was evaluated by means of bicoordinate plots (utilization plots) associated with analyses of covariance developed by Raubenheimer and Simpson (28). These authors suggested that the use of ANCOVA is more appropriate than use of ratio variables for analysis of nutritional indices. Thus, a one-way ANCOVA was performed using frass produced as the dependent variable and food intake as the covariate. Reproductive potential and ovarian metabolites data were assessed by ANOVA procedure (at $P < 0.05$) followed by Student-Newman-Keuls (SNK) test for means' separations. All analyses were performed using SPSS (Version 15.0).

RESULTS

Feeding activity.

The effect of feeding the 5th instar larvae of *L. migratoria* on sorghum leaves sprayed with *ME-Rc* is presented in Table 1. The results show that individuals fed on treated leaves exhibited a decline in food intake, compared to untreated control ones, which varied in a dose-dependent manner. *ME-Rc* functioned as antifeedant by reducing significantly the amount of food ingested by *L. migratoria* nymphs which decreased by 39.09% using the highest (2%) *ME-Rc* tested concentration (Table 1). The analysis of variance with tested concentrations as classification criteria showed significant differences among tested treatments as expressed by the heterogeneous groups obtained using SNK test (Table 1).

Not only the larvae treated with *ME-Rc* showed a decline in their food intake but the larvae exhibited also a decreased digestibility after treatment. In fact, larvae fed *ME-Rc*-treated sorghum leaves were less able to digest and absorb nutrients from the provided food. This was tested by utilization plots (Fig. 1) and analysis of covariance, with food intake as the covariate for faecal production (Table 2).

A highly significant effect of the covariate was observed. This showed that mass of frass depended on the amount of food ingested by larvae. Results given in Table 2 also show that food intake and faecal production varied depending on *ME-Rc* concentrations. Indeed, the lowest amount of faeces was noted in the control group although the highest weight of ingested food was noted with this treatment indicating that tested insects were able to digest untreated food more efficiently than that treated with *ME-Rc*.

Table 1. Effect of *ME-Rc* on the mean fresh weight of consumed sorghum leaves by the 5th instar larvae of *Locusta migratoria* (mean ± SD)

Treatment	Mean food intake (g fresh weight)
Control	1.97 ± 0.18 a (1.874- 2.067)*
ME-Rc-C₁	1.58 ± 0.15 b (1.486- 1.679)
ME-Rc-C₂	1.53 ± 0.17 b (1.437- 1.631)
ME-Rc-C₃	1.2 ± 0.1 c (1.110- 1.304)
F-value	42.992
***P*-value**	< 0.001

Means followed by different letters are significantly different according to SNK test at $P < 0.05$.
* 95% lower and upper confidence limits are shown in parenthesis.
ME-Rc: Methanolic extract of *Ruta chalepensis*.

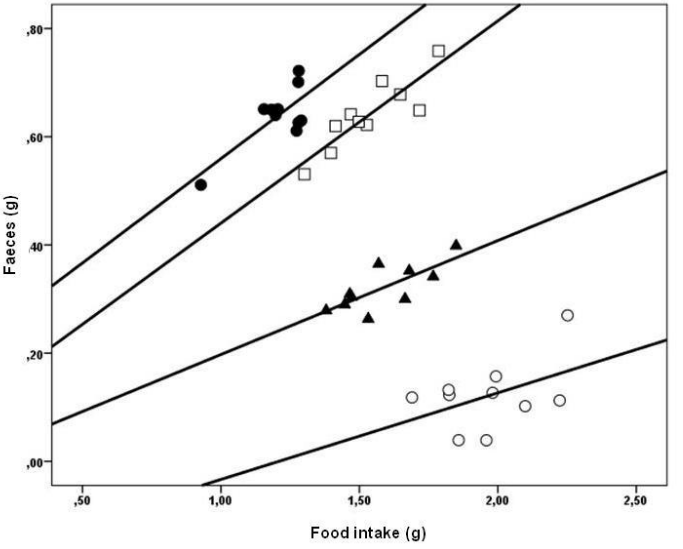

Fig. 1. Relationship between food intake and production of faeces of *Locusta migratoria* during the 5th instar larvae after treatment with different *ME-Rc* concentrations.
— o —: *Control*, —▲—: *ME-Rc-C₁*, —□—: *ME-Rc-C₂*, — ● — *ME-Rc-C₃*:
C₁: 0.5%, *C₂*: 1%, *C₃*: 2%.

Table 2. Table of the analysis of covariance for faeces production adjusted by food intake of *Locusta migratoria*, taken during the 5th instar larvae after treatment with different *ME-Rc* concentrations

Source of Variation	df	Sum of Squares	Means of Squares	F	Pr > F
Food intake	1	0.054	0.054	28.72	< 0.001
***ME-Rc*-Concentrations**	3	0.075	0.025	189.77	< 0.001
Error	35	0.066	0.002		

Reproductive potential.

Data presented in Table 3 shows that *ME-Rc* treatment had significantly increased ($P < 0.05$) preoviposition duration and decreased ($P < 0.05$) the number of eggs per ootheca. This adverse influence of *ME-Rc*-treated food on the reproductive potential of *L. migratoria* was also demonstrated by the significant reduction both in fecundity and fertility. The highest decrease was observed using the highest concentration tested (*ME-Rc-C_3*). Indeed, fecundity and fertility were lowered by 21.4 ± 4.96% and 40.09 ± 9.12%, respectively as compared to the untreated control insects. The corrected sterility rate reached 39.03 ± 5.77% and 45.78 ± 7.9%, respectively, for treatments based on *ME-Rc-C_2* and *ME-Rc-C_3* (Table 3).

Table 3. Effect of *ME-Rc* on reproductive potential parameters of *Locusta migratoria* (mean ± SD)

Treatment	TFOp (in days)	NE/Ot	Fecundity (%)	Fertility (%)	CS (%)
Control	12.33 ± 2.51 a	46 ± 6.92 a	100	73.32 ± 6.75 a	-
ME-Rc-C_1	23 ± 2.64 b	28.3 ± 7.63 b	27.66 ± 2.19	57.14 ± 2.04 b	21.76 ± 5.2
ME-Rc-C_2	24.33 ± 4.16 b	26.6 ± 4.5 b	27.61 ± 10.51	44.48 ± 2.51 c	39.03 ± 5.77
ME-Rc-C_3	22 ± 1.73 b	22.66 ±5.68 b	21.4 ± 4.96	40.09 ± 9.12 c	45.78 ± 7.9

For each parameter, means followed by different letters are significantly different according to SNK test at $P < 0.05$.

*TFOp: Time of first oviposition; NE/Ot: Number of eggs per ootheca; CS: Corrected sterility.

Weight of adult females.

Fig. 2 shows the fresh body weight of *L. migratoria* adult females in presence of various concentrations of *ME-Rc* in their diet. Results revealed that the fresh body weight of *ME-Rc*-treated females was significantly reduced ($P < 0.05$) compared to that of control ones. The analysis of variance considering concentrations as classification criteria showed significant differences among treatments from the 8th day where SNK test allowed their distribution within heterogeneous groups as illustrated in Fig. 2. Treatment of newly emerged females resulted in a 40.1% decrease in their fresh weight with the highest concentration of *ME-Rc* applied as compared to untreated control insects observed 12 days after their emergence. Indeed, the average weight recorded in 12 day-old females treated with the highest concentration was 1.49 ± 0.27 g whereas that measured in control females of the same age was 2.49 ± 0.06 g (Fig. 2).

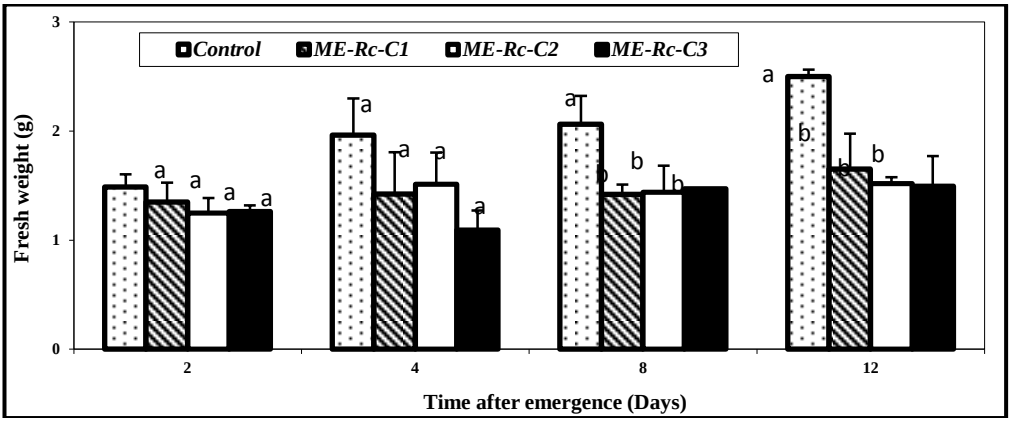

Fig. 2. Effect of *ME-Rc* on fresh weight of adult females noted at various times after their emergence (mean ± SD). For each date, means followed by different letters are significantly different according to SNK test at $P < 0.05$. *ME-Rc-C1*: 0.5%, *ME-Rc-C2*: 1%, *ME-Rc-C3*: 2%.

Morphometric parameters of ovaries.

In control females, the weight of ovaries increased during sexual maturation with a maximum value of 437 ± 38.5 mg noted at 12 days after emergence (Fig. 3). However, with *ME-Rc* applied on newly emerged adults, the ovary weight was significantly ($P < 0.05$) lowered with all tested concentrations compared to controls of the same age. Results also indicated that the fresh body weight did not differ significantly ($P > 0.05$) between control and treated females during the previtellogenesis phase (2 and 4 days after emergence), a period during which oocytes are still immature and terminal oocytes are translucent (Fig. 3).

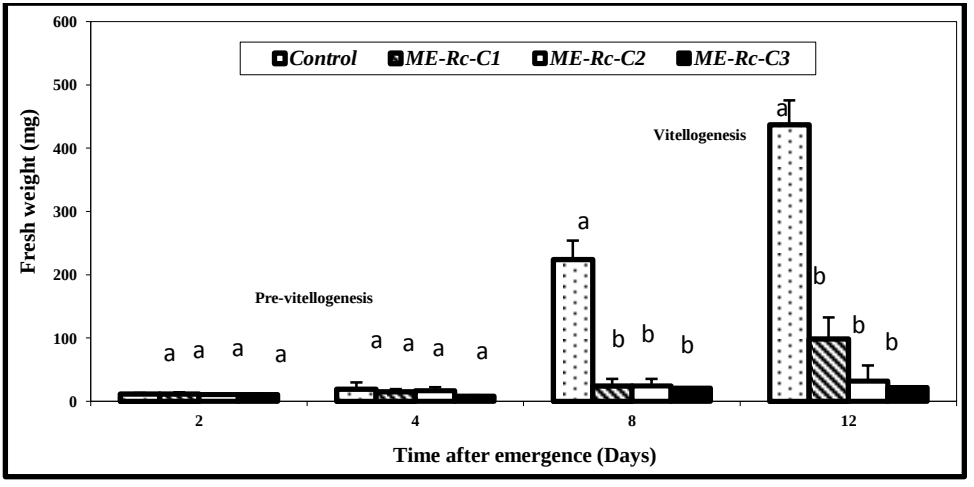

Fig. 3. Effect of *ME-Rc* on fresh weight of ovaries noted at various times after emergence (mean ± SD). For each date, means followed by different letters are significantly different according to SNK test at $P < 0.05$. *ME-Rc-C1*: 0.5%, *ME-Rc-C2*: 1%, *ME-Rc-C3*: 2%.

A significant difference was also recorded in the size of terminal oocytes between control and treated females during the first gonadotrophic cycle (Table 4). An important rise in the terminal oocyte length was observed in control locusts between the 2nd and 12th day after emergence, when it increased from 0.3 ± 0.06 to 6.56 ± 0.31 mm. However, oocyte growth was much slower and more gradual in *ME-Rc*-treated insects, in which terminal oocyte length reached only 1.88 ± 0.71 mm on day 12 using the highest concentration (Table 4). The volume of basal oocytes was also increased during sexual maturation in control individuals reaching 6.2 ± 1.08 mm^3. *ME-Rc* based treatment led to a significant decrease ($P < 0.05$) in the volume of basal oocytes in a dose-dependent manner from the 4th day after emergence.

Morphometric measurements also involved the ovarian index established in order to compare the development of the ovaries in control and treated insects. Results given in Table 4 indicate that treatment of newly emerged females with *ME-Rc* had significantly ($P < 0.05$) affected the growth of the terminal oocytes resulting in a low ovarian index (< 1). ANOVA revealed that the significant differences among treatments occurred from the 4th day of imaginal life. Indeed, during the first two days, the ovaries were still at the juvenile stage and there was no accumulation of reserve in oocytes.

Table 4. Morphometric measurements of ovaries in control and *ME-Rc*-treated *Locusta migratoria* females noted at various times after emergence (mean ± SD)

Parameter	Treatment	2 DAE	4 DAE	8 DAE	12 DAE
Length of terminal oocyte (mm)	Control	0.3 ± 0.06 a	0.97 ± 0.06 a	5.65 ± 0.22 a	6.56 ± 0.31 a
	ME-Rc-C1	0.37 ± 0.11 a	0.55 ± 0.12 b	2.29 ± 0.07 b	2.99 ± 0.1 b
	ME-Rc-C2	0.28 ± 0.03 a	0.52 ± 0.16 b	2.29 ± 0.07 b	2.35 ± 0.3 bc
	ME-Rc-C3	0.56 ± 0.5 a	0.59 ± 0.21 b	1.12 ± 0.36 c	1.88 ± 0.71 c
Volume of terminal oocyte (mm^3)	Control	0.04 ± 0.06 a	0.36 ± 0.06 a	3.04 ± 0.22 a	6.2 ± 1.08 a
	ME-Rc-C1	0.04 ± 0.01 a	0.14 ± 0.01 b	1.12 ± 0.02 b	2.07 ± 0.5 b
	ME-Rc-C2	0.03 ± 0.02 a	0.11 ± 0.01 b	0.78 ± 0.2 bc	1.5 ± 0.48 b
	ME-Rc-C3	0.04 ± 0.01 a	0.11 ± 0.05 b	0.42 ± 0.26 c	1.24 ± 0.71 b
Ovarian index	*ME-Rc-C1*	1.2 ± 0.18 a	0.56 ± 0.08 a	0.4 ± 0.01 a	0.45 ± 0.02 a
	ME-Rc-C2	0.95 ± 0.07 a	0.53 ± 0.14 a	0.4 ± 0.01 a	0.35 ± 0.03 ab
	ME-Rc-C3	1.93 ± 1.8 a	0.6 ± 0.17 a	0.19 ± 0.06 b	0.28 ± 0.1 b

For each date, means followed by different letters are significantly different according to SNK test at $P < 0.05$. DAE: Days after emergence.

Biochemical composition of ovaries.

Table 5 illustrates changes occurring in ovarian metabolites concentrations in *L. migratoria* adult females upon treatment with *ME-Rc*. Analysis of the data revealed that treatment with *ME-Rc* caused a significant ($P < 0.05$) decrease in protein amounts in ovaries from the 8th day of imaginal life. Indeed, the quantity of ovarian proteins recorded in female 8 day-old and treated with the highest concentration tested was 0.58 ± 0.22 mg/ovaries. However, in control females of the same age the level of these proteins was estimated at 6.62 ± 1.05 mg/ovaries (Table 5). Moreover, during the previtellogenesis phase, no significant difference was noticed ($P > 0.05$) in the amount of proteins between control and treated females. Results revealed that total carbohydrates of ovaries sampled

from *ME-Rc*-treated females decreased significantly ($P < 0.05$) comparatively to the untreated control insects. Indeed, the analysis of variance considering concentrations as classification criteria showed a significant difference among treatments from the 4th day as indicated by the heterogeneous groups generated using SNK test (Table 5). The amount of carbohydrates determined in the ovaries dissected 12 days after emergence on *ME-Rc*-treated females was 0.22 ± 0.09 mg/ovaries with the highest concentration used. At the same age, the total carbohydrates noted in the control group were estimated at 1.74 ± 0.16 mg/ovaries (Table 5).

Table 5. Ovarian metabolites amounts (mg/ovaries, mean ± SD) in control and *ME-Rc*-treated *Locusta migratoria* females noted at various times after emergence.

Parameter	Treatment	2 DAE	4 DAE	8 DAE	12 DAE
Proteins	Control	0.79 ± 0.25 a	1.15 ± 0.45 a	6.62 ± 1.05 a	9.39 ± 1.83 a
	ME-Rc-C1	0.54 ± 0.2 a	0.57 ± 0.13 a	0.65 ± 0.1 b	0.91 ± 0.14 b
	ME-Rc-C2	0.54 ± 0.27 a	0.58 ± 0.16 a	0.72 ± 0.21 b	1.01 ± 0.24 b
	ME-Rc-C3	0.52 ± 0.14 a	0.54 ± 0.1 a	0.58 ± 0.22 b	0.72 ± 0.16 b
Carbohydrates	Control	0.28 ± 0.11 a	0.59 ± 0.06 a	0.41 ± 0.17 a	1.74 ± 0.16 a
	ME-Rc-C1	0.27 ± 0.11 a	0.21 ± 0.03 b	0.26 ± 0.06 ab	0.29 ± 0.06 b
	ME-Rc-C2	0.09 ± 0.03 a	0.17 ± 0.02 b	0.17 ± 0.08 ab	0.20 ± 0.1 b
	ME-Rc-C3	0.12 ± 0.02 a	0.15 ± 0.01 b	0.08 ± 0.02 b	0.22 ± 0.09 b

For each date, means followed by different letters are significantly different according to SNK test at $P < 0.05$. DAE: Days after emergence.

DISCUSSION

Research in recent years has been turning more towards new environment-friendly alternatives for pest control. Among these, botanical derived insecticides have attracted the greatest attention and have been explored extensively (2). Insect reproduction and its hormonal regulation represent potential targets for the development of biorational insecticides. In this perspective, the discovery of molecules able to interfere with the development and reproduction physiology of phytophagous insects has been the focus of several investigations in recent years (12).

To find natural insecticide, we examine in the present study the insecticidal activities of *R. chalepensis* extract on the feeding activity and the reproduction physiology of *L. migratoria*. The results showed that the methanolic extract of *R. chalepensis* leaves affected *L. migratoria* reproduction by significantly reducing its fecundity and fertility thereby inducing partial sterility in females. *ME-Rc* based treatments also caused disturbances in ovarian development and in the incorporation of the haemolymph metabolites (proteins and carbohydrates) in the oocyte resulting in a significant reduction in their concentrations in the ovaries. Antifeedant effect was also observed after treatment with *ME-Rc*.

Previous studies documented the antifeedant properties of *R. chalepensis* against different orders of insects. Indeed, Emam et al. (14) denoted larvicidal and antifeedant activities of the aqueous ethanolic extract of *R. chalepensis* leaves against *Spodoptera littoralis* larvae with LC_{50} of 0.89 mg/ml. The authors found

that two alkaloid compounds namely furocoumarin and quinolone were in part responsible for the insecticidal activity displayed by the aqueous ethanolic extract of *R. chalepensis* and they concluded that these two compounds could be used to control *S. littoralis* larvae. Similarly, Mancebo et al. (25) denoted that substances present in the common rue *R. chalepensis* extract exhibited antifeedant activity against *Hypsipyla grandella* 3th instar larvae. Antifeedant effect induced by *R. chalepensis* extracts was also demonstrated for the Colorado potato beetle, *Leptinotarsa decemlineata* 4th instar larvae and adults (18). In other studies, Richter et al. (29) showed that ethanolic extracts of *R. chalepensis* leaves affect molting duration in the last nymphal instar of the American cockroach *Periplaneta americana*. However, Mancebo et al. (25) demonstrated that the common rue extract did not affect the development of immature stages and the pupal weight of *H. grandella*. Also, Barbosa et al. (8) tested aqueous leaf extracts of *R. graveolens* on pests of commercial tomato crops at the concentration of 5% under field conditions and recorded a decrease in the number of *Bemisia tabaci* adults, mines and adults of *Tuta absoluta* and sum of pests (sum of total pests found) in the treated plots compared to the control ones. Yael et al. (33) reported that both ether and methanol extracts of *R. chalepensis* display remarkable larvicidal activities and could be considered as potential source of bio-insecticides. Indeed, these authors found that ether extracts of *R. chalepensis* displayed larvicidal activity against *Aedes aegypti* which varied in a dose-dependent manner where LC_{50} was estimated at 6.4, 2.2, and 1.8 µg/ml for 12, 18 and 24 h exposure times, respectively.

The antifeedant properties displayed by *R. chalepensis* extracts against *L. migratoria* seem to be related to alkaloids detected in plant foliage (14). In general, alkaloids and their glycosides seem to be most often involved as feeding repellents or deterrents. Alkaloids extract from the aerial part of *Pergularia tomentosa* (Asclepiadaceae) caused significant antifeedant and growth inhibitory effects against the 5th instar larvae of *L. migratoria* (5). In the same way, Bagari et al. (7) noted that alkaloids from *Nerium oleander* (Apocynaceae) interfere with the metabolic activities of *Schistocerca gregaria* and reduce the digestibility. Similarly, Sandoval-Mojica and Capinera (30) showed that alkaloids, the active ingredient in sabadilla, induced an antifeedant effect toward *S. americana*. These authors suggested that alkaloids seem to be perceived in the American grasshopper by the stimulation of a specialized deterrent receptor. Electrophysiological studies have shown that contact chemoreceptors on *S. americana* tibia and tarsus are stimulated by alkaloids, and have demonstrated an association between the neuron activity and the antifeedant response.

The results of the present study also showed that *ME-Rc* had significantly decreased the reproductive potential and metabolite concentrations in insect ovaries. There are several reports in the literature demonstrating the influence of some plant extracts on the reproduction of *L. migratoria*. In fact, research conducted by Acheuk et al. (4) showed that methanolic extract of *Haplophyllum tuberculatum*, administered orally to newly emerged females at 1500 µg/female, had significantly delayed the first oviposition and reduced fecundity and fertility. Additionally, it was observed that the plant extract reduced the haemolymph protein levels and

blocked their uptake by oocytes. Vitellogenesis was affected by the treatment during the first gonadotropic cycle. In fact, *H. tuberculatum* extract was shown able to affect the development and the maturation of terminal oocytes, including a precocious resorption of terminal oocytes. In the same way, Abdellaoui et al. (3) reported that gibberellic acid (GA_3), a plant growth regulator, was responsible for the extension of the pre-ovipositional phase and the egg laying rhythm, the delay in the development of ovaries and the reduced length of terminal oocyte in *L. migratoria*. The same authors showed that GA_3 caused disturbances in the incorporation of the haemolymph metabolites (proteins, carbohydrates, and lipids) in the oocyte resulting in a significant decrease in their concentrations in the ovaries.

The different reproduction potential disturbances observed in the treated females appear to be a consequence of the reduction of food intake previously demonstrated. These changes probably result in inadequate energy reserves for egg-yolk formation. It has been reported in several other studies that undernourishment or ingestion of inappropriate host plant profoundly affects the basic systems of the body, including the reproductive system of insects (6, 7). Thus, the adverse effect of *ME-Rc* expressed by the significant decrease in food consumption can limit the activity of the fat body leading to lowered synthesis of vitellogenin and consequently, the inhibition of vitellogenesis. Indeed, Raccaud-Schoeller (27) reported that vitellogenesis occurs at the expense of fat body reserves and a correlation has been demonstrated in various insects between reproductive cycles and the contents of fat body lipids, proteins and carbohydrates. According to our findings, *ME-Rc* showed considerable antifeedant and antigonadotropic activities and complementary studies are required to explain with more details the effect of this extract on *L. migratoria* reproduction events particularly on the endocrine system (ecdysteroids and juvenile hormone) involved in its development and reproduction.

RESUME

Abdellaoui K., Miladi M., Ben Marzouk I., Bahloul N., Acheuk F., Chaira N. et Ben Halima-Kamel M. 2016. Effets antiappétant et antigonadotrope de l'extrait méthanolique de *Ruta chalepensis* sur *Locusta migratoria.* Tunisian Journal of Plant Protection 11: 91-104.

Le criquet migrateur *Locusta migratoria* est l'un des ravageurs les plus importants en raison des dégâts considérables qu'il peut occasionner sur les cultures en Afrique et en Asie. Dans le but d'identifier de nouveaux produits plus respectueux de l'environnement pour la gestion des criquets migrateurs, des expériences ont été menées afin d'évaluer les effets de l'extrait méthanolique de *Ruta chalepensis* (*ME-Rc*) sur l'alimentation et les différentes phases de la reproduction de *L. migratoria*. Les résultats ont montré que *ME-Rc* a entraîné une baisse significative de la prise de nourriture et de la digestibilité des insectes. Le traitement des femelles a provoqué un prolongement de la période de préoviposition et une diminution significative de la fécondité et de la fertilité. *ME-Rc* a également affecté la croissance et le développement des ovocytes comme démontré à travers les mesures du poids frais des ovaires, de la longueur et du volume des ovocytes terminaux et de l'index ovarien. De plus, les traitements à base de *ME-Rc* ont également provoqué des perturbations au niveau de l'incorporation des métabolites

hémolymphatiques (protéines et carbohydrates) dans les ovocytes entraînant une réduction significative de leurs concentrations au niveau des ovaires.

Mots clés: Antiappétance, *Locusta migratoria*, métabolites ovariens, reproduction, *Ruta chalepensis*

ملخص

عبدالاوي، خميّس ومريم ميلادي وإنصاف بن مرزوق وندى بهلول وفاطمة العشّاق ونزار شعاير ومنية كامل-بن حليمة. 2016. تأثيرات المستخلص الميثانولي لنبتة *Ruta chalepensis* على السلوك الغذائي وتكاثر الجراد المهاجر *Locusta migratoria*. Tunisian Journal of Plant Protection 11: 91-104.

يعتبر الجراد المهاجر *Locusta migratoria* واحد من أهم الآفات بسبب الأضرار الكبيرة التي يمكن أن يسببها للمحاصيل في أفريقيا وآسيا. ومن أجل البحث عن مواد جديدة أكثر رفقا بالبيئة لمقاومة حشرة الجراد، أجريت تجارب لتقييم مدى تأثير المستخلص الميثانولي لنبتة *Ruta chalepensis* (*ME-Rc*) على السلوك الغذائي وتكاثر الجراد المهاجر. أثبتت النتائج المتحصل عليها بأن *ME-Rc* تسبب في تقليص شهيّة الأكل وإعاقة هضم الوجبة الغذائية لدى اليرقات. من ناحية أخرى، تسبب *ME-Rc* في إضعاف قدرة الحشرة على التكاثر بشكل واضح ويتبين ذلك من خلال تمدد فترة ما قبل الوضع وتراجع في نسبة الخصوبة. بالإضافة إلى ذلك، تسبب المستخلص الميثانولي لهذه النبتة في تراجع نمو البويضات لدى أنثى حشرة الجراد حيث تم تسجيل تراجع واضح في وزن ومؤشر نمو المبيض وفي طول وحجم البويضات النهائية. كما تسبب *ME-Rc* أيضا في اضطراب دمج المكونات الكيميائية للسائل الدموي (البروتينات والكربوهيدرات) في البويضات مما أدى إلى تراجع كبير في كمياتها في المبيض.

كلمات مفتاحية: أيض المبيض، تكاثر، مقلص شهية الأكل، *Ruta chalepensis*، *Locusta migratoria*

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