Effects of Latex from *Pergularia tomentosa* and the Aggregation Pheromone, Phenylacetonitrile, on *Locusta migratoria* Larvae

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ABSTRACT

Miladi, M., Abdellaoui, K., Regaieg, H., Omri, G., Acheuk, F., Ben Halima-Kamel, M. **2018**. **Effects of latex from *Pergularia tomentosa* and the aggregation pheromone, phenylacetonitrile, on *Locusta migratoria* larvae. Tunisian Journal of Plant Protection 13 (si): 87-98.**

Despite being a serious risk to human health and environment, chemical insecticides remain the most used for locust control. Searching for alternative control methods, effective and compatible with the environment, has become of increasing interest. Plant latex is an endogenous fluid secreted from highly specialized laticifer cells and has been suggested to act as a plant defense system. The aim of the present investigation was to study the insecticidal potentialities of *Pergularia tomentosa* latex at different concentrations, alone or in combination with the penylacetonitrile (PAN), on the 4th instar larvae of *Locusta migratoria*. The obtained results showed that the latex revealed an interesting insecticidal activity against *L. migratoria* larvae, resulting in a mortality reaching 96.49 %, 6 days after treatment. Toxicity bioassays revealed that PAN, associated with the latex, is able to accelerate and to increase the mortality rate. Pheromone-based treatment affected the health of treated insects by significantly reducing their respiratory rhythms. PAN was shown able to alter, quantitatively and qualitatively, the larval blood cells as expressed by the significant decrease in the number of the differential haemocyte counts (prohemocyte, plasmatocytes and granulocytes) and the important cell lysis.

Keywords: Latex, *Locusta migratoria*, *Pergularia tomentosa*, phenylacetonitrile, toxicity

Locust plague is one of the world's most critical plagues and remains a major constraint to food security and social stability for many rural populations living from agriculture with climatic elevated risk. The migratory locust *Locusta migratoria* is the most widespread locust species (Lomer et al. 2001). Nymphs in the gregarious phase show an aggregating behavior and move in bands to seek food, whereas adults swarm and migrate over long distances

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Accepted for publication 11 February 2018

causing significant crop and pastures damage (Magor et al. 2008). To limit damage to crops and pastures, treatment is required with large amounts of broad-spectrum chemical pesticides. Although effective in reducing the locust population and their incidences, pesticides had some potentially negative environmental effects. In addition, frequent applications of insecticides have led to the occurrence of resistance in some field locust populations (He et al. 2004; Yang et al. 2009). In order to reduce both locust damage and insecticide input, it is important to develop alternative control methods as part of an integrated pest management (IPM) program. Research in recent years has been turning more towards selective bio-rational pesticides. Among these, botanical insecticides have attracted the greatest attention and have been reviewed extensively (Abdellaoui et al. 2013; Ma et al. 2014; Rattan 2010). Botanical insecticides, known as plant secondary metabolites, are expected to be possible alternatives to the traditional synthetic insecticides and have proved to be suitable candidates that fit reasonably well in locust management programs (Acheuk et al. 2012).

Among the natural compounds produced by plants, the latex appears to directly or indirectly influence the patterns of growth and reproduction of associated phytophagous (Chavan Bhagyashri et al. 2015). Latex is the milky sap of plants secreted by the specialized plant cells called laticifers, upon tissue injury and do not have any role in the primary metabolism of plants (Agrawal and Konno 2009). It has been reported in 12500 plant species representing 22 families (Evert 2006). The latex contains a variety of chemicals and proteins, such as various terpenoids, alkaloids, rubber, and cardenolides as well as various proteins and enzymes such as proteases, chitinases, and glucosidases (Konno 2011). It may include also various toxic compounds: for example, the neurotransmitter dopamine in the Persian poppy (*Papaver bracteatum*), narcotic alkaloid morphine in the opium poppy (*Papaver somniferum*), and insecticidal compounds such as the glycosidase inhibitors 1,4-dideoxy-1,4-imino-d-arabinitol (d-AB1) and 1-deoxynojirimycin (DNJ) in mulberry (Hagel et al. 2008). In addition, cysteine protease in latex of papaya (*Carica papaya*) and wild fig (*Ficus virgatalatex*) is toxic to caterpillars of herbivorous insects (Konno et al. 2004). Asclepiadaceae (milkweeds) is a large family of about 230 genera and almost 2000 species distributed through the tropics and temperate areas of the world (Arribere et al. 1998). The family is renowned for cardenolides-containing plants, such as *Asclepias, Pergularia, Gomphocarpus* and *Calotropis* (Nenaah 2013) which usually develop secretory tissues (laticifers) that produce white corrosive latex (Goyder 2006).

Pergularia tomentosa, is a perennial herb which extends in the Saharian and Sub-Saharian countries of North Africa (Babaamer et al. 2012). The plants are 25 cm tall, usually with many leaves and branches having pale green-white stems that are mostly ascending. Nutritional composition of this plant species and its antifungal, molluscicidal and insecticidal activities were recently reported. According to numerous reports, *P. tomentosa* leaves are a rich source of flavonoids and cardenolides and can be a good source of natural antioxidant, antibacterial, cytotoxic, and allelopathic compounds (Cherif et al. 2016; Hosseini Kahnouj et al. 2017; Lahmar et al. 2017). Despite *P. tomentosa* has several traditional uses and various biological effects, very little information is available

concerning their effects on the arthropod fauna and only few studies refer to the insecticidal activities of this plant (Acheuk and Doumandji-Mitiche 2013; Paul et al. 2011).

In the same way of research, Hassanali et al. (2005) discovered that Phenylacetonitrile (PAN), a pheromone produced by the adult male of the desert locust, significantly affected the behavior of *Schistocerca gregaria* larvae and increased their sensitivity to insecticides. Bal and Sidati (2013) have demonstrated that the addition of small amounts of PAN allows to half the quantities of insecticides used in the control of the locust larvae, while maintaining the same efficiency. The present investigation is an attempt to explore the effects of the latex of *P. tomentosa* and the aggregation pheromone, PAN, against fourth instar larvae of *L. migratoria*. For this purpose, we studied (i) the latex activities against *L. migratoria* larvae at various concentrations, and (ii) the effect of latex and PAN in binary combination on some physiological parameters of treated larvae (respiratory activity, and immune system).

MATERIALS AND METHODS

Insects.

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 as previously described (Abdellaoui et al. 2013). 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 latex extraction.

The latex of *P. tomentosa* was collected during spring seasons (March-April 2017) from the region of Hergla (N: 36.1°, E: 10.3°), Tunisia. The crude latex was collected from the healthy aerial parts of *P. tomentosa* by cutting the petiole of youngest leaves, and left to flow into sterile Eppendorf tubes. The extract was gently agitated during collection to overcome the tendency of the coagulation-like effect of the materials. After being collected, lattice was immediately brought to the laboratory and stored at + 4°C until use in the experiments.

The aggregation pheromone, phenyl-acetonitrile.

Phenylacetonitrile or benzyl cyanide is an aromatic organic compound of formula $C_6H_5CH_2CN$. The PAN (~ 98%, Sigma) is the main component (~ 80%) of the pheromone issued by the gregarious male adults of desert locust (Amwayi et al. 2012).

Treatments.

The bioassay for insecticidal activity of fresh latex (FL) of *P. tomentosa* against *L. migratoria* was conducted on newly ecdysed (< 6 h post molting) 4^{th} instar larvae using four concentrations 1, 5, 50, and 100% (v/v) prepared in distilled water and denoted, respectively as FLC1, FLC2, FLC3, and FLC4. The FL was sprayed directly on *Sorghum vulgare* leaves which were subsequently offered as a mono-specific diet for larvae of the migratory locust. In the control experiment, the insects received the same quantity of distilled water used as solvent for preparing the different concentrations. Control and experimental larvae (n = 60 for each concentration) were placed in separate cages (30 cm^3) under the same conditions described above for the mass rearing. The mortality was assessed daily via direct observation for a period of 6 days

(according to the duration of the 4th instar larvae under the mass rearing condition), and when no antennal movements were observed, the insects were considered dead. Insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls (Abbott 1925). Probit analysis (Finney 1971) was conducted to estimate the LC_{50} and LC_{90} values with their 95% confidence limits.

Other experiments were conducted to assess the effect of the FL and PAN in binary combination. The larvae were firstly exposed to PAN for 6 h and then treated by the FL at the lowest concentrations used (FLC1 and FLC2) as described previously. PAN has been associated at the concentration of 2% prepared in paraffin oil. PAN solution was applied onto Whatman No.1 filter paper disks (2 cm in diameter) which were then introduced into 2-liter plastic boxes containing the larvae. Controls were exposed with solvent alone and each treatment was replicated three times.

Differential haemocyte counts (DHCs).

This trial was conducted to investigate the effect of the latex and PAN on the immune system of *L. migratoria* larvae. Four treatments were applied: untreated larvae, larvae exposed to PAN (2%) for 6 h, larvae treated topically with the FLC1 and FLC2, and larvae exposed to the PAN for 6 h then treated with the FL. About 72 h after treatment, larvae (n = 5 for each concentration) were sampled and bled with a sterile needle on the first hind leg. A 5 µl-sample of hemolymph from each treated and control larvae were collected with a calibrated microcapillary tube and the haemolymph from two individuals was never mixed. Differential hemocyte counts (DHCs) were evaluated according to Guzo and Stoltz (1987). The obtained hemolymph from each treated or control larva was applied to a microscope slide and smeared to a thin film. The smears were first stained with diluted May-Grunwald solution for 3 min, and washed several times with distilled water, and then dipped in tap water. They were stained for a second time with diluted Giemsa for 10 min then washed again in distilled water. The slides were examined and photographed with a Leica LAS EZ (V 3.1.0) microscope. The analysis concerned the prohaemocytes, plasmatocytes, and granulocytes which are easy to identify.

Effect on the respiratory rhythm.

This experiment was carried out on 3-day-old 4th instar larvae. The insects were treated by ingestion with the latex alone or after exposure to PAN (2%) for 6 h. The effect on the respiratory rhythm is achieved by counting the opening rhythm of the metathoracic spiracle under a binocular microscope for one minute, after immobilizing the insect and releasing its hind legs.

Statistical analyses.

Results are expressed as means ± standard deviation. The significance between control and treated series was estimated using Student Newman Keuls (SNK) test at $P \leq 0.05$. All data were statistically analyzed by SPSS (Version 13.0.).

RESULTS

Effect of fresh latex on larval mortality.

The results reported in Fig. 1 show that the FL of *P. tomentosa* exhibited insecticidal activity against *L. migratoria*. Treatment of newly emerged 4th instar larvae resulted in a significant mortality which reached 96.49 ± 6.07% 6 days after treatment in insects fed directly on sorghum leaves treated with undiluted

fresh latex (FLC4). The influence of FL is widely correlated to the concentration used and the analysis of variance considering FL concentration as classification criteria revealed a significant difference among treatments (F = 12.7, df = 3, P = 0.0021).

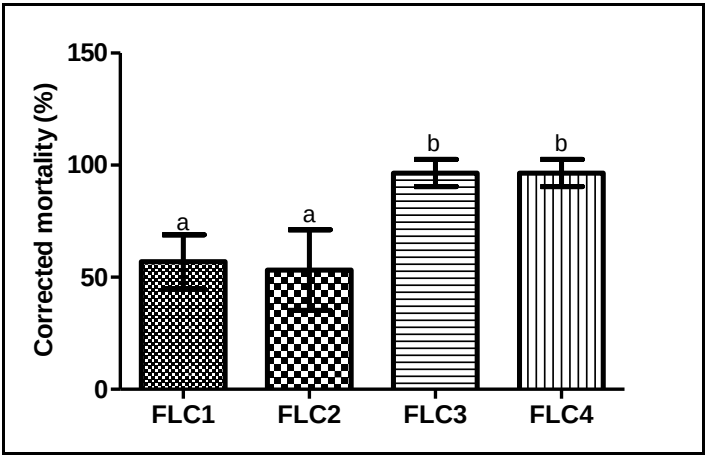

Fig. 1. Corrected mortality (mean ± SD, %) of *Locusta migratoria* larvae noted 6 days after treatment by the fresh latex of *Pergularia tomentosa*. Mean value represents three replicates, each containing 20 insects. Means followed by the same letter were not significantly different based on SNK-test at $P < 0.05$.

The evaluation of Fl toxicity was also estimated by LC_{50} and LC_{90} values, and their 95% confidence limits expressed as percentage after 6 days of treatment (Table 1). The corresponding LC_{50} and LC_{95} values were respectively 8.56% and 52.49%.

Table 1. Toxicity of *Pergularia tomentosa* fresh latex to newly emerged 4th instar larvae of *Locusta migratoria*

LC_{50} [a, b]	LC_{90} [a, b]	df	$\chi 2$
8.56 (5.28-32.7)	52.49 (29.84-206.07)	13	494.6

[a] Units LC_{50} and LC_{95}= %.
[b] 95% lower and upper fiducial limits are shown in parenthesis.

Effect of PAN/FL combination.

Toxicity bioassays also showed that the combination of PAN with FL (PAN/FL) significantly increased the larvae mortality (F = 34.58, df = 4, $P <$ 0.0001). Indeed, the mortality rates observed in the larvae treated with the fresh latex alone at the lowest concentrations used (FLC1 and FLC2) were 56.92 ± 12.1 and 53.21 ± 18.05%, respectively, 6 days after treatment. These same concentrations caused mortality rates of 92.98 ± 6.84 and 96.29 ± 6.41%, respectively, when the larvae were exposed for 6 h to the aggregation pheromone (PAN) at the concentration of 2% before feeding on FL-treated leaves (Fig. 2).

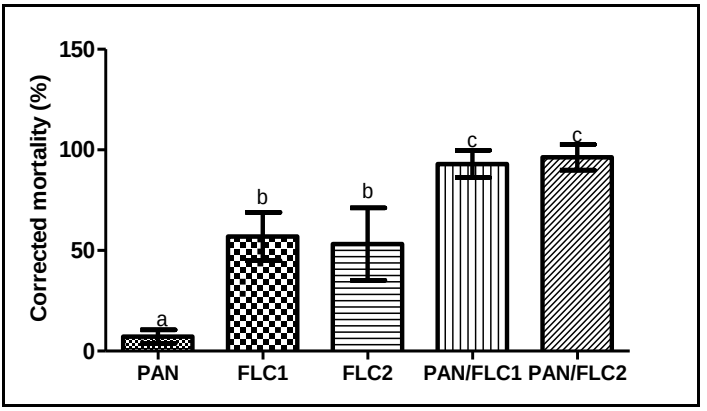

Fig. 2. Corrected mortality (mean ± SD %) of *Locusta migratoria* larvae noted 6 days after treatment by the fresh latex of *Pergularia tomentosa* in combination with the aggregation pheromone, phenylacetonitrile (PAN/FL). Mean value represents three replicates, each containing 20 insects. Means followed by the same letter were not significantly different based on SNK-test at $P < 0.05$.

Bioassay was also designed to determine the LT_{50} and LT_{90}, effective times to cause mortality of 50 and 90% of treated insects. The lethal time bioassay results showed that the PAN pheromone seemed able to accelerate the mortality of the larvae that became more sensitive to the latex treatment. The LT_{50} values for the fresh latex, applied alone, were respectively 4.8 and 4.5 days. When the larvae were initially exposed to the pheromone, the time required to cause 50% mortality of the larvae became shorter and reached 1.93 days (Table 2).

Table 2. LT_{50} and LT_{90} values (days) of the fresh latex of *Pergularia tomentosa* alone (FL) or in combination with the aggregation pheromone, phenylacetonitrile (PAN/FL) for *Locusta migratoria* 4th instar larvae

Treatments	LT_{50} (days)*	LT_{90} (days)*	*df*	χ^2
FLC1	4.88 (4.58-5.18)	9.1 (8.33-10.17)	16	6.02
FLC2	4.52 (4.19-4.82)	9.08 (8.28-10.19)	16	7.32
PAN/FLC1	2.87 (2.15-3.47)	5.64 (5.05-6.53)	16	11.86
PAN/FLC2	1.93 (1.6-2.21)	4.63 (4.2-5.24)	16	3.96

*95% lower and upper fiducial limits are shown in parenthesis. FLC1 = 1%, FLC2 = 5%. PAN/FL: Larvae exposed to the PAN for 6 h then treated win the FL. FL: fresh latex, PAN: Phenylacetonitrile.

Differential hemocyte counts.

Data illustrated in table 3 show the effect of *P. tomentosa* fresh latex, alone or in combination with the aggregation pheromone, phenylacetonitrile, on the DHC of the 4thinstar larvae of *L. migratoria* noted 72 h after treatment. In this study, the DHC was expressed in relative numbers of prohemocytes, plasmatocytes, and granular cells. Haemocytes were distinguished on the basis of morphological characteristics and staining affinity. As summarized in Table 3, quantitative determination of larval

blood cells recorded in treated and normal larvae showed that the FL significantly reduced the number of circulating hemocytes (prohemocytes, plasmatocytes, and granulocytes) as compared to control series. The effect became more apparent by exposing the larvae to PAN before treatment with FL. Indeed, the combined treatment (PAN/FLC2) induced the highest reduction for the three cell classes. Results also showed that PAN, applied alone, significantly reduced the DHC (Table 3). The analysis of variance revealed a significant difference among treatments for prohemocytes (F = 48.13, df = 6, P < 0.0001), plasmatocytes (F = 75.42, df = 6, P < 0.0001), and granulocytes (F = 89.16, df = 6, P < 0.0001).

Table 3. Differential haemocyte counts (DHCs) (cell/µl, mean ± SD) of *Locusta migratoria* larvae determined at 72 h post-treatment with *Pergularia tomentosa* fresh latex (FL) alone or in combination with phenylacetonitrile (PAN/FL)

Haemocyte	Control	PAN	FLC1	FLC2	PAN/FLC1	PAN/FLC2
Prs.	446.9 ± 89.8 a	75.1 ± 8.3 c	140.7 ± 10.5 b	160.7 ± 8.5 b	81.6 ± 14.4 c	62.6 ± 14.9 c
Pls.	462.9 ± 69.1 a	98.6 ± 4.6 bc	124.8 ± 1.1 bc	138.5 ± 6.5 b	105.4 ± 37.9 bc	78.1 ± 9.1 c
Grs.	537.4 ± 31.7 a	119.7 ± 22.7 b	129 ± 33.2 b	107.8 ± 33.4 b	97.2 ± 7.1 b	62.1 ± 2.5 c

For each haemocyte category, means followed by the same letter were not significantly different based on SNK-test at P < 0.05. Prs: prohemocytes, Pls: plasmatocytes, Grs: granulocytes, FL, fresh latex, PAN: phenylacetonitrile.

Qualitatively, the application of FL and PAN, in addition to abnormal counts, also caused great abnormalities to the different blood cells. These abnormalities were manifested by distortion of the cytoplasmic and nuclear membrane, rupturing of cell wall, enlargement of cells and abnormal staining of the haemocytes (Fig. 3).

Effects on the respiratory activity.

The obtained results concerning the respiratory rhythm of the 4th instar larvae of *L. migratoria* are shown in Fig. 4. It can be deduced that the fresh latex with the two concentrations FLC1 and FLC2, alone or in a binary combination with PAN (2%), could significantly decrease the respiratory activity of the larvae compared to the untreated control (F = 142.1, df = 5, P < 0.0001). The analysis of the variance followed by the SNK-test gave three significantly different groups, which proved that PAN was able to improve the efficiency of FL by further reducing the respiratory rhythm of *L. migratoria* larvae. Indeed, we counted 39.25 ± 1.47 and 35.37 ± 2.48 opening of the metathoracic spiracle per minute for FLC1 and FLC2, respectively. However, these values decreased significantly after exposure of the larvae to PAN pheromone to reach 23.8 ± 4.16 and 23.2. ± 3.54 for both concentrations, respectively (Fig. 4).

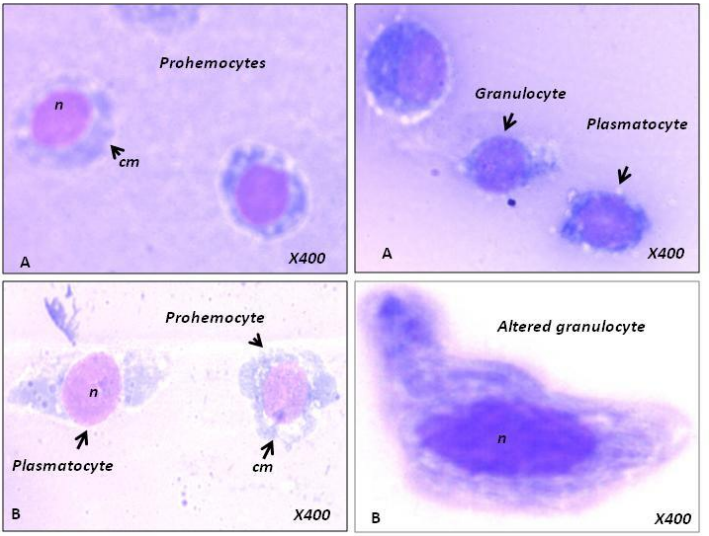

Fig. 3. Light microscope observation of circulating haemocytes of normal *Locusta migratoria* larvae (A) and treated with PAN/FLC2 (B). ×400 original magnifications. Note the disorganization of the cytoplasmic and nuclear membrane and the abnormal staining of the haemocytes. cm: cytoplasmic membrane, n: nucleus.

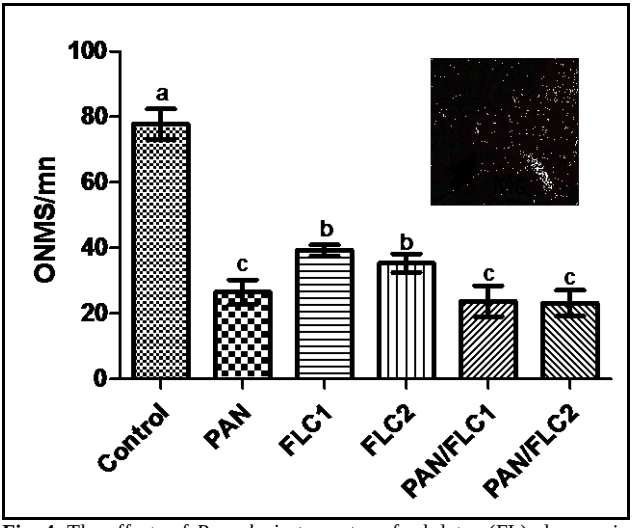

Fig. 4. The effects of *Pergularia tomentosa* fresh latex (FL) alone or in combination with the aggregation pheromone, phenylacetonitrile (PAN/FL) on the respiratory activity (mean ± SD %) of *Locusta migratoria* larvae after 3 days of treatment. Means followed by the same letter were not significantly different based on SNK-test at $P < 0.05$. Ms: metathoracic spiracle, ONME/mn: opening number of metathoracic spiracle per minute.

DISCUSSION

Botanical insecticides may be the future of locust control programs. Plant derivate active compounds, have potential uses as growth and reproduction inhibitors, repellents and as oviposition deterrents (Shahi et al. 2010). In the literature, it is well known that *Asclepia* latex, the protoplasmic content derived from the laticiferous cell which characterizes this genus, contains specialized substances including the poison, asclepione (Hayes 1947).

Nevertheless, only a few researchers have focused on the valorization of Asclepiadaceae plant latex as bio-insecticide. During the present study, the latex of *P. tomentosa* was tested against one of the most redoubtable enemies of culture *L. migratoria* in 4th instar larval stage alone and in combination with the aggregation pheromone PAN. This treatment significantly affected the respiratory rhythm, reduced the number of circulating hemocytes and caused significant mortality.

Some researchers have already discussed the biological activities of *P. tomentosa*. Indeed, Acheuk and Doumandji-Mitiche (2013) showed that alkaloids extracted from the aerial part of this plant exhibited a potent larvicidal effect against the 5th instar larvae of *L. migratoria* with a dose-dependent relationship. The mortality rate reaches 100% with the highest dose 240 µg/larvae 10 days after treatment. Likewise, Hussein et al. (1999) showed that the ethanolic extract of *P. tomentosa* killed test snails within 24 h after treatment. According to these authors, the rapid death of treated snails indicates the presence of highly sensitive target(s) which may be exploited in discovering new specific and effective molluscucide.

We also demonstrated that the exposure to PAN reduced the concentration of fresh latex from 100% to 1% while preserving the same efficiency. Results found in this research are similar to those of other authors who indicated that the addition of small quantities of phenylacetonitrile could make it possible to decrease by half the quantities of insecticides used in the control of desert locust nymphs, while preserving the same effectiveness (Bal and Sidati 2013). Moreover, Ramos et al. (2010) found that the proteins contained in the latex of *Calotropis procera* showed very significant adverse effects on the growth and development of *Callosobruchus maculatus*. Similarly, Nenaah (2013) reported that latex protein of *C. procera* exhibited considerable toxic and feeding deterrent effects against adults of *Sitophilus oryzae* and *Rhyzopertha dominica* in a dose-dependent manner.

Furthermore, our finding showed that the treatment of the 4th instar larvae of *L. migratoria* with *P. tomentosa* latex, alone or in combination with the PAN, significantly disturbed their haemogram by reducing the number of the differential hemocyte counts. This situation has already been mentioned by Guzo and Stoltz (1987) in *Schistocerca gregaria* treated by *Metarhizium anisopliae*. This decrease is probably the result of the depletion of these cells following the phagocytic activity induced against the treatment. Similarly, Duressa et al. (2014) demonstrated that the locusts lost over 60% of the circulating hemocytes within 30 min following β-1,3-glucan injection. Our results are also consistent with those of Aylin et al. (2017) demonstrating that azadirachtin used at 100 ppm is able to reduce total hemocyte counts and to induce significant alterations in differential hemocyte counts. According to these authors, azadirachtin decreased

the counts of circulating hemocytes due to the induction of autophagic or apoptotic pathways resulting in cell death. It was reported in many studies that azadirachtin leads to apoptosis and autophagy in insect cell lines originated from ovarian tissues (Huang et al. 2010). The decrease in the rate of opening rhythm of the methathoracic spiracle reminds the results of Moussa (2003) who showed that the use of neem (*Azadirachta indica*) oil reduces the respiratory rate of *L. migratoria.*

Although the results obtained are very interesting, further studies are still needed to evaluate with more details the possible interferences of PAN with the behavior and physiology of *L. migratoria* larvae and adults. We can also consider in future experiments a chemical study of *P. tomentosa* latex to determine its major active compounds.

RESUME

Miladi M., Abdellaoui K., Regaieg H., Omri G., Acheuk F. et Ben Halima-Kamel M. 2018. Effets du latex de *Pergularia tomentosa* et de la phéromone d'agrégation, phénylacétonitrile, sur les larves de *Locusta migratoria*. Tunisian Journal of Plant Protection 13 (si): 87-98.

Bien qu'ils constituent un risque sérieux pour la santé humaine et l'environnement, les insecticides chimiques restent les plus utilisés dans la lutte antiacridienne. La recherche de méthodes alternatives de contrôle, efficaces et compatibles avec l'environnement, est devenue indispensable. Le latex végétal est un fluide endogène sécrété par des cellules laticifères hautement spécialisées et il a été suggéré qu'il agissait comme un système de défense des plantes. Le but de la présente étude est d'étudier les potentialités insecticides du latex de *Pergularia tomentosa* à différentes concentrations, seul et en combinaison avec le pénylacétonitrile (PAN), sur les larves du quatrième stade de *Locusta migratoria*. Les résultats obtenus ont montré que le latex révélait une activité insecticide intéressante contre les larves de cet insecte, entraînant une mortalité pouvant atteindre 96.49 %, 6 jours après le traitement. Les bio-tests de toxicité ont montré que le PAN, associé au latex, semble accélérer et augmenter le taux de mortalité. Le traitement à base de cette phéromone a affecté la santé des insectes en réduisant considérablement leur rythme respiratoire. Il a été également démontré que le PAN semble altérer, quantitativement et qualitativement, les cellules sanguines des larves qui se manifestent par une diminution significative du nombre des hémocytes (prohémocytes, plasmatocytes et granulocytes) et une importante lyse cellulaire.

Mots clés: *Locusta migratoria*, Latex, *Pergularia tomentosa*, phénylacétonitrile, toxicité

ملخص

الميلادي، مريم وخميس عبداللاوي وهاجر رقيق وغفران عمري وفاطمة عشاق و منية بن حليمة الكامل. 2018. مفعول لبن نبتة *Pergularia tomentosa* وفرمون التجمع penylacetonitrile على يرقات الجراد المهاجر *Locusta migratoria*. Tunisian Journal of Plant Protection 13 (si): 87-98.

على الرغم من أنها تشكل خطرا جسيما على صحة الإنسان والبيئة، لا تزال المبيدات الكيميائية للحشرات الأكثر استخداما في مكافحة الجراد. لذلك أصبح من الضروري البحث عن طرق مكافحة بديلة فعالة ومنسجمة مع المحيط. اللبن النباتي هو سائل تفرزه خلايا متخصصة للغاية ويساهم في حماية النباتات من الأمراض. يهدف هذا العمل إلى اختبار مفعول لبن نبتة *Pergularia tomentosa* وفرمون التجمع penylacetonitrile على فيزيولوجيا الجراد المهاجر *L. migtatoria*. أظهرت النتائج المتحصل عليها أن لهذا المستخلص فاعلية كبيرة على يرقات الطور الرابع للجراد حيث تسبب في نسبة موت عالية تصل الى 96.49% في خلال 6 أيام. كما أثبتت الاختبارات أيضا بأن استخدام الفرمون مع اللبن النباتي يساهم في تسريع وارتفاع نسبة موت اليرقات. وقد أثر هذا الفرمون على صحة الحشرات ويتبين ذلك من خلال

اضطرابات واضحة في التنفس وتغيرا، كميا ونوعيا، في الخلايا الدموية لليرقات حيث سجل انخفاض كبير في عدد الخلايا وبعض التشوهات الخلوية.

كلمات مفتاحية: جراد، سمية، فرمون التجمع، لبن نباتي، مهاجر، *Pergularia tomentosa*

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