Toxicity of the active fraction of *Pergularia tomentosa* and the aggregation pheromone phenylacetonitrile on *Schistocerca gregaria* fourth-instar nymph: effects on behavior and acetylcholinesterase activity

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ABSTRACT

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Chemical insecticides remain the most used approach in locust control although they present a serious menace to human health and the environment. The search for alternative control methods, efficient and environmentally friendly, has become indispensable. The aim of this work is to study the effect of the aggregation pheromone, phenylacetonitrile, alone or in combination with the active fraction of *Pergularia tomentosa* on *Schistocerca gregaria* fourth-instar nymph. Toxicity bioassays showed that the combination of phenylacetonitrile with the active fraction of *P. tomentosa* significantly increased nymph mortality. Results also showed that the aggregation pheromone caused significant mortality especially after 6 hours of exposure. The pheromone also caused neurotoxic effects on *S. gregaria* nymph due to the disturbance of the acetylcholinesterase activity. We also noted the presence of cannibalism phenomenon. Phenylacetonitrile seems to have an effect on phase ploypheniism of *S. gregaria* imagos that exhibit specific traits to the solitarious phase.

Keywords: Behavior, botanical insecticide, desert locust, enzyme, pheromone

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The desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae) is potentially the most destructive pest insects in many semi-arid territories of Africa and the Near East (Seidelmann et al. 2005). This specie is able to transform between two phases, solitaria and gregaria, which differ in various behavioral, morphological and physiological characteristics (Pener and Simpson 2009; Tanaka and Nishide 2013). During solitary phase, the locust populations are likely to present no obvious economic threat to the cultivated vegetations (Sword et al. 2010). However, nymphs in the gregarious phase show an aggregating behavior and move in bands to seek food, whereas adults swarm and migrate over long distances causing significant crop and pastures damage (Magor et al. 2008). The consequences of the invasions can be disastrous for food security of many states in affected areas (Abbassi et al. 2003; Chen 2010). To limit damage to crops and pastures, treatment is required with large amounts of broad-spectrum chemical pesticides. Due to fast action, low cost, easy application and efficiency, synthetic insecticides have become an important part of locust and grasshoppers management in modern agricultural systems. However, after decades of use, their negative side effects, such as toxicity to humans and animals, environmental contamination, and toxicity to non-target insects have become apparent and interest in less hazardous alternatives of locust control is therefore being renewed. Plant species with known insecticidal actions are being promoted and research is being conducted to find new sources of botanical insecticides. Plant species have the ability to synthesize a variety of secondary metabolites that are not essential for their growth and development, but are important in the protection against phytophagous insects and microbial pathogens. Many secondary metabolites have insecticidal, repellent as well as antifeedant activity. Furthermore, they cause reproduction retardation and act as insect growth disruptors (Barney et al. 2005; Al-Mazra'awi and Ateyyat 2009).

Asclepiadaceae (milkweeds) is a large family of about 230 genera and almost 2000 species distributed through the tropics and temperate areas of the world (Arribére et al. 1998). Among these species, *Pergularia tomentosa*is a perennial shrub that occur in the Tunisia flora (Gill 1992). The plant was reported to have effective antifungal activity (Hassan et al. 2007), insecticidal activity against *Locusta migratoria* (Acheuk and Doumandgi Mitiche 2013) and antifeedant effect on *Spodoptera littoralis* (Green et al. 2011).

In the same way of research and in an effort to identify novel pest-control products that are both environmentally acceptable and effective for the management of the desert locust, the study of the pheromonal bouquet of *S. gregaria* is currently one of the most actively investigated areas in chemical ecology, partly owing to its interesting perspectives for the development of new bio-pesticides. A series of studies led to the identification of a number of volatile aromatic compounds released by gregarious desert locusts with phenylacetonitrile (PAN, also named benzyl cyanide) as the dominant component (Torto et al. 1994). Hassanali et al. (2005) discovered that phenylacetonitrile significantly affected the behavior of *S. gregaria* nymphs and increased their sensitivity to insecticides. Bal and Sidati (2013) have demonstrated that the addition of small amounts of PAN reduces the quantities of insecticides used for the control of nymphs, while maintaining the same efficiency.

In this paper, we studied (i) the toxicity of the active fraction of *Pergularia tomentosa* (AFP) alone or in binary combination with PAN against *S. gregaria* nymphs, and (ii) the effect of the PAN and on some behavioral and physiological parameters of treated nymph such as cannibalism, phase ployphenism and neurotoxicity. The attempt results give us additional information on the mode of action of PAN.

MATERIALS AND METHODS

Locust rearing.

Mass rearing of *S. gregaria* was carried out in the Laboratory of Entomology at the Higher Agronomic Institute of Chott-Mariem as previously described (Abdellaoui et al. 2013). Nymphs and adults were held in 50 × 50 × 50 cm cages at 30-32 °C, 50-70% RH, and under a 12 h:12 h, L:D photoperiod. Locusts were fed daily on fresh *Brassica oleracea* leaves supplemented with wheat bran.

Plant material and the active fraction extraction.

The Asclepediaceae *P. tomentosa* was collected during spring seasons (March-April 2017) from the region of Hergla (N: 36.1°, E: 10.3°), Tunisia. The active fraction preparation was carried out in the Laboratory of Insect Physiology and Molecular Ethology (KU Leuven, Belgium). The methanolic crude extract (18 g) was subjected to column chromatography on a silica gel column (63-200 µm, 70-230 meshes). The column was initially eluted with hexane, followed by EtOAc and hexane mixtures (EtOAc: hexane, 25:75, 50:50, 75:25, 100:0). Then, the eluting solvent mixture was changed to MeOH: EtOAc (25:75, 50:50, 75:25, 100:0, v:v) and finally to 20% aqueous acetic acid: MeOH (5:95, 10:90). 5 ml aliquots from each fraction were concentrated under reduced pressure in a Savant SpeedVac Concentrator (Thermo Scientific), the dried residues were re-dissolved in 5 ml EtOH and tested for their insecticidal activity against 3rd instar nymph locust. Only the MeOH, the EtOAc (50:50) fraction, which gives mortality rate >70% has been recovered and the solvent was evaporated. The dried active fraction of *P. tomentosa* (AFP) was kept at 4°C.

The aggregation pheromone phenylacetonitrile.

Phenylacetonitrile (PAN) or benzyl cyanide (98%) was purchased from Sigma-Aldrich. It is an aromatic organic compound of formula $C_6H_5CH_2CN$.

Treatments.

The bioassay for insecticidal activity of the active fraction of *P. tomentosa* (AFP) against *S. gregaria* was conducted on newly ecdysed (< 6 h post molting) 4th instar nymph using three concentrations 0.06, 0.24 and 0.96 % chosen in accordance with amounts used in preliminary tests in the laboratory. Locusts were inoculated by applying 5 µl of the corresponding concentration under the pronotal shield. In the control experiment, the insects received the same quantity of ethanol used as solvent for preparing the different concentrations. Control and experimental nymphs (n = 60 for each concentration) were placed in separate cages (30 cm^3) under the same conditions described above for the mass rearing. Insect mortality was calculated according the Abbott correction formula (Abbott 1925). Probit analysis (Finney 1971) was conducted to estimate the LC_{50} values with their 95% confidence limits after 8 daysof treatment. Other experiments were conducted to assess the effect of the AFP and PAN in binary combination. Firstly, both products were applied simultaneously by treating nymphs topically with a homogeneous mixture of PAN (at the concentration of

0.5, 1 and 2%) and the AFP (at the LC_{50}) (v:v) formulated in ethanol. In other tests, insects were first exposed to the PAN (0.5, 1 and 2%) for 2, 4 and 6 hours and then treated topically by the AFP (LC_{50}) as described earlier.The mortality was then assessed daily via direct observation for a period of 8 days.

Effect of PAN on cannibalistic behavior.

Four replicates of 20 fourth-instar nymphs that had molted 12 hours earlier were exposed to PAN at the concentrations of 0.5, 1 and 2% for 6 hours and transferred into 30 cm × 30 cm × 30 cm cages as described above. For control, a similar number of replicates treated with paraffin oil were deployed. Fresh food consisting of *B. oleracea* leaves (25 g/cage) was provided early in the morning (8 am). The mortality was assessed daily and dead nymphs were immediately removed and inspected. Number of nymphs that were cannibalized (evidence of having been mauled and partially fed by conspecifics e.g. nymph without abdominal parts) was recorded. The percentage of cannibalized nymphs was computed.

Comparison of the effects of PAN and antennectomy.

The purpose of these experiences is to verify if the gregarious male pheromone has a disruptor behavioral effect on the conspecific fourth-instar nymphs and to check if the insects are able to detect the pheromone in their environment. Replicate groups (three each) of 10 fourth-instar nymphs were either treated with PAN, antennectomised, or left untreated (control) and the number of nymphs engaged in different behaviors (feeding, roosting, moving) at 8 am and 8 pm compared for 3 successive days after treatment was recorded (Bashir and Hassanali 2010).

Morphometric measurements.

Digital calipers were used to measure the following classical morphometric phase characteristics for adults to determine E/F and F/C ratios (E = Length of fore wing, F = Length of hind femur, C = Maximum head width) for female and male imago originating from control (exposed to the paraffin oil) and PAN-exposed fourth-instar nymphs at the concentration of 2% for 6 hours. Locusts were held in a number of 20 individuals/cage to maintain the same phase characteristics. F/C and E/F ratios were reported on morphometric chart according to Duranton and Lecoq (1990). They distinguish the gregarious from the solitary and *transiens* (*dissosians* and *congregans*) forms. For each group, 20 males and 20 females were measured.

Enzymatic assays.

Acetylcholinesterase (AChE) assays was conducted on the fourth-instar nymphs (< 6h post-molting, n = 12) sampled from control and treated groups after 2, 4 and 6 hours of exposure. Heads were separately homogenized in a glass homogenizer in 100 mM K-phosphate buffer (250 mmol/l sucrose, 50 mmol/l Tris-HCl, 1 mmol/l EDTA, and 1 mmol/l DTT at a 1/4 w/v ratio, pH 7.5). The homogenates were centrifuged at 15000 g for 20 min to generate the S9 fraction. After centrifugation, the supernatants were collected and stored at -80 °C until analysis. All procedures were carried out at 4 °C. Proteins in the S9 fraction were quantified according to the Bradford method (1976) using Coomassie Blue reagent. The AChE activity was carried out following the method of Ellman et al. (1961) as previously described (Abdellaoui et al. 2018).

Data analysis.

Results are expressed as means ± standard deviation (SD). For multiple comparisons, a parametric one-way analysis of variance (ANOVA) was performed on data along with Tukey-test using Graph Pad Prism (Version 6.0.).

RESULTS

Effect of the active fraction of *P. tomentosa* on fourth-instar nymph mortality.

The results reported in Fig. 1 showed that the AFP exhibited insecticidal activity against *S. gregaria*. Treatment of newly emerged fourth-instar nymph resulted in a significant mortality with the highest concentration 0.96% which reached 54.19 ± 11.05% 8 days after treatment. The influence of AFP is widely correlated to the concentration used and the analysis of variance considering AFP concentrations as classification criterion revealed a significant difference among treatments (F = 15.19, df = 2, P = 0.0013).

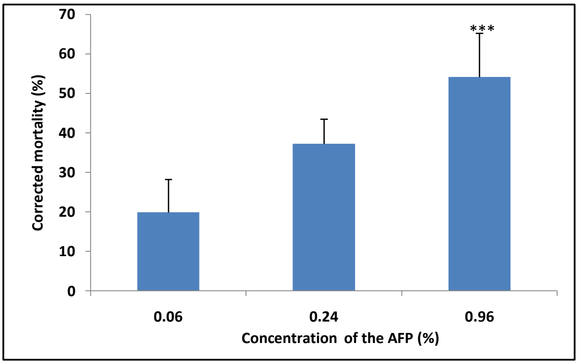

Fig.1. Effect of the active fraction of *P. tomentosa* (AFP) on the corrected mortality of *S. gregaria* fourth-instar nymph. Mean value (± SD, %) represents three replicates, each containing 20 insects. Significant differences (P< 0.001) are indicated by asterisks *** according to Tukey-test.

The evaluation of the AFP toxicity was also estimated by LC_{50} value, and its 95% confidence limits expressed as percentage for an 8-day period (Table 1). The corresponding median lethal concentration was 0.8 % (95%, *cl* = 0.59-1.27%). This concentration was chosen for the subsequent interaction experiments.

Table 1. Toxicity of *P. tomentosa* active fraction to newly emerged 4th instar nymph of *S. gregaria*

Treatment	LC_{50}[a, b]	Chi square (χ^2)	*df*	*P* value
Topical application	0.81 (0.59 - 1.27)	46.36	10	$P < 0.0001$

[a] Units LC_{50} = %

[b] 95% lower and upper fiducial limits are shown in parenthesis.

Effect of PAN/AFP combination on nymphal mortality.

Experiments were conducted on fourth-instar nymphs of *S. gregaria* in order to determine the percent mortality at each concentration of the PAN alone and combined with the AFP at the LC_{50}. As summarized in Table 2, when the PAN is applied alone by topical application, the mortality rates ranged from 23.81 ± 8.9 to 44.26 ± 15.9% at 8 days after treatment for the average concentration 0.5 and 2%respectively. The analysis of variance considering concentrations as classification criterion showed a significant difference among treatments ($F = 6.96$, $df = 2$, $P = 0.015$). The activity of PAN against nymphs becomes more evident when the locusts are exposed to the pheromone and the mortality rates become higher even with the lowest tested concentration. Indeed, the percentage of corrected mortality reached 79.64 ± 28.29% after 6 hours of exposure at the concentration of 1%. ANOVA showed a significant difference among treatments for 2 and 6 hours of exposure ($F = 10.18$, $df = 2$, $p = 0.005$; $F = 5.9$, $df = 2$, $P = 0.02$, respectively). The analysis of variance also showed that the mortality rates did not differ significantly between the PAN concentrations after 4 hours of exposure ($F = 1.81$, $df = 2$, $P = 0.22$) as indicated by the homogeneous groups generated using Tukey-test (Table 2).

Toxicity bioassays also showed that the application of the aggregation pheromone PAN in a binary combination with the AFP at the LC_{50} significantly increased the nymphal mortality. Indeed, the mortality rates observed in the nymphs treated with the AFP alone at the highest used concentration (0.96%) was 54.19 ± 11.05% for an 8-day observation period. However, when the LC_{50} of the AFP was applied simultaneously with the PAN at the concentration of 2%, the percentage of corrected mortality reached 79.64 ± 16.35%. Data also revealed that when the nymphs were first exposed to the aggregation pheromone before receiving the AFP, the mortality rates become higher and reached92.72 ± 10.48% after 6 hours of exposure. The analysis of variance, with the PAN concentrations as classification criterion, showed a significant difference among treatments for all the tested exposure times ($F = 4.67$, $df = 2$, $P = 0.04$; $F = 10.88$, $df = 2$, $P = 0.004$; $F = 13.88$, $df = 2$, $P = 0.002$), respectively for 2, 4 and 6 hours of exposure (Table 2).

Table 2. Percentage of corrected mortality (mean ± SD) of *S. gregaria* fourth-instar nymph treated by the AFP in combination with the aggregation pheromone, phenylacetonitrile (PAN/AFP) for an 8-day observation period.

Treatment	Topical application		PAN-Exposure		
			2 h	4 h	6 h
PAN alone	0.5%	23.81 ± 8.90	10.17 ± 8.17	28.39 ± 8.49	36.42 ± 12.48
	1%	13.44 ± 9.43	37.94 ± 19.49	33.92 ± 12.48	79.64 ± 28.29 *
	2%	44.26 ± 15.9 *	56.16 ± 13.6 **	49.37 ± 9.21	64.19 ± 4.24
	$F_{(2,9)}$	6.96	10.18	1.81	5.90
	P value	0.015	0.005	0.22	0.02
PAN/AFP (LC_{50})	0.5%	64.82 ± 17.80	36.7 ± 14.69	36.70 ±14.69	53.52 ± 15.12
	1%	56.79 ±17.00	64.71 ± 9.61 *	57.43 ± 18.28	89.08 ± 9.08 **
	2%	79.64 ±16.35	57.99 ± 15.51	85.44 ± 10.49 **	92.72 ± 10.48 **
	$F_{(2,9)}$	1.848	4.67	10.88	13.38
	P value	0.2126	0.0405	0.004	0.002

Mean value represents three replicates, each containing 20 insects. Significant and highly significant differences ($P< 0.05$ and $P< 0.01$) are indicated by asterisks * and ** respectively (Tukey-test).For the topical application, the PAN and AFP were applied simultaneously. For the exposure tests, nymphs were first exposed to the PAN for 2, 4 and 6 hours and then treated topically by the AFP (LC_{50}).

Effect of PAN on cannibalistic behavior.

We investigated in this study the relationship between the PAN treatment and cannibalism behavior in *S. gregaria* fourth-instar nymphs. It appears from the results grouped in Fig. 2 that cannibalistic behavior was significantly higher (*F*= 8.16, *df* = 2, *P* = 0.0031) in PAN-treated groups compared with control. Indeed, the percentages of nymphs that were cannibalized were 17.5 ± 5 and 22.5 ± 5% respectively to the lowest and highest tested concentration of PAN. For the medium concentration (1%), the number of cannibalized nymphs did not exceed 10 ± 8.16%. However, the cannibalistic behavior did not exceed 3.33 ± 2.71% in the control group exposed to the paraffin oil (Fig. 2). As illustrated in Fig. 3, the cannibalism results in apartially fed by conspecifics e.g., nymphs without abdominal orthoracic parts (Fig. 3).

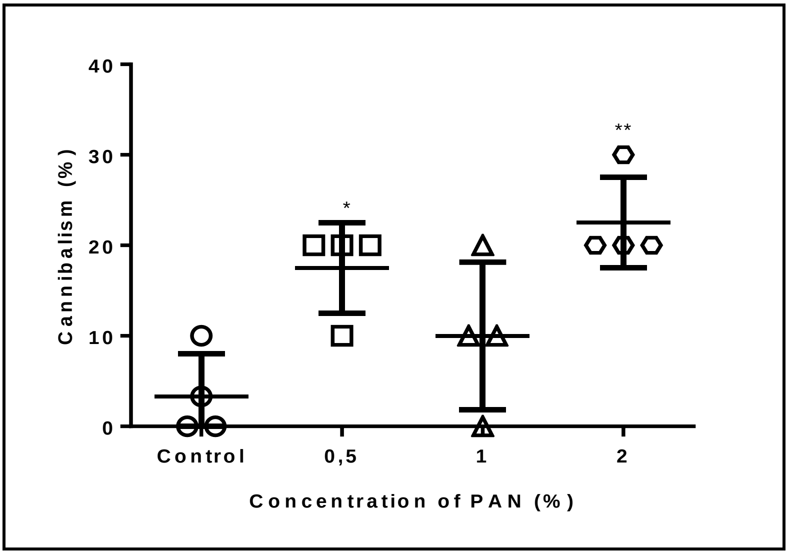

Fig. 2. Effect of the PAN on cannibalistic behavior of *S. gregaria* fourth-instar nymphs. The data are represented as means ± SD of four replicates each containing 20 insects. Significant and highly significant differences ($P< 0.05$ and $P< 0.01$) are indicated by asterisks * and ** respectively (Tukey-test).

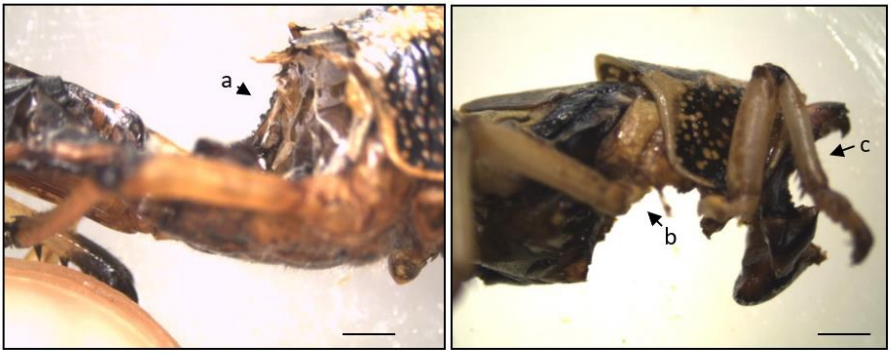

Fig. 3. Cannibalistic behavior of *S. gregaria* fourth-instar nymphs exposed to the aggregation pheromone for 6 hours. — = 0.1cm. Nymphs cannibalized in the abdomen (a), thorax (b) and head (c) parts.

Comparison of the effects of PAN and antennectomy.

The results regrouped in Fig. 4 showed the effects of PAN and antennectomy on the fourth-instar nymphs behavior as reflected in the percentages engaged in feeding, roosting or moving, in the mornings and evenings. Obtained results revealed that the PAN-exposed nymphs demonstrated similar behavioral as those antennectomised. Indeed, we noted that both groups are in moving at the two different periods of the day (8 am and 8 pm). However, the control nymphs showed a normal circadian cycle characterized by an engagement in feeding at 8 am and roosting at 8 pm (Fig. 4a).

Fig. 4b summarised the percentages of nymphs engaged infeeding, roosting or moving in the mornings for three consecutive days. The obtained results showed that 72.22 ± 10.18% of the control nymphs are engaged in feeding and only 2.22 ± 1.92% are in roosting which showed normal circadian pattern of behavior. For both PAN-exposed and antennectomised nymphs, the majority of the nymphs are observed moving in the experimental cages with 54.44 ± 9.62% and 38.88 ± 5.09% respectively. These results were also evidenced by the ANOVA showing a significant difference among treatments in the fourth-instar nymph behavior (F = 8.46, df = 2, P = 0.017; F = 8.13, df = 2, P = 0.019; F = 40.61, df = 2, P = 0.0003) respectively for moving, roosting and feeding).

Effect of PAN on morphometric measurements of adults.

The morphometric measure-ments, F/C and E/F ratios used in the determination of the phase polyphenism of the desert locust were calculated on male and female imagoes originating from control and PAN-exposed fourth-instar nymphs at the concentration of 2% for 6 hours. Results were reported on morphometric chart as illustrated in Fig. 5. These results showed that PAN shifts morphometric variables of adult locusts towards solitarious values compared with the control ones especially in the case of males. In Fig. 5b, the F/C ratio was higher, whereas the E/F ratio was lower indicating a shift toward a solitariousness. Furthermore, the majority of the treated locust females were in *transiens dissocians* phase progressing towards solitarious phase as shown in Fig. 5b. However, control individuals present a morphomertry *transiens*-type with a gregarious tendency (Fig. 5a).

Acetylcholinesterase activity.

The effect of the exposure to PAN at different concentrations on the AChE enzymatic activities of *S. gregaria* fourth-instar nymph is shown in Fig. 6. Results revealed that the PAN significantly increased the AChE activity. The analysis of variance considering concentrations as classification criteria showed a significant difference among (F = 7.37, df = 3, P = 0.0046; F = 72.61, df = 3, P< 0.0001; F = 19.59, df = 3, P< 0.0001) respectively for 2, 4, and 6 hours of exposure. The ANOVA also showed that the effect of the PAN on the AChE activity varied as a function of the exposure time (F = 62.4, df = 2, P< 0.0001; F = 32.89, df = 2, P< 0.0001; F = 8.61, df = 2, P = 0.0081) respectively for the 0.5, 1, and 2%. The AChE values of the control group ranged from 121.6 ± 35.83% to 964.9 ± 209.5% and 257.8 ± 67.76% nmol/min/mg proteins after 2, 4 and 6 hours of exposure to paraffin oil respectively.

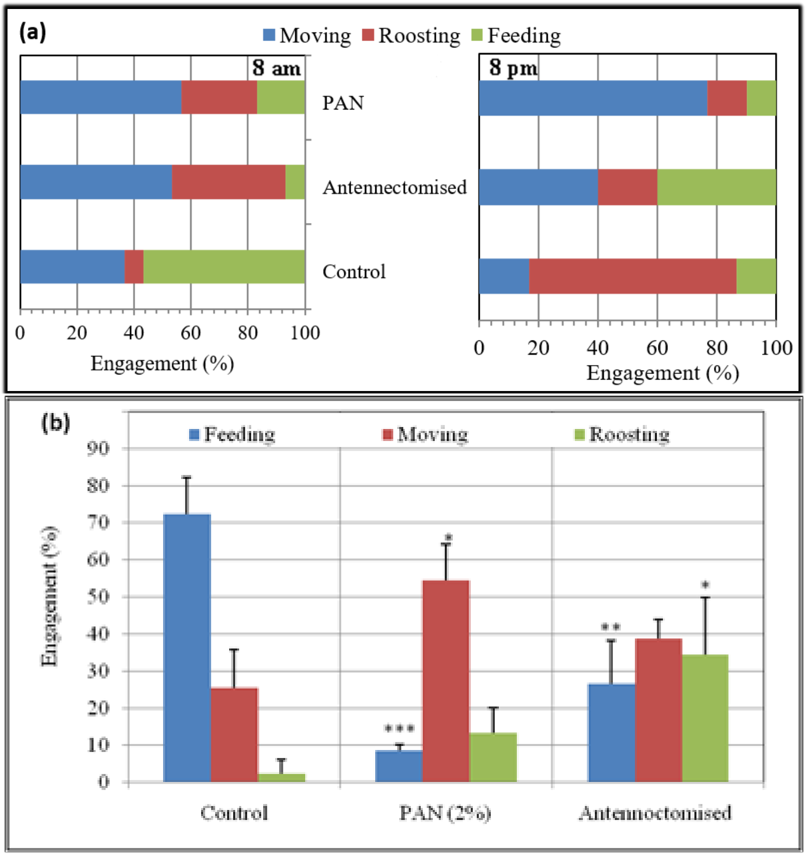

Fig. 4. Percentages of fourth-instar nymphs engaged in feeding, moving or roosting after PAN exposure and antennectomised (a). Nymphs engagement observed in the mornings (8am) and evenings (8pm); (b) cumulative percentages of nymphs engagement observed at 8 am for three consecutive days. Significant differences ($P< 0.05$, $P< 0.01$ and $P< 0.001$) are indicated by asterisks *, ** and *** respectively (Tukey-test).

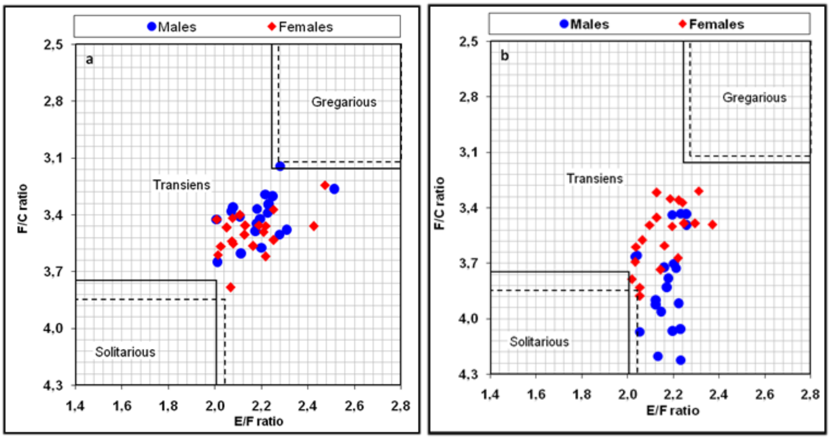

Fig. 5. Dispersion of morphometric parameters in control and treated locusts. a: F/C and E/F ratios of control locusts. b: F/C and E/F ratios of locusts originating from PAN-exposed nymphs.E = length of fore wing, F = hind femur length, C = maximum head width.

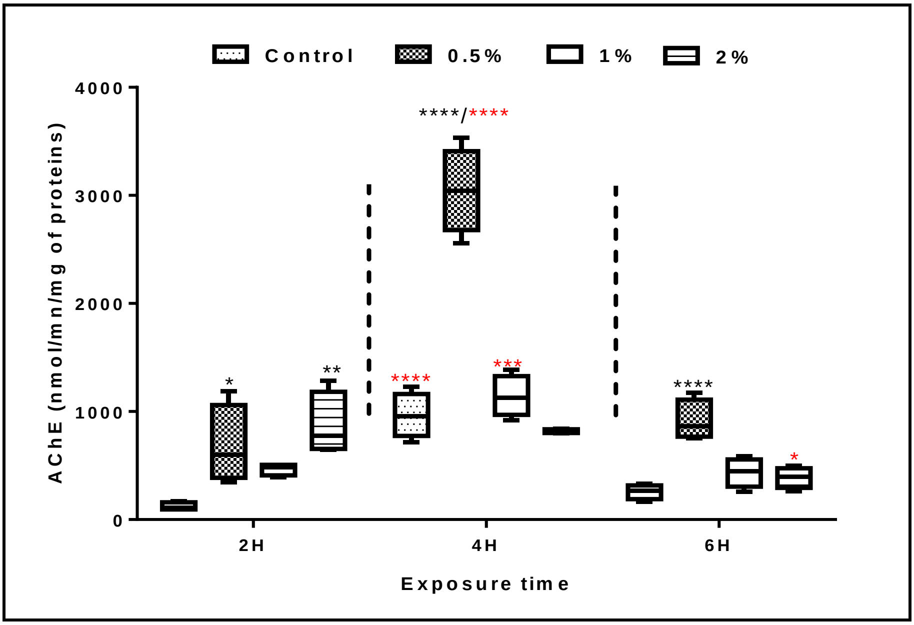

Fig. 6. Effects of PAN on the AChE activity (means ± SD, n = 12) of *S. gregaria* fourth-instar nymph. Comparison was made between concentrations (black asterisks) and between the exposure times for each concentration (red asterisks). *, **, and *** indicated significant differences at *P*< 0.05, *P*< 0.01 and *P*< 0.001) respectively, (Tukey-test).

DISCUSSION

Considering negative effects of synthetic pesticides, research in recent years has been turning more towards new environment-friendly alternatives for pest control. Botanical insecticides, known as plant secondary metabolites, have proved to be suitable candidates in locust management programs (Acheuk et al. 2012). Experiments were conducted to assess the effect of the active fraction of *P. tomentosa* (AFP) alone and in combination with the aggregation pheromone (PAN) on *S. gregaria* fourth-instar nymph. Results revealed that treatment of newly emerged nymphs by the AFP resulted in a significant mortality with a dose response relationship. The percentage of corrected mortality becomes higher and reached 92.72 ± 10.48% when the nymphs were first exposed to the pheromone before receiving the AFP. A previous study conducted by Acheuk and Doumandji-Mitiche (2013) showed that alkaloids extracted from the aerial part of *P. tomentosa* exhibited a potent insecticidal effect against the Fifth-instar nymph of *L. migratoria* with a dose-dependent relationship. Similarly, methanol crude extracts of *P. tomentosa* deterred feeding of *Spodoptera littoralis* in a binary-choice bioassay. The anti-feeding activity in *P. tomentosa* was attributed to cardenolides compounds (Green et al. 2011) which are responsible of several physiological effects such as spasms, regurgitation and slowed growth in non-adapted species of insect (Dussourd and Hoyle 2000).

Although semiochemicals have been successfully used in the field for the management of some pests, they are not generally considered to be sufficiently reliable in their action when used alone. They are more effective when used as part of integrated control strategies (Smart et al. 1994). The use of the PAN in locust management programs has been discussed by several authors. Indeed, Bal and Sidati (2013) showed that the addition of small quantities of phenylacetonitrile could make possible to decrease by half the quantities of insecticides used in the control of desert locust nymphs, while preserving the same efficiency. Moreover, Bashir and Hassanali (2010) reported that major adult pheromone constituent PAN, in addition to its pheromone character and its effects on communication between individuals, could also have some toxicity on nymphs and can make them more sensitive to insecticides and biological agents treatment. In the same way, Miladi et al. (2018) studied the effects of the latex of *P. tomentosa* and the PAN against *L. migratoria* nymphs. The authors demonstrated that the exposure to PAN reduced the concentration of fresh latex from 100 to 1 % while preserving the same efficiency.

The results of the present investigation also showed that PAN significantly affect the fourth-instar nymphs behavior by disturbing their circadian cycle as reflected in the percentages of nymphs engaged in feeding, roosting or moving in the mornings and evenings. The PAN exposure appears to interfere with the functioning and the physiology of the antennae and the insect will be unable to locate the diet. Indeed, the antennae function primarily as sense organs and they are richly endowed with olfactory sensilla. They are the primary olfactory organs of all insects. Olfaction is a sense that can be used at a distance as well as locally for detecting a variety of odorants in the environment that facilitate the research of feeding sites(Patel et al. 2013).Therefore, any perturbation at this level can be lethal to the target organism. Similarly, Hassanali et al. (2005)showed that when nymphsof *S. gregaria* are exposed to PAN vapor for 6hours, their antennal electrophysiological response to odors substantially depressed (by >90%) and recovery of antennal sensitivity occurs only gradually.

Our results also showed that the PAN may be involved in the control of phase-related morphometric ratios of F/C and E/F. Nymphs treated with PAN show a shift toward solitary morphometric ratios at adult stage suggesting that PAN has a solitarising effect on the progeny of treated locusts. Heifetz et al. (1996) reported that loss of olfactory communication between the gregarious individuals is also consistent with behavioral phase change of crowd-reared nymphs resulting from disruption of antennal chemoreception. These finding are consistent with those of Mahgoub et al. (2011) who indicated that the PAN induced solitary behavior. Treated nymphs become confused and disoriented, some lost their appetite completely, while others turned cannibal. Similarly, Bashir and Hassanali (2010) showed that long term contact of field gregarious hopper bands with PAN makes them relatively hyperactive. Few days later, their movements became random and they stopped marching as coherent groups. They started to roost for longer periods on vegetations and they fragmented into smaller and smaller groupings and individuals. There were clear signs of increased predation and cannibalism at the roosting sites. These authors suggest that cannibalistic behavior may be secondary consequences of the reduced food intake. In addition, PAN could interfere with the metabolic function in nymphs resulting in impaired development that could in turn disrupt the formation and function of olfactory organs such as antennae and inhibits perception by nymphs of their own aggregation pheromone (Ochieng 1997). However, pheromonal communication may be the principal mechanism modulating the aggregation behavior of the desert locust (Obeng-Ofori et al. 1993).

Finally, the obtained results also demonstrated that PAN was found to induce a disturbance in in AChE activity. The AChE is a key enzyme that terminates nerve impulses by catalyzing hydrolysis of the neurotransmitter acetylcholine in the nervous system (Aygun et al. 2002).

From this experiment, it may be concluded that the addition of the aggregative pheromone PAN enhanced the toxicity of the LC_{50} of the active fraction of *P. tomentosa*. The compound seemed to present anti-insect properties by disturbing behavior and reducing the neurotoxicity as evidenced by an increase in AChE activity. This can reflect positively on reducing cost and environment hazards especially that the quantity of the pheromone used in desert locust manipulation is very low. However, the mode of action of PAN on desert locust nymphs and its interference with the nervous system is still imperfectly known and requires further detailed study like possible interaction with some central nervous system neurotransmitters such as serotonin and GABA that accompany phase change in the locust.

RESUME

Miladi M., Abdellaoui K., Ben Hamouda A., Boughattas I., Tlili H., Mhafdhi M., Acheuk F. et Ben Halima-Kamel M. 2018. Toxicité de la fraction active de *Pergularia tomentosa* et de la phéromone d'agrégation phénylacétonitrile contre les larves du quatrième stade de *Schistocerca gregaria*: Effets sur le comportement et l'activité de l'acétylcholinestérase. Tunisian Journal of Plant Protection 13 (2): 201-216.

Les insecticides chimiques restent l'approche la plus utilisée dans la lutte antiacridienne bien qu'ils représentent une menace pour la santé humaine et l'environnement. La recherche de méthodes alternatives de lutte, efficaces et respectueuses de l'environnement, est devenue indispensable. Le but de ce travail est d'étudier l'effet de la phéromone d'agrégation, phénylacétonitrile, seule ou en combinaison avec la fraction active de *Pergularia tomentosa* sur les larves de *Schistocerca gregaria*. Les essais biologiques de toxicité ont montré que la combinaison du phénylacétonitrile avec la fraction active de *P. tomentosa* a augmenté significativement la mortalité larvaire. Les résultats ont également montré que la phéromone d'agrégation a entraîné une mortalité significative, en particulier après 6 heures d'exposition. La phéromone a également provoqué des troubles du comportement (locomotion et alimentation) et a augmenté l'activité de l'acétylcholinestérase chez les larves de *S. gregaria*. Nous avons également noté la présence du phénomène de cannibalisme. Le phénylacétonitrile semble avoir un effet sur le polymorphisme phasaire chez les imagos de *S. gregaria* qui ont présenté des caractères morphométriques spécifiques à la phase solitaire.

Mots clés: Comportement, criquet pèlerin, enzyme, insecticide botanique, phéromone

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ملخص

الميلادي، مريم وخميس عبد اللاوي وأمال بن حمودة وعتاب بوغطاس وهيثم تليلي ومنى المحافظي وفاطمة عشاق ومنية كامل-بن حليمة. 2018. سمية المستخلص الفعال لنبتة *Pergularia tomentosa* وفيرمون التجميع phenylacetonitrile على يرقات الطور الرابع ليرقة الجراد الصحراوي *Schistocerca gregaria*: التأثيرات السلوكية ونشاط الأنزيم acetylcholinesterase.
Tunisian Journal of Plant Protection 13 (2): 201-216.

تعتبر المبيدات الحشرية الكيميائية الطريقة الأكثر استخداما في مكافحة الجراد على الرغم من أنها تشكل تهديداً على صحة الانسان والبيئة. لذلك أصبح البحث عن طرق مكافحة بديلة فعالة ومنسجمة مع المحيط أمر لا غنى عنه. يهدف هذا البحث إلى دراسة مدى تأثير فيرمون التجميع phenylacetonitrile بمفرده أو بالاشتراك مع المستخلص الفعال لنبتة *P. tomentosa* على الطور الرابع ليرقات الجراد الصحراوي *S. gregaria* . أظهرت الاختبارات الحيوية أن الجمع بين المستخلص الفعال لنبتة *P. tomentosa* و فيرمون التجميع phenylacetonitrile يأدي إلى زيادة كبيرة في معدل الوفيات لدى الطور اليرقي المذكور. كما أثبتت النتائج أيضاً أن التعرض إلى phenylacetonitrile بصفة منفردة لمدة 6 ساعات يأدي بدوره إلى ارتفاع في نسبة الوفيات كما يتسبب أيضاً في اضطرابات سلوكية وزيادة نشاط أنزيم acetylcholinesterase. لاحظنا أيضاً أن لهذا الفيرمون تأثير على ظاهرة التحول المرحلي لليرقات.

كلمات مفتاحية: أنزيم، جراد صحراوي ، سلوك، فيرومون، مبيدات الحشرات النباتية

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