

Crude *Artemisia judaica* as a Natural Repellent Against the Egyptian Yellow Fat-tailed Scorpion *Androctonus australis*

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ABSTRACT

Scorpion envenomation is a global life-threatening hazard. Therefore, there is a pressing need to find repellents against these animals for safer residential and human activity areas. The primary objective of this study was introducing a reliable natural repellent as a safer alternative to current pesticides that are mainly chemicals imposing health hazards and risks of environmental contamination. It is also aimed to investigate the effects of some repellents on the circadian clock, in addition to the involvement of pectines as the main chemoreceptors in regulating circadian locomotor activity under repulsive environmental stress in *Androctonus australis* scorpion. The repellency of crude litters of seven plants; artemisia, rosemary, cinnamon, ginger, peppermint, clove and cedar was investigated. Thus, sand substrate of a designed circular arena (\varnothing 120 cm) was covered with one candidate plant in each trial. A video-tracking system supported with infrared camera was used for data acquisition and analysis of circadian locomotor activities and spatial preference of intact and pectine-amputated scorpions, under free-running continuous darkness conditions (DD). Results illustrated the normal circadian locomotor activities in scorpions. They revealed a significant repulsive effect of *Artemisia judaica* ($p < 0.005$), where animals spent ($85\% \pm 5.81$) of total time away from the substrate covered with *Artemisia* in comparison to the other plants. Pectine-amputation appeared to result in a significant loss of discrimination between the substrate covered with *Artemisia* and normal sand ($p < 0.005$). Furthermore, *Artemisia* appeared to have no significant effect, neither on the average daily locomotor activity nor on the free-running period of the circadian clock. It could be concluded that *Artemisia* represents a safe natural repellent for scorpions. Under the current conditions, this repulsive environmental stressor has no significant influence on the scorpion circadian output.

Key words: *Artemisia judaica*, scorpion, plant repellents, circadian, *Androctonus australis*

INTRODUCTION

Important epidemiological studies indicate that scorpion envenomation (SE) is a public health problem, sometimes a life-threatening hazard, especially in tropical and subtropical regions of Central and South America, North Africa, the Middle East, and South Asia (Isbister and Bawaskar, 2014). In the world, approximately 1700 species of scorpions are described, only about thirty of them are recognized as potentially dangerous for humans (Chippaux and Goyffon, 2008; Stockmann and Ythier, 2010). SE can vary widely from just local pain or inflammation to inducing severe and sometimes fatal complications. The morbidity and mortality of scorpion envenomation is directly related to the sting-treatment interval (Ganesh and Kumaravel, 2016). Risk is elevated for children especially in rural areas and during summer. Therefore, there is a pressing need to find repellents against scorpions for safer residential and human activity areas.

Current countermeasures against insect and invertebrate pests rely heavily on chemical pesticides that impose health hazards and risks of environmental contamination (Krieger, 2001). Therefore, there is an urgent need to develop or find safe alternatives. Many spices, herbs and their extracts were found to have pesticide activities frequently existing in their extracted essential oils. Extracts of artemisia, rosemary, cinnamon, ginger, peppermint, and others have shown

repellent and pesticide activities (Priestley *et al.*, 2006; Williamson, 2007; Williamson *et al.*, 2007; Gillij *et al.*, 2008; Khandagle *et al.*, 2011).

The primary objective of the current study is to identify an environmentally friendly, easy to use, natural plant with a repulsive effect against scorpions. Additionally, it aims to investigate the role of pectines; scorpions' primary chemo- and mechanoreceptors, in receiving chemical stimuli and determining spatial navigation preferences as well as the possible involvement of the circadian clock in regulating locomotor activity under repulsive environmental stress.

MATERIALS AND METHODS

Experimental animals

Adult scorpions, *Androctonus australis* (Linnaeus, 1758), were used in this study. They were captured from the western coastal desert of Egypt. Sixty scorpions; average weight is 4 ± 0.5 g, were housed in the animal house of Zoology Department at Suez Canal University, Ismailia, Egypt. They were kept at a relative humidity of 40-60% and a temperature of $25 \pm 2^\circ\text{C}$ under natural day/night cycles of (LD 13:11). Each scorpion was kept separately in a plastic jar (height 20 cm \times radius 14 cm) with 2-3 cm of sand. All animals were fed on live cockroaches of *Periplaneta americana*; every 2 weeks.

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Plant treatment

Seven commercial crude plants (*Artemisia*, Cedar, Cinnamon, Clove, Ginger, Peppermint, and Rosemary) were selected to investigate their repulsive potential against scorpions. Powders of aerial parts were applied except cedar which was used in an oil form (Table 1). In each trial, an amount of 40 g of plant candidate, was spread evenly over the sand in one half of the arena; leaving the other half with pure sand (Fig. 1 B2). In set of experiments, 80 g of *A. judaica* were used to cover all sand substrate, forming a complete repulsive habitat as an environmental stressor (Fig. 1 B3).

Table (1): List of plant candidates, sources and weights used in the experiments.

Plant	Source	Weight
<i>Artemisia (Artemisia judaica)</i>	Dried branches & leaves	40 & 80 g
Cedar wood tree (<i>Calocedrus sp.</i>)	Oil	40 g
Cinnamon (<i>Cinnamomum zegalanicum</i>)	Dried grinded bark	40 g
Clove (<i>Eugenia aromatica</i>)	Dried fruits	40 g
Ginger (<i>Zingiber officinale</i>)	Dried aerial branches	40 g
Peppermint (<i>Mentha piperita</i>)	Dried branches & leaves	40 g
Rosemary (<i>Rosemarinus officinarum</i>)	Dried leaves	40 g

Video tracking system

Scorpion navigational behavior was recorded using surveillance IP infra-red cameras with a convenient resolution of (640×480 pixels) which are connected to a computer where video tracking data was saved (Fig. 1). Locomotor activity and spatial navigation were recorded for 7-14 days in each trial. Data of control groups was recorded for longer periods (up to 42 days) to allow a thorough monitoring of the scorpion circadian rhythms.

Experimental design

Experiments were conducted in a lightproof room under free-running conditions of continuous darkness (DD) and room temperature of 25±2°C. Special arenas were designed where each arena is composed of a wooden surface (diameter = 120cm) covered with a sand layer (3 cm). The arena is securely bordered with a circular plastic wall (height = 30cm, circumference = 377cm). Total arena area is 1.1304 m². The experimental design is illustrated in Figure (1).

In this study, 60 scorpions divided into 4 main groups were used; with at least five replicates for each experiment. Scorpions were fed one day before each trial and each scorpion was used once in the experiments to avoid any confounding memory effects. Scorpion locomotor activities were monitored where each animal was allowed to move freely inside the arena. Substrate was completely swept and renewed after every trial. The four experimental groups and their objectives were as follows:

- 1) A control group was used for monitoring the normal circadian locomotor patterns on sand substrate. Two crumpled pieces of cardboard were used as scorpion shelters on each side (Fig. 1 B1).
- 2) A group, was subdivided into seven subgroups, each to investigate the repulsive effect of one of the seven candidate plants. In each trial, an amount of 40 g of the experimental plant was spread evenly over the sand in one half of the arena; leaving the other half with pure sand. Scorpion shelters were placed at the sides of the arena (Fig. 1 B2).
- 3) A third group to investigate the influence of an effective natural repellent on the potential circadian clock modulation in a continuous repulsive environment. These scorpions were tested in an arena fully covered with 80 g of *A. judaica* (the efficient repellent out of the 7 candidates tested). (Fig. 1 B3).
- 4) A final group to investigate the potential role of pectines in determining the scorpion's spatial preference and its possible involvement in circadian clock modulation. Pectines were cut from their base. Two days after removing the pectines, each amputated-scorpion was introduced into the arena with (*Artemisia*/Sand substrate); one half-covered by 40 g of *A. judaica* as a repulsive plant. The other half was only a sand substrate, with a shelter on each side (Fig. 1 B4).

Data Analysis

The video behavioral data was analyzed using (Smart Video Tracking Software, Panlab). Total locomotor activity per hour and percentage of spatial preference of the total time spent by the scorpion on each substrate were calculated. Microsoft Excel 2010 and IBM SPSS version 21 were used for data analysis. Student t-test and one-way analysis of variance (ANOVA) followed by Post-hoc Tukey test were used for further statistical analysis at the significant level of p<0.05. Data is represented as mean±SD. Actograms of circadian activity and Chi-square periodogram analyses of the free-running period (τ , tau) of the locomotor activity were produced using Actogram J (Schmid *et al.*, 2011); freely available at (<http://actogramj.neurofly.de/>), which works as a plugin in ImageJ software, version 1.50d; a freeware for image processing in life sciences (<http://imagej.nih.gov/ij/>).

RESULTS

The scorpion circadian locomotor activities

As illustrated in Figure (2A), under continuous darkness and on sand substrate, intact scorpions exhibited their normal circadian locomotor pattern; being active during the subjective night and rest during the subjective day. This endogenous rhythm persisted

under these DD conditions. The free-running period τ (Tau) reached (24 ± 0.68) hours (Fig. 2B). The average daily total activity of scorpions was (74.66, $SD=12.7$) meter/day with an average distance travelled (6.22, $SD=1.06$) meter/hour during the subjective night. On the sand substrate, control scorpions wandered evenly in the arena spending nearly about 50% of their time in each half with an average percentage of (51.8, $SD=4.15$) of their two-week period spent in the arena.

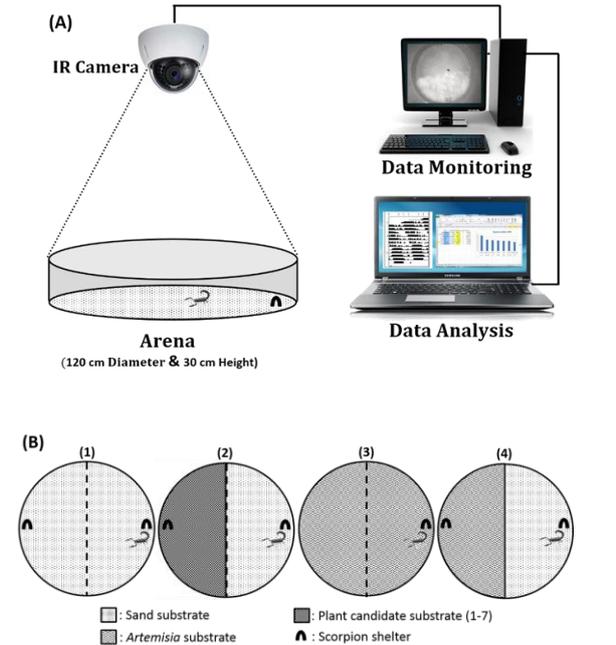


Figure (1): The experimental design illustrating (A) locomotor activity data acquisition setup, and (B) arena layout.

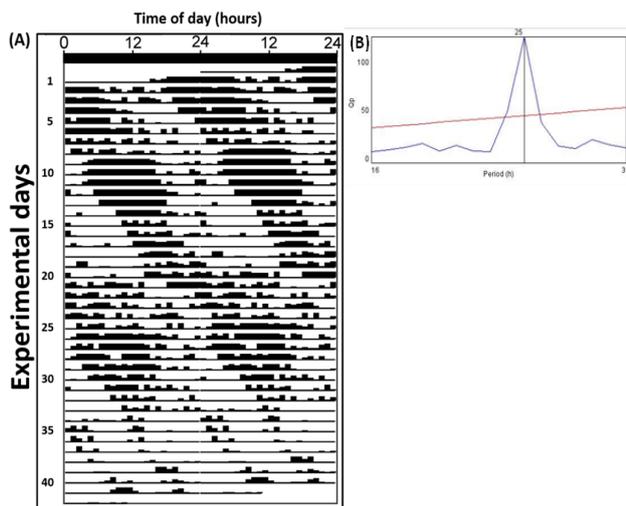


Figure (2): A representative double-plotted actogram (A) of the scorpion *Androctonus australis* locomotor activity illustrating the free-running rhythm on sand substrate along 42 recording days, (B) Chi-square periodogram showing the calculated period length τ (Tau) of 25 hours. Black bar above the figure indicates the continuous darkness conditions (DD).

Comparison of the repulsive effect of plant candidates

The percentage of time spent by each scorpion subgroup inside the arena away from the plant-covered substrate during two-week test period is given in (Fig. 3). One-way ANOVA statistical analysis revealed a significant effect of plant treatment on scorpions' spatial preference ($F_7, 32=4.82, p<0.001$). Post-hoc comparisons using the Tukey test showed that *Artemisia* was the only plant to have a significant repulsive effect on scorpions compared to control with a mean score of (33.01, $SD=16.35$), with scorpions spending about 85% of their time away from the *Artemisia* substrate. The same test revealed that, the treatments of rosemary, cinnamon, ginger, cedar oil, clove and peppermint did not differ significantly from the control, with the scorpions showing indifferent spatial preference or avoidance for the arena's substrate covered with those plants and spending nearly about 50% of their time in each half.

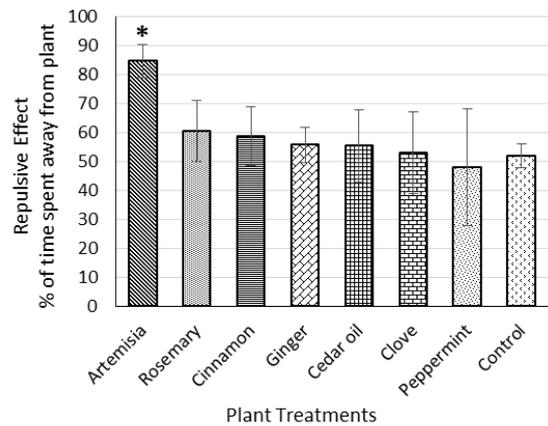


Figure (3): Repulsive effect of plant treatments on the spatial preference of *Androctonus australis* scorpion. The percentage of time spent away from the plant-covered substrate was calculated during two-week video recording period, under free-running continuous darkness conditions (DD). Control scorpions were tested in arenas with sand substrate only. Data represents the average of five replicates as mean \pm SD.

* indicates a significant repulsive effect of *Artemisia* on scorpions ($p<0.05$).

Role of pectines in determining the spatial preference

Because of its significant repulsive effect, *Artemisia* was used to investigate the role of pectines in spatial preference. A one-way ANOVA followed by a post-hoc Tukey test confirmed the significant repulsive effect of *Artemisia* on scorpions with intact pectines ($F_2, 12=64.1, p<0.0001$) which spent about 85% of their time away from *Artemisia*. However, the test revealed a non-significant difference between the spatial preference of scorpions with amputated pectines and intact scorpions (Fig. 4). Both subgroups; intact (as a negative control) and amputated scorpions, spent about

50% of their time indifferently on either the sand substrate or the *Artemisia* substrate, respectively.

Effect of *A. judaica* on daily total locomotor activity

To investigate the possible effect of repellents on the total daily locomotor activity of scorpions, the average daily total distance covered in meter/hour of control scorpions on sand substrate was compared with scorpions on arenas with *Artemisia* substrate.

Student's Two-Sample t-test revealed no significant effect of *Artemisia* on the average daily total activity of scorpions ($t_9=0.53$, $p=0.30$; NS), with scorpions on sand travelling (75.83, SD=13.84) meter/day, against (73.07, SD=13.54) meter/day for scorpions in arenas with *Artemisia*. However, the locomotor activity of pectine-amputated scorpions in arenas with *Artemisia*/Sand substrate was significantly decreased. ANOVA test followed by a Tukey test revealed a significant drop in their locomotor activities to (46.66, SD=6.59) meter/day, ($F_2, 13=9.73$, $p<0.005$) (Fig. 5).

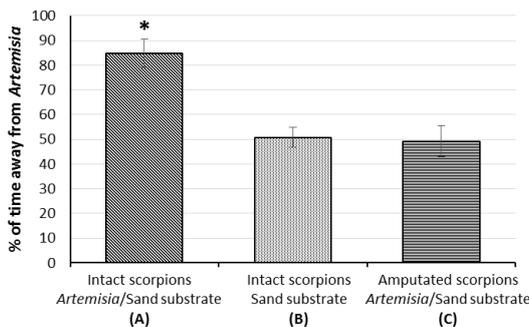


Figure (4): Role of pectines in determining the scorpion *Androctonus australis* spatial preference. In a two-week video recording period, intact scorpions (B) spent as long as amputated scorpions (C) on either sand or *Artemisia*/Sand substrates, respectively. Note: scorpions with intact pectines showed a significant avoidance response to *Artemisia* on *Artemisia*/Sand substrate (A).

* indicates a significant avoidance response of intact compared to amputated scorpions ($p>0.05$).

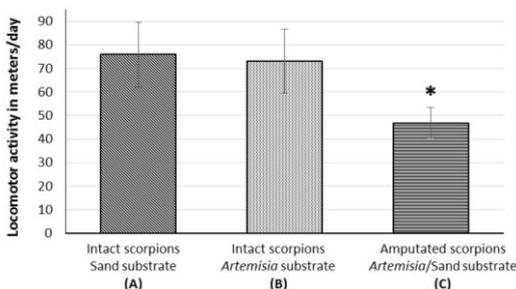


Figure (5): Average of daily total locomotor activity of scorpion *Androctonus australis* on sand (A), *Artemisia* (B) substrates in comparison with amputated-pectine scorpions on *Artemisia*/Sand substrate (C). Note: total locomotor activity decreased significantly in (C).

* indicates a significant drop in locomotor activity of scorpions with amputated pectines compared to the two other groups ($p<0.05$).

It was noticed, however, that the locomotor activity dampened gradually during the two-week recording period and sometimes interspersed with gaps of low activity, some scorpions even had 1-2 days of inactivity. In addition, under DD free-running conditions, the phase of locomotor activity during the subjective night exhibited a daily delay of about 1-2 hours per cycle, so that, in the second week the activity was gradually phase shifted (Figs. 6 & 7).

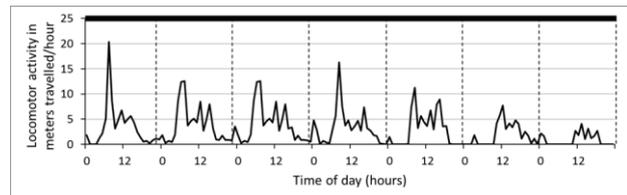


Figure (6): Daily average of total locomotor activity of a control scorpion *Androctonus australis* on sand substrate represented by distance travelled in meter/hour during the second week of recordings. Black bar above the figure represents DD conditions, whereas dotted vertical lines represent day ends.

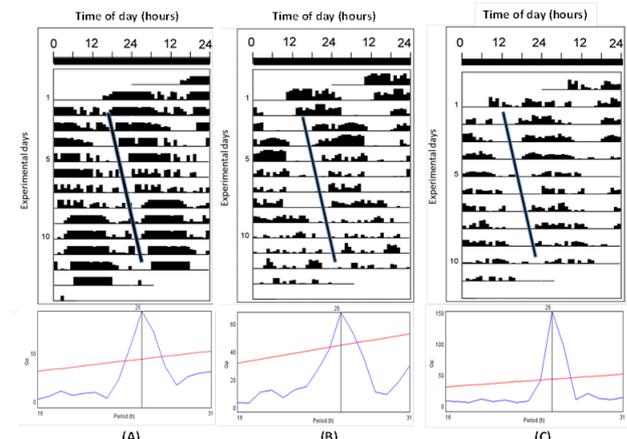


Figure (7): Representative double-plotted actograms of the scorpion *Androctonus australis* circadian locomotor activities comparing the free-running rhythms of (A) intact scorpion on sand, (B) intact scorpion on *Artemisia*, and (C) pectine-amputated scorpion on *Artemisia*/Sand substrates. The Chi-square periodograms (lower traces) show the calculated period length τ (Tau) of 25 hours in all cases. It shows the persistence of the circadian rhythms. Black bars above the figures indicate the continuous darkness (DD).

Effect of *A. judaica* on circadian free-running period

In order to investigate whether the plants in the surrounding environment would have any effect on the scorpion circadian clock and if the pectines, as major sensory organs, could contribute to or affect the clock input, we compared the free-running period τ of scorpions under DD conditions in arenas on sand only, *Artemisia* only and *Artemisia*/Sand substrates. A one-way ANOVA followed by a post-hoc Tukey test revealed no significant effect of *Artemisia* on the free-running period of scorpions compared to controls (F_2 ,

13=0.18, $p=0.84$; NS), with the average free-running period length τ being longer than 24 hours; ($\tau=24.75$, $SD=0.58$) for intact scorpions on sand only, ($\tau=24.83$, $SD=0.51$) for intact scorpions on *Artemisia*, and ($\tau=24.68$, $SD=0.46$) for pectine-amputated scorpions on *Artemisia*/Sand substrates. Actograms and periodograms of the free-running scorpions' circadian rhythms are demonstrated in (Fig. 7).

DISCUSSION

The current study proved that powders of aerial parts of *A. judaica* could be applied as a natural repellent plant against scorpions providing safer residential and human activity areas.

Repulsive effect of *A. judaica*

Using a crude plant as a natural repellent is safe from the environmental point of view, for minimizing the risks of disrupting the ecological balance or the target animal population. On the contrary, using pesticides creates and complicates ecological problems, affecting population numbers and leaving dangerous chemical residues in the environment.

The current results demonstrated a repulsive effect of crude litters of *A. judaica* against scorpions. Scorpions spent about 85% of total time away from *Artemisia* substrate (Fig. 3). Previous studies indicated that *Artemisia* and other plants are promising candidates for providing a natural repellent. *Artemisia* extracts were found to have repellent and pesticide activities (Özek *et al.*, 2014). *A. judaica*'s essential oil reduced egg laying and F1 progeny in the cowpea weevil, *Callosobruchus maculatus* (Abd-Elhady, 2012). The extracted oil from *A. annua* was applied as an anti-mosquito repellent (Yimer and Sahu, 2014). The same extract was found to be a feeding deterrent for the codling moth, *Cydia pomonella*, that helps reducing apple infestation (Durden *et al.*, 2011), the lesser mulberry pyralid, *Glyphodes pyloalis* and the elm leaf beetle, *Xanthogaleruca luteola*, accompanied by negative effects on the biochemical metabolism (Khosravi *et al.*, 2010; Shekari *et al.*, 2008). A mixture of essential oils from the leaves of *Artemisia princeps* and seeds of *Cinnamomum camphora* has synergistic repellent and insecticidal activities against the storage pests, *Sitophilus oryzae* and *Bruchus rugimanus* (Liu *et al.*, 2006). The repellent activities of *Artemisia* are suggested to be due the presence of some special chemicals in the plant's defense system, like, flavonoids, terpenes, tanins and sterols (Martin *et al.*, 2003, Zapata *et al.*, 2009). These chemicals might have evolved in some plants to play a direct role in defense. Flavonoides, for example were thought to have evolved for defense against herbivory (Treutter, 2006).

Based on the idea that flavonoids, terpenes and tanins may serve a defensive function for the plant, it could be suggested that scorpions might be able to link the presence of *Artemisia* with reduced number of other

insects, especially herbivores that might have been repelled away by the *Artemisia*'s defense chemicals. In other words, scorpions might be able to realize that the chances of capturing prey is lower near *Artemisia*, so they move away searching for an area with less repulsive plants with defensive chemicals and hence higher chances to find prey. Moreover, scorpions might have learned that insects that would be able to feed on *Artemisia* and resist its repulsive chemicals, could be less palatable and their bodies might harbor high concentrations of the *Artemisia*'s defense chemicals. This does not exclude the possibility that *Artemisia* might be able to repel the scorpions via other unknown pathways, probably through the nervous system.

Role of pectines in spatial navigation

The pectines have been argued to be the main chemosensory organs in scorpions (Gaffin and Brownell, 1997; Vinnedge, 2013; Wolf, 2008). They scan the substrate for foraging, localizing potential mates, and tactile discrimination of suitable substrates for spermatophore deposition, avoiding predators and finding suitable shelters (Brownell and Polis, 2001). Therefore, they are proposed to be prime candidates in determining spatial preference in response to chemicals in the surrounding environment. Considering the chemicals emanating from *Artemisia* substrate, results of this study revealed that scorpions with amputated pectines has significantly lost their ability to discriminate between the half of the arena covered with *Artemisia* and the half with sand only (Fig. 4) compared to scorpions with intact pectines, which spent 85% of their total time away from *Artemisia*. These results suggest that the chemoreceptive pectines play an important role in spatial navigation and foraging decisions.

The pectines are connected to the neural and muscular system and to other organs and structures (Root, 1990; Farley, 2001). So, the amputation of pectines could affect other parts of the animal's body (Mineo and Del-Claro, 2006), and this might be a possible reason for the unstable locomotor activities for scorpions during the next days after removal of their pectines. Also the current results revealed that the locomotor activity of scorpions with amputated pectines was interspersed with gaps of low activity and sometimes 1-2 days of inactivity (Fig. 7C). This reduced activity might be a consequence of injury or pain resulted from removing the pectines. While the few inactivity days might be a way to conserve energy after fully exploring the arena and finding no potential preys. The activity resumes later to scan the arena again for the possibility of finding novel preys.

Effect of *A. judaica* on average daily total locomotor activity

The results showed that *A. judaica* did not significantly affect average daily locomotor activity, however, scorpions with amputated pectines in arenas

with *Artemisia*/Sand substrate moved significantly less per day (Fig. 5). It is suggested that the injury sustained due to pectines removal could be the reason why the scorpions with amputated pectines moved less on arenas half-filled with *Artemisia*, but not the *Artemisia* itself. Since locomotor activity of intact control scorpions in arenas fully covered with *Artemisia* is not significantly different from controls.

Effect of *A. judaica* on circadian free-running period

The *Artemisia* substrate represents an environmental stressor. Therefore it is interesting to evaluate the contribution of the pectines sensory input on the scorpions' circadian clock input. We compared the free-running period τ of scorpions under DD conditions in arenas half treated with *Artemisia* with control scorpions in arenas with sand only. The present work showed that the circadian rhythm persisted and no significant difference between locomotor activities patterns of normal scorpions and amputated-pectines scorpions either on sand or *Artemisia* substrates. In addition, no significant effect of the *Artemisia* on the free-running period of scorpions, ($t_8=0.57$, $p=0.58$; NS), Figures (6 and 7). The free-running period was similarly longer than 24 hours (about 25 hours) in all scorpion cases, control on sand and on *Artemisia* as well as on *Artemisia*/Sand substrates. These results are consistent with Mineo and Del-Claro (2006) who reported that scorpions having pectines covered with paraffin showed no adverse impact upon its behavior during navigation. Also no significant difference of circadian locomotor activity was observed between intact scorpions and amputated-pectines scorpions in presence of sand substrate where a very close free-running period (24.75 h) was recorded in amputated scorpions under DD conditions (Baz, 2009).

In conclusion, the crude Egyptian plant *A. judaica* can be successfully used as a natural repellent against the expansion of scorpions toward the residential and human activity areas. It also evidenced that *A. judaica* had no significant effects on the scorpion circadian behavioral activities. Further experimentation on the repellent effect of extracts of different aerial parts of this plant is needed to facilitate its production for commercial purposes.

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نبات الشيح "ارتميزيا جودايقا" طارد طبيعي ضد العقرب المصري الأصفر عريض الذيل "اندروكتونص أستراليس"

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الملخص العربي

إن التعرض للدغات العقارب السامة يمثل خطراً يتعرض له الإنسان حول العالم ، ومن ثم فمن الضروري إيجاد مواد طاردة للعقارب لتأمين أماكن الإقامة والنشاط البشري. إن الهدف الرئيسي من هذه الدراسة هو تقديم مادة طبيعية طاردة يُمكن الاعتماد عليها مع الوضع في الاعتبار تقييم مدي إمكانية تأثيرها على إيقاع النشاط اليومي ، بالإضافة إلى التحقق من دور الزوائد المشطية (pectines) كمستقبلات كيميائية وميكانيكية في تنظيم إيقاع النشاط الحركي اليومي "circadian locomotor activities" تحت تأثير المادة الطاردة وذلك في العقرب المصري "اندروكتونص اوستراليس . *Androctonus australis*" لذلك تم التحقق من تقييم تأثير أجزاء من ٧ نباتات وهي كالتالي: الشيح ، إكليل الجبل ، القرفة ، الزنجبيل ، النعناع ، القرنفل والسيدر ، حيث تم تصميم حلبة دائرية (قطرها = ١٢٠سم) مع تغطية أرضيتها في كل تجربة بأحد النباتات محل الدراسة. ولقد تم استخدام نظام تتبع للفيديو من خلال كاميرات تعمل بالأشعة تحت الحمراء لتسجيل البيانات ، ثم تحليل نشاط الحركة اليومي ومدي تفضيل العقارب السليمة وكذلك المُستأصل زوائدها المشطية للأماكن المختلفة داخل الحلبة ولقد أجريت التجارب في ظروف معملية تمثل ظلام مستمر (DD). أظهرت النتائج نشاط حركي يتسم بإيقاع يومي طبيعي في العقارب ، ولقد أثبت التحليل الإحصائي وجود تأثير منفرد واضح لنبات الشيح حيث قضت العقارب حوالي (٨٥%±٥.٨١) من إجمالي وقت التجربة بعيداً عن الأرضية المُغطاة بالشيح بالمُقارنة بما قضته العقارب مع باقي النباتات الأخرى. ومن جانب آخر أظهرت العقارب مُستأصلة الزوائد المشطية عدم وجود فارق ذو دلالة احصائية في التفضيل بين الأرضية المُغطاة بالشيح مقارنة بالنتائج في الأرضية الرملية العادية ، وكذلك أوضحت النتائج أن نبات الشيح لم يسبب تأثيراً ملحوظاً على متوسط النشاط الحركي اليومي ولا على نمط إيقاع الساعة البيولوجية اليومية في العقارب. وخُصت هذه الدراسة الي استنتاج أن نبات الشيح يُمثل مادة طبيعية آمنة طاردة للعقارب ، وأن هذه المادة الطاردة لم يثبت لها أي تأثير جانبي على إيقاع النشاط اليومي للعقرب تحت الظروف المعملية الحالية للتجربة.