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First Attempts to Repel Scale Insects Using Plant Extracts: Effect on The Date Palm Scale *Parlatoria Blanchardi* Targ. (Hemiptera: Diaspididae)

Mahfoud Babaousmail[1]*, Mohamed-Azzedine Idder[1], Abdellah Kemassi[2]

[1] Laboratory of Date Palm Research, University of Kasdi Merbah Ouargla, BP. 155 Ouargla 30000, Algeria.

[2] Department of Biology, University of Ghardaia, BP. 455 Ghardaia 47000 Algeria.

ABSTRACT

Date palm, (Phoenix dactylifera L.), is a crop that plays a central role in the economy and the social life in the Middle East and North Africa. Date palm scale (Parlatoria blanchardi), is one of the most dangerous insect enemies of this crop. The aim of the present paper was to assess the repellent activity of some spontaneous plants against the crawlers of the first instar larvae of Parlatoria blanchardi. Methanol extracts of Castor bean Ricinus communis, Bitter apple Citrullus colocynthis and Syrian rue Peganum harmala were subjected to a phytochemical screening. Next, a bioassay was conducted to test their repellent effect on the crawlers compared to the control. The results indicated the presence of Tanins, Alkaloids, Polyphenols, Flavonoids and Saponins in the three extracts, whereas Terpenoids were not detected. The repellent bioassay showed that the mean number of crawlers found in negative control leaflets was significantly higher $P<0.05$ than most of the extracts' concentrations. Some concentrations of C. colocynthis and P. harmala, however, lost their efficacy after 11 days of treatment. Overall results may contribute to develop new strategies to control the scale insects' populations not only by toxicity but also by repellency.

Keywords: *Biopesticide, Date Palm Scale, Behavior, Crawler, Repellent.*

Corresponding author: Mahfoud Babaousmail
e-mail✉ babaousmail.mahfoud @ gmail.com
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1. INTRODUCTION

Mealybugs and scales (Order Hemiptera, Superfamily Coccoidea) comprise a group of small herbivorous insects with sucking mouthparts that colonize a wide variety of plants (Pellizzari et al. 2010). Scale insects affect crop quality and yield by excreting honeydew which encrusts the plant parts like leaves and fruits (Ben-Dov 1994). Date palm scale (DPS) *Parlatoria blanchardi* Targioni-Tozzetti1892 (Hemiptera: Diaspididae) species is a primary pest of the date palm *Phoenix dactelyfera* crop (Abbas et al. 2014; El-Shafie 2017). This species may infest all parts of the date palm with the heaviest infestations at the base of the older leaves (Swaminathan and Verma 1991; Idder et al. 2015). Heavy infestation may reduce the quality of the date fruits, and result in serious yield losses (Smirnoff 1957).

The date palm scales as the other scales are among the most difficult arthropod pests to manage because of their morphological traits that protect them from insecticides (Frank 2012). They are immobile for all, and just remain in the crawler stage. Adult and the nymphal instars are covered with a waxy coating which prevents insecticide from contacting their body beneath (Raupp et al. 2001). Hence, it is important to find a way to control scale insects by repelling the crawlers which may prevent them from settling in the host plant.

Plant extracts contain many secondary metabolites that act as repellents, feeding deterrents and toxins, which have a role in defense against herbivores, pests and pathogens (Maia and Moore 2011; Lee 2018). Yet, extracts and essential oils of plants with their phytochemicals are a promising source to control pests with less desirable properties compared to the conventional insecticides (Isman 2015).

Ricinus communis (Euphorbiaceae), *Citrillus colocynthis* (Cucurbitaceae) and *Peganum harmala* (Zygophyllaceae) are spontaneous plants that have been mainly used by the indigenous people for medicinal purposes and also to repel insects (Niroumand et al. 2016). In the present paper, it was aimed to find out the main phytochemicals present in the extracts prepared from these plants, and assess their possibility of repelling the crawlers of the date palm scale.

2. MATERIAL AND METHODS

Preparation of extracts

The extracts were prepared from 3 spontaneous plants, collected in April 2016 from the M'zab region, Algeria. The parts used included: Aerial parts of *Peganum harmala* (Zygophyllaceae), leaves and stems of *Citrullus colocynthis,* and leaves of *Ricinus communis.* The plants were rinsed with distilled water, air dried in shed for 7 days, and ground to the powder with an electrical blender. Methanol extract was prepared by mixing 100 g of powder with 400ml of methanol and 200ml of distilled water, in round bottom flasks and refluxed for 7 h in 40°C. The solutions were cooled at room temperature, and were filtered with a Whatman number 1 filter paper then concentrated using a rotary evaporator. The extracts were conserved at 5°C.

Phytochemical screening

The prepared extracts were subjected to the standard phytochemical screening to detect the presence of: Saponins, Tanins, Terpenoids, Polyphenols, Flavonoids and Alkaloids. The screening was carried out following the protocols described by (Harborne 1984).

Bioassays

Date palm (*Phoenix dactelyfera L.*) leaves of Ghars variety were collected from the experimental farm of the University of Kasdi Merbah in Ouargla, Algeria and brought to the laboratory. Using a pair of scissors, a sufficient quantity of palm leaflets pieces that measured 60 mm x 12mm was prepared from highly infested and non-infested leaves by DPS *Parlatoria blanchardi.* Each piece of the non-infested leaflet was treated using a hand-sprayer held 10-20 cm from the leaflet surface. Next, it was placed under a piece of the highly infested leaflet forming a Plus (+) shape, and stapled together in the middle to provide an easy movement of the crawlers between the leaflets (Fig. 1). Then, it was put in a 9cm Petri dish that contained moistened cotton which was watered when it was necessary during the experiment to keep the leaflets fresh. The Petri dish was kept open without a tip to avoid the high humidity that could inhibit the development of DPS (Idder-ighili et al. 2015).

The non-infested leaflets were assessed daily using a dissecting microscope at a magnification of ×4 and ×10 to check the presence of the first instar larvae crawlers. The number of crawlers has been recorded every 3 days from day 7 until the day 15 of the bioassay. The treatments included: the three extracts with different concentrations (8.8, 17.5, 35, 70 mg/ml) and distilled water as a control. Each treatment was replicated 5 times, but reduced to 4, due to the desiccation of some leaflets. The bioassay was carried out in April 2017 under the general laboratory conditions (26°C ±4, 24% RH, and a photoperiod of 16:8 [L: D] h).

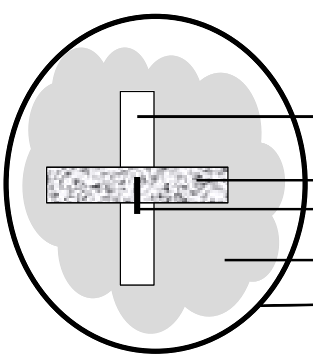

Figure 1: Repellent bioassay.

Data analysis

The collected data were analyzed using one-way analysis of variance (ANOVA). The statistical significant differences of the treatment were determined by Tukey post-hoc test to detect significant differences at the 0.05 percent level. All statistical analysis was carried out using SPSS 22.0 for Windows.

3. RESULTS

Phytochemical screening

The preliminary phytochemical screening results of the hydro-methanolic extracts of (*R. communis* leaves, *C. colocynthis* leaves and stems and the aerial parts of *P. harmala)* have been presented in Table1.

Table 1: Results of the preliminary phytochemical screening

Phytochemicals	*C. colocynthis*	*P. harmala*	*R. communis*
Tanins	+	+	+
Alkaloids	+	+	+
Polyphenols	+	+	+
Terpenoids	-	-	-
Flavonoids	+	+	+
Saponins	+	+	+

(+) present (-) absent

The results revealed that the three extracts contained Tanins, Alkaloids, Polyphenols, Flavonoids and Saponins whereas Terpenoids have not been detected in the three extracts.

Bioassay results

After seven days of the experience set-up, the crawlers have started to move from the highly infested leaflets to the non-infested ones. The movement was mainly observed in the control treatment (Distilled water), with an average of M= 4 larvae per leaflet, whereas the mean of larvae didn't exceed M=1 in all of the other treatments

Table 2: Post-hoc Tukey test to determine the significance of repelling DPS crawlers by treatments (the methanol extract of *R. communis P. harmala* and *C. colocynthis* at different concentrations) compared with control (distilled water) ,15 days after the treatment.

Treatment	Concentration mg/ml	Mean difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Peganum harmala	8,8	25,000*	5,297	0,002	6,16	43,84
	17,5	23,000*	5,297	0,006	4,16	41,84
	35	16,500	5,297	0,140	-2,34	35,34
	70	19,250*	5,297	0,041	0,41	38,09
Ricinus communis	8,8	24,500*	5,297	0,003	5,66	43,34
	17,5	24,250*	5,297	0,003	5,41	43,09
	35	25,000*	5,297	0,002	6,16	43,84
	70	24,500*	5,297	0,003	5,66	43,34
Citrullus colocynthis	8,8	24,250*	5,297	0,003	5,41	43,09
	17,5	17,500	5,297	0,092	-1,34	36,34
	35	25,000*	5,297	0,002	6,16	43,84
	70	18,750	5,297	0,052	-0,09	37,59

*. The mean difference is significant at 0.05.

After 15 days of spraying the non-infested leaflets, the number of crawlers in the non-infested leaflets has been recorded. Then, one-way analysis of the variance between the groups was conducted to evaluate the repellency of treatments towards the crawlers of DPS. There was a statistically significant difference at the $p<0.05$ level [$F (2, 42) =3.26$, $p=0.002$].

Post-hoc pairwise comparisons using Tukey test indicated that the mean number of the crawlers moved to the non-infested leaflets was significant in all leaflets treated by *R. communis* P< 0.05, but for *C. colocynthis* and *P. harmala*, the significance in the treatments was dependent on the concentration. There was no statistically significant difference between the control treatment and the leaflets treated by *P. harmala* at the concentration of 35mg/ml ($p=0.14$) and *C. colocynthis* at the concentrations of 17.5 and 70 mg/ml $p=0.092$, $p=0.052$; respectively (Table 2).

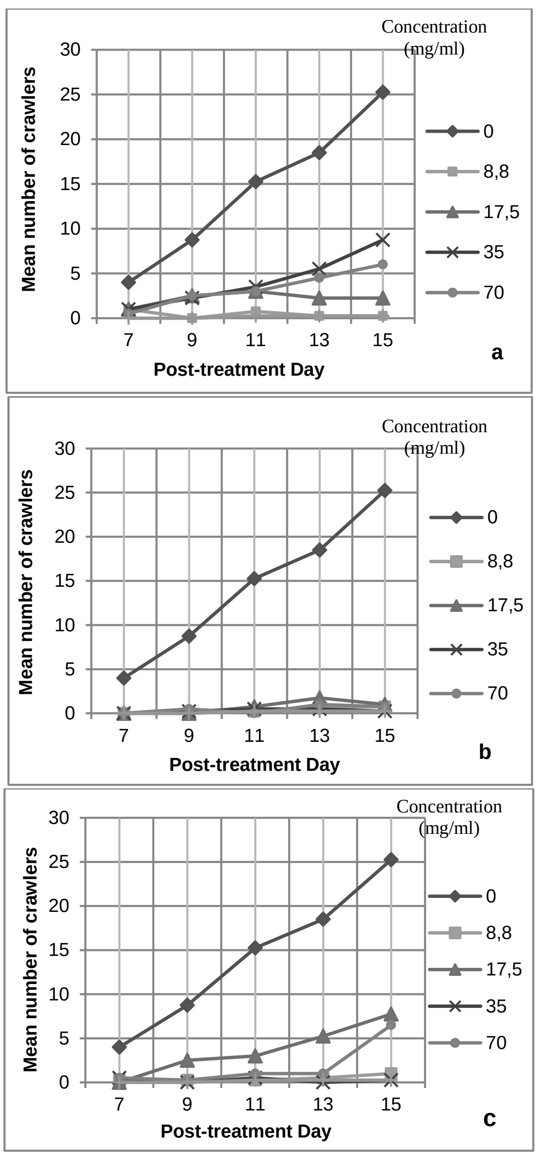

Figure 2: The repellent effect of methanol extracts of plants tested. The lines represent the means of crawler larvae found in treated non-infested leaflets, from day7 to day 15, by each plant extract (**a** *P. harmala,* **b** *R. communis,* **c** *C. colocynthis*) compared with the control.

From the data shown in (Fig. 2), it is apparent that the number of crawlers in control treatment was increasing with time till the day 15 (end of bioassay), it reached M=25.5 whereas the highest mean recorded in the other treated leaflets was M=8.5 in case of the leaflets treated by *P. harmala* at the concentration of 35mg/ml.

Regarding to the leaflets treated by the plant extracts, the evolution of mean crawlers number was between M=0 and M=1.75 during the bioassay in *R. communis* treatments (Fig. 2b), however, a remarkable increasing was observed from the day 11 at the higher concentrations of 35 and 70 mg/ml in case of *P. harmala* (Fig. 2a) and at the concentrations of 17.5 and 35 mg/ml of *C. colocynthis* (Fig. 2c).

During this bioassay, M=1 was the highest mean number of crawlers recorded in the non-infested leaflets treated by the lower concentration of 8.8mg/ml of the three plant extracts, this revealed that there was no clear correlation between the concentration of the extracts and their repellent effects towards the DPS crawlers of *Parlatoria blanchardi*.

4. DISCUSSION

In the present work, the crude extracts of *C. colocynthis, P. harmala* and *R. communis* were screened for their secondary metabolite composition rather than their possibility of repelling the DPS crawlers.

The phytochemical screening results indicated the presence of Tannins, Alkaloids, Polyphenols, Flavonoids and Saponins in the extracts (Table 1); this result was consistent with similar previous studies. The presence of the cited phytochemicals was reported in *P. harmala* by Fatma et al. (2016), in *C. colocynthis* (Uma & Sekar 2014; Al-Snafi 2016) and in *R. communis* (Mamoucha et al. 2017; Mahmoud et al. 2014; Nemudzivhadi and Masoko 2015). Contrarily, Terpenoinds have not been detected in the three extracts (Table 1). Many factors may affect the variation of the secondary metabolites' composition of the plant extracts such as the environmental conditions, geographic variations and the extraction methods (Figueiredo et al. 2008). This evidence might explain the differences between the phytochemical screenings results of the same plant species.

A remarkable result that was emerged from the bioassay data was that the highest number of DPS crawlers' which have moved to the non-infested leaflets was recorded in leaflets treated by distilled water as control (15 days after treatment, P was significant at P 00.5) compared to most of the concentrations of the three plant extracts treatments (Table 2). Hence, there is a relationship between the behavior of crawlers (whether or not to move to the non-infested leaflets), and the leaflets' treatments.

It was demonstrated by (Renard et al. 1998) that the behavior of Mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) crawlers were affected by the variety of their host plant (cassava *Manihot esculenta)*; the crawlers spent less walking time and then fixed quickly on a preferred cassava variety, needed more time to fix in less preferred one, and attempted to escape on non-host plants. The findings of this study suggested that the tested extracts have affected the crawlers' decision in settling in the non-infested leaflets, due to the odors that could be released by the phytochemicals detected in the extracts. There have been several studies that supported this hypothesis. It was demonstrated by Calatayud and Rü (2006) that mealybug scales *Phenacoccus manihoti* are able to perceive the odors present in the thin air layer above the leaf surface (the boundary layer), which consequently affects their decision in choosing the fixation site on leaves of the host plant (Renard et al. 1998).

The number of DPS crawlers was increased remarkably from day 11 at the higher concentrations of 35, 70 mg/ml in case of *P. harmala* (Figure 2a) and at 17.5, 35 mg/ml in case of *C. colocynthis* (Figure 2c). This finding could be explained by the instability of the compounds induced in repelling DPS crawlers. The stability of plant derivate compounds were affected when exposed to the elements such as air, light, and elevated temperatures (Turek and Stintzing 2013).

The missing of the correlation between the concentration and the repellent activity of extracts might be due to the distribution heterogeneity of the phytochemical compounds in each spray, so they did not exhibit the same effect on the crawlers. This problem has been widely indicated in dose-response bioassays (Robertson et al. 2017).

5. CONCLUSIONS

In summary, the Date palm leaflets sprayed with the tested extracts were less preferred by the DPS crawlers to settle in compared to the control treatment. It has been suggested that the phytochemicals detected in the extracts were responsible of disrupting the behavior of crawlers in recognition of the host plant.

In order to develop a new control strategy that prevents the fixation of the scale insects on the host plants, further deeper behavioral studies are needed to understand the relationship between the scale insect crawlers and their host plants.

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7. CONFLICT OF INTEREST

The authors declare that there was no conflict of interest regarding the publication of this article

8. AUTHORS' CONTRIBUTIONS

Study concepts and design: I. M.A., Conducting the experiment: B. M; Statistical analysis of the data: K. A., manuscript writing: B. M.; manuscript editing: K.A., manuscript revision: I.M.A., manuscript final version approval: B.M. and K. A. and I. M. A. All authors read and approved the manuscript.

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