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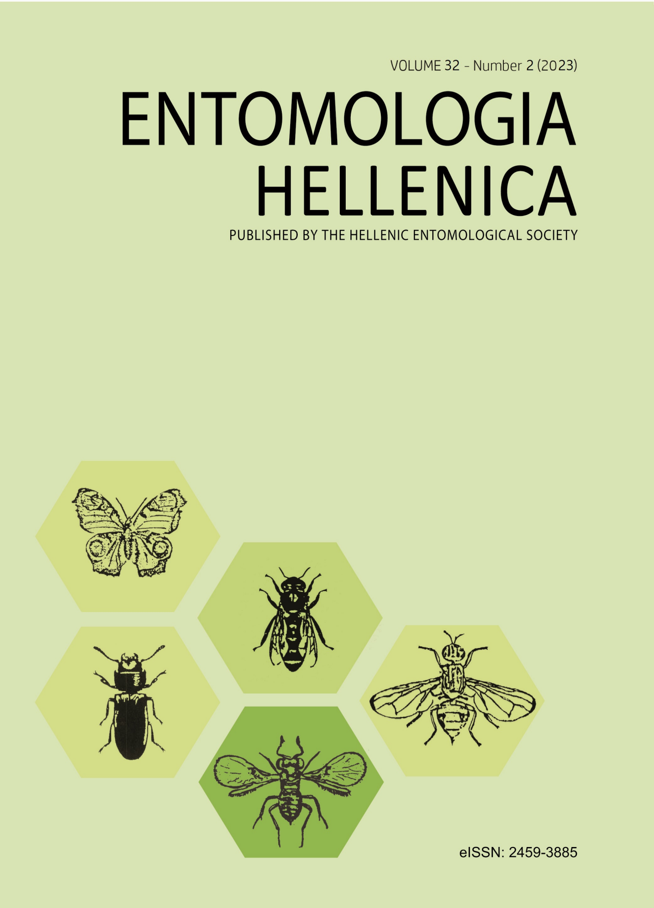

Aphicidal activity screening of plant extracts from Pistacia lentiscus (Anacardiaceae)

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Aphicidal activity screening of plant extracts from *Pistacia lentiscus* (Anacardiaceae)

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ABSTRACT

The excessive use of chemical pesticides has generated many problems to the human health and the environment. To reduce these problems, the use of biopesticides deriving from plants has a lot of advantages. In this context, the aim of this study was to investigate the effect of *Pistacia lentiscus* L. extracts on insect pests. In vitro tests of five essential oil concentrations (EOs) and five aqueous extracts were performed with respect to their efficacy against the rosy apple aphid *Dysaphis plantaginea* (Passerini). The obtained results revealed that the concentration of 9% was the most toxic against aphids (mortality rate of 50.77%), while the concentration 5% was the most repellent (repellency percentage -RP- 53.33%) among the examined aqueous extracts. Concerning the EOs, the 10000ppm treatment was the most efficient with a mortality rate of 71.13% and an RP of 66.67%.

KEY WORDS: Essential oils, aqueous extracts, *Dysaphis plantaginea*, aphid mortality, aphid orientation.

Introduction

Aphids are included amongst the most destructive and economically important plant pests on earth, causing severe financial losses worldwide (Yan et al., 2018). The rosy apple aphid, *Dysaphis plantaginea* (Passerini), is a key pest in western European apple orchards and one of the most problematic pests in organic apple production; the common strategy to control this aphid species in conventional apple production is application of aphicides just before flowering, very often followed by a second application after flowering or early in the summer (Cross et al., 2007). However, the massive and indiscriminate use of pesticides in crop protection, food preservation, and insect and pest control has led to acute or chronic poisoning issues in humans and domestic animals, while it has resulted in extensive ecological adverse effects (Gupta, 2016). Therefore, the use of alternative methods including biopesticides is encouraged to limit the negative effects of chemical plant protection products. In comparison to pesticides, biopesticides are an eco-friendly nontoxic natural type of pesticides, derived naturally like from plants, bacteria, and minerals which have the capacity to control pests without affecting the environment, so they constitute a major component of integrated pest management programs (Acharya et al., 2017). Besides, biopesticides are more or less naturally available, efficacious, easily dissociated and disintegrated, and bestowed

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with various modes of action and activity for effective management of agriculture pests (Dar et al., 2022). Moreover, they offer a unique opportunity for developing countries to explore and develop their own natural biopesticide resources in crop protection (Copping & Menn, 2000).

Plant extracts have been used for millennia as a means of pest control in agriculture, and the gradual banning of entire groups of chemical pesticides following the new European regulations has intensified interest in these natural substances (Lannou, 2022). They are often a source of novel chemical structures that differ from those more likely to be devised by traditional pesticide chemists (Duke et al., 2013). In fact, public and private research sectors around the globe are now working to develop new solutions to meet the environmental challenges of modern agriculture and better support human health and the environment (Siegwart & Lavoir, 2022).

On the other hand, *Pistacia lentiscus* L. (Anacardiaceae) includes numerous wild and cultivated species, widely distributed in the Mediterranean and Middle Eastern areas (Milia et al., 2021). Previous studies demonstrate different biological activities of *P. lentiscus* essential oils (EOs), including fumigant toxicity against moths (Bachrouch et al., 2010), repellency against coleopteran pests (Bougherra et al., 2015), antioxidant and anti-acetylcholinesterase activities (Aissi et al., 2016). In this context, the aim of the present study was to evaluate the aphicidal effect of different extracts from *Pistacia lentiscus* against *Dysaphis plantaginea* aphids, employing in vitro approaches.

Materials and Methods

Used materials and preparation of solutions. In our study, the rosy aphid colonies and the apple leaves (cultivar Royal *Gala*) that were subsequently used in the toxicity and repellency tests, were collected from an apple tree orchard from the region of Khenchela (northeastern Algeria).

The leaves of *P. lentiscus* that were used for the preparation of the aqueous extracts and EOs, were provided from the region of Mila (northeastern Algeria).

EOs were prepared using the hydro distillation technique. Fresh leaves of *P. lentiscus* were submitted to steam hydro distillation for three hours, according to the method described by Zeraib et al. (2014). The prepared essential oils were dehydrated over anhydrous sodium sulfate and stored in sealed glass vials at 4-5°C until further use. Subsequently, six solutions (100, 500, 1000, 5000, 10000 ppm and the control) were obtained by adding distilled water containing 2% Tween 20.

Concerning the aqueous extracts, the infusion method was adopted. Fresh infusions were prepared by macerating the crude powder of *P. lentiscus* leaves for a few minutes in cold or boiling water (Handa, 2008). In the present study, plant powder was diluted in heated distilled water. The obtained mixtures were stirred for a short while before being left for two hours. Next, the mixtures were filtered. Five concentrations were then obtained (1, 3, 5, 7 and 9 %), in addition to the control (distilled water alone).

Toxicity test. Under laboratory conditions, 36 Petri dishes were prepared (three replications for each treatment among the 12 tested solutions). Leaf discs of the same size were dipped in each concentration for 2-5 seconds. Each treated leaf was separately kept in a Petri dish with ten aphids. The number of deceased aphids was recorded 24 hours after treatment. The mortality percentage was corrected through Abbots' formula (1925):

$$\text{Corrected mortality rate} = [(\text{Tmp}-\text{Cmp})/(100-\text{Cmp})] * 100$$

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where:

Tmp = mortality percentage on treated leaf disc;

Cmp = mortality percentage on control leaf disc.

Repellency test. A total of 30 Petri dishes prepared with three replications for each concentration (five concentrations of EOs and five of the aqueous extracts). Each Petri dish was divided into two equal parts. For each replication, two leaf discs free from aphids were used, one disc, untreated, used as a control, while the second disc had been dipped in a treatment solution for 2-5 seconds. In the center of each dish, ten aphids were placed. The number of aphids moving to each side of the dish was recorded after 24h. According to the following equation, the percentage of repellency (PR) was calculated as by (Singh et al., 2012):

$$PR = [(NC-NT)/(NC+NT)] * 100$$

where:

NC = number of insects oriented towards the control;

NT = number of insects oriented towards the treated disc disc

Statistical analysis. ANOVA one way analysis (followed by Student-Newman-Keuls test) at the significance threshold of 0.05, was performed in order to compare the average aphid mortality of each treatment, using SPSS software (10th version). The same software was used to determine the Lethal Concentration 50 (LC_{50}) of the studied plant extracts, by *Probit analysis*.

Results and Discussion

Toxicity test

Concerning the essential oils, we noted that the corrected mortality rate of aphids was equal to or more than 50% for all treatments except for the rate of 1000ppm (28.50 %), and the difference was not significant between treatments ($p > 0.05$) according to ANOVA statistical analyses (Table 1). In addition, the estimated LC_{50} of the studied EOs was 349.33ppm.

TABLE 1: Corrected mortality percentages of aphids on different concentrations of *Pistacia lentiscus* EOs

Treatments (ppm)	**Corrected mortality after 24h (Mean ± Standard-Error)**
100	50.00 ± 16.05a
500	64.47 ± 06.79a
1000	28.50 ± 09.64a
5000	56.67 ± 07.41a
10000	71.13 ± 07.79a
Significance	*0.098**

** Significant at P<0.05*

For the aqueous extracts, the lowest aphid mortality was recorded on apple leaves treated by the concentrations 3%, 5% and 7% (3.70, 7.03 and 6.67%, respectively), while the highest for the concentration 9% (50.77 %). Moreover, ANOVA showed significant differences between the different concentrations (Table 2). On the other hand, LC_{50} for the aqueous extracts was 52.44 %.

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TABLE 2: Corrected mortality percentages of aphids on different aqueous extracts of *Pistacia lentiscus*

Treatments (%)	Corrected mortality after 24h (Mean ± Standard-Error)
1	13.70 ± 12.60a
3	3.70 ± 3.70a
5	7.03 ± 3.53a
7	6.67 ± 6.67a
9	50.77 ± 10.87b
Significance	*0.013**

* *Significant at P<0.05*

Previous studies have revealed that different types of plant extracts negatively affect the biology of aphids, including essential oils from some Asteraceae species on green peach aphid *Myzus persicae* (Hemiptera: Aphididae) (Czerniewicz et al., 2018), the oils of *Artemisia monosperma* and *Citrus sinensis* against the oleander aphid, *Aphis nerii* (Hussein et al., 2021), *C. limon* peel aqueous extract against aphids *Macrosiphum roseiformis* (Gupta et al., 2017), aqueous leaf extracts of *Calotropis gigantea* and *Croton laccifera* on the cowpea aphid *Aphis craccivora* (Thakshila et al., 2022), in addition to other kinds of plant extracts against *A. fabae* (Abdel-Rahman et al., 2019) and *Macrosiphum rosae* (Noureldeen et al., 2022).

The insecticidal effect is attributed to some compounds contained in the extracts. Plants produce a diversity of secondary metabolites that protect plants under biotic stress, as they act as antioxidants, osmoprotectants, antimicrobials, and repellents (Jha & Mohamed, 2022). For instance, the results of Sotelo-Leyva et al. (2023) indicated that the extract of *Ficus petiolaris* showed a toxic activity against the aphid *Melanaphis sacchari*, and its xanthotoxin compound showed strong aphicidal activity at low concentrations. Furthermore, Yan et al. (2018) found that among nine tested alkaloids from *Lycoris radiata* against *Aphis citricola*, N-allylnorgalanthamine possessed the highest aphicidal activity, and this compound showed an obvious inhibitory effect on AChE in *A. citricola* in a time- and dose-dependent manner.

Repellency test

Figure 1 shows the repellency percentage for all EOs, after 24h of insect releasing. It is noticed that the 10000ppm treatment recorded the maximal RP with 66.67 % repellency.

Concerning the aqueous extracts, the highest repellency rate was recorded for the 5% treatment with RP 55.33%, followed by the treatments 1% and 3% with 13.33% RP for both (Figure 2).

Plant secondary metabolites act either on the behaviour (repellent or antifeedant effects, scent masking) or on the physiology of individual herbivorous insects (inhibition of development, growth or reproduction) (Bardin, 2022). Due to their repellent properties, essential oils extracted from aromatic plants, could represent a valid, eco-friendly alternative to chemical repellents (Bougherra et al., 2015). In this study, the EOs of *P. lentiscus* recorded the best results of repellency compared to aqueous extracts. Essential oil from the leaves of *P. lentiscus*, growing in the Oran region in the west of Algeria, revealed that its main compounds were terpinene-4-ol, α-terpineol, α-pinene, limonene, β-myrcene,

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p-cymene, α-phellandrene and β-caryophyllene (Hamiani et al., 2016). Bougherra et al. (2015) mentioned that *P. lentiscus* EOs exert a broad-range unspecific repellency among three coleopteran pests, and they found that β-caryophyllene (a component of this oil) was able to exert the highest repellency rates against *Sitophilus zeamais*. However, previous studies conducted in Algeria (Dob et al., 2006), Tunisia (Aissi et al., 2016) and Morocco (Zrira et al., 2003) highlighted that the geographic sources of *P. lentiscus* significantly influence the chemical composition of their EOs.

It is noticed that the bioactivity of EOs is due to several compounds, mainly terpenoids, including monoterpenes and sesquiterpenes, and to a lesser extent phenylpropanoids (Siegwart & Lavoir, 2022).

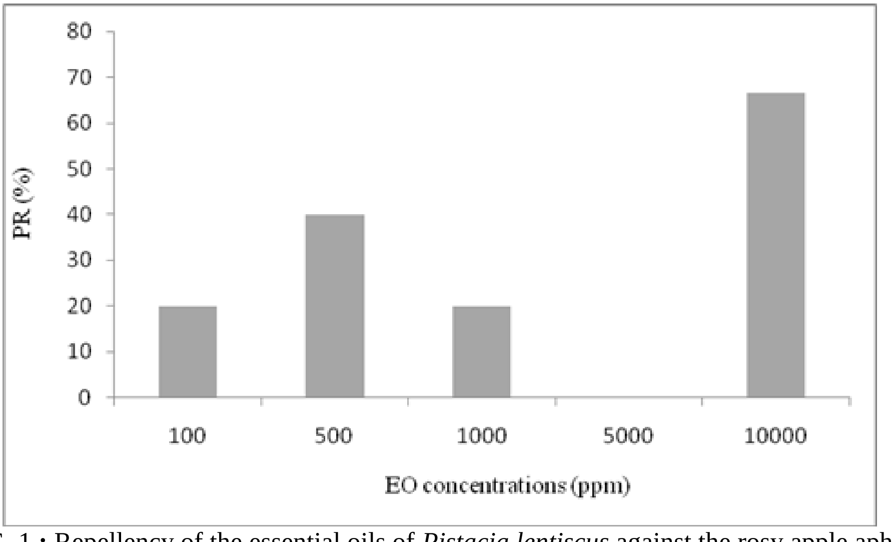

FIG. 1.**:** Repellency of the essential oils of *Pistacia lentiscus* against the rosy apple aphid

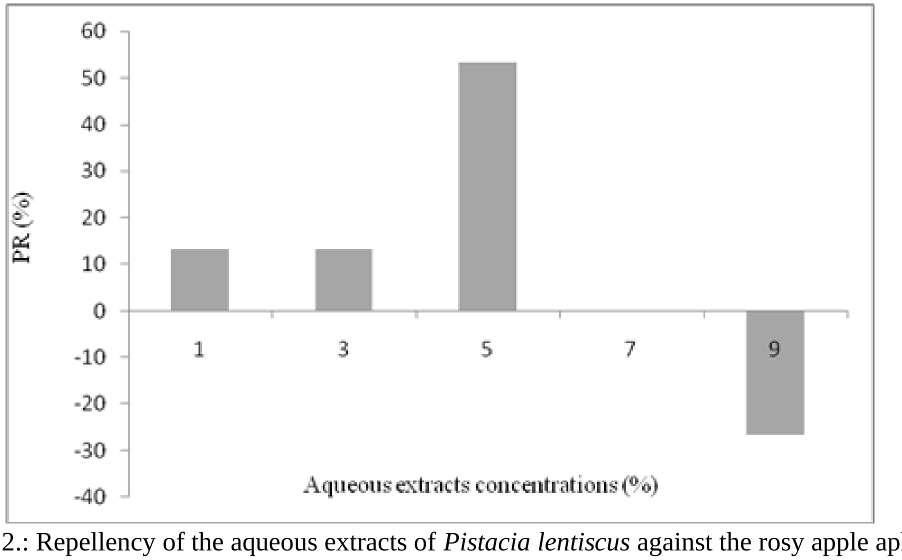

FIG. 2.: Repellency of the aqueous extracts of *Pistacia lentiscus* against the rosy apple aphid

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Conclusion

The aphicidal effect screening of different extracts of *P. lentiscus* against *D. plantaginea* allowed us to conclude that the EOs were more effective as compared to the aqueous extracts. The 10000ppm treatment was the most efficient and most repellent (71.13% and 66.67% respectively). Therefore, different types of *P. lentiscus* extracts may constitute interesting candidates as constituents of the integrated management of *D. plantaginea*, in order to reduce the volume of chemical pesticides and their side effects. Further large-scale field experiments with these compounds are needed to confirm the obtained results of the present study.

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Έλεγχος αφιδοκτόνου δραστικότητας φυτικών εκχυλισμάτων του *Pistacia lentiscus* (Anacardiaceae)

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ΠΕΡΙΛΗΨΗ

Η υπερβολική χρήση χημικών φυτοφαρμάκων έχει δημιουργήσει πολλά προβλήματα στην ανθρώπινη υγεία και το περιβάλλον. Για τη μείωση των προβλημάτων αυτών, η χρήση βιοπαρασιτοκτόνων που προέρχονται από φυτά έχει πολλά πλεονεκτήματα. Στο πλαίσιο αυτό, στόχος της παρούσας μελέτης ήταν να διερευνηθεί η επίδραση εκχυλισμάτων του *Pistacia lentiscus* L. σε επιβλαβή έντομα. Πραγματοποιήθηκαν *in vitro* δοκιμές πέντε συγκεντρώσεων αιθέριων ελαίων (EOs) και πέντε υδατικών εκχυλισμάτων σε σχέση με την αποτελεσματικότητά τους έναντι της αφίδας *Dysaphis plantaginea* (Passerini). Τα αποτελέσματα έδειξαν ότι όσον αφορά στα υδατικά εκχυλίσματα, η συγκέντρωση 9% ήταν η πιο τοξική κατά του εντόμου (ποσοστό θνησιμότητας 50,77%), ενώ η συγκέντρωση 5% η πιο απωθητική (ποσοστό απωθητικότητας -RP- 53,33%). Για τα EO, η δόση των 10000 ppm ήταν η πιο αποτελεσματική με ποσοστό θνησιμότητας 71,13% και RP 66,67%.

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