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IN VITRO SCREENING OF THE INSECTICIDAL ACTIVITY OF *Salvia microphylla* (Lamiaceae)

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Abstract

Aphids are important pests in fields and orchards. The use of chemical insecticides as the main control method resulted in many ecological and health problems. Thus, the development of alternative methods, such as botanical bio-pesticides becomes indispensable in recent years. In this context, we studied the insecticidal and repellent effects of different concentrations of essential oils (EOs) and aqueous extracts from Salvia microphylla (Lamiaceae) against Aphis craccivora aphids, under laboratory conditions. The results showed that higher concentrations of extracts (10000 ppm for EOs and 10% for aqueous solutions) proved significant aphicidal activity, where mortality rates of 100% and 74% were noticed, respectively. Furthermore, aqueous extract at 10% concentration recorded the highest repellent effect (repellency rate = 71.8%). Therefore, the use of extracts from S. microphylla may constitute an important tool in integrated pest management.

Key words: *essential oils, aqueous extracts, Aphis craccivora, toxicity, repellency.*

INTRODUCTION

Insects account for 20-30% of the loss of production, but in some cases, they provoke a total loss (De Geyter et al., 2007). They damage field crops by sucking, chewing, or boring into different plant parts (Singh & Kaur, 2018). Among their important groups, aphids (Homoptera: Aphididae) cause plant damage through insect feeding and transmission of virus diseases (Weigand & Bishara, 1991).
To control these pests, the spray of chemical pesticides was the most used method. Pesticides are synonymous with modern agriculture and provide the most effective and economically efficient means of controlling thousands of species of insect pests, weeds, fungi, and nematodes that compete for our food and fibre (Saxena et al., 2014). The worldwide annual consumption of pesticides is around 3 million tonnes, whereas the annual cost is approximately $100 billion (Kumar et al., 2022).
However, many problems appeared during the last few years due to the inappropriate employment of these chemical products. The continual addition of large amounts of persistent pesticides to the environment has caused widespread destruction of soil fertility and endangered the ecological security of food, and farmers (Saxena et al., 2014). Besides, uninterrupted use has resulted in the development of resistant strains of various insect pests (Singh & Kaur, 2018).
Therefore, there is a pressing need for new and unconventional insect pest management techniques. The agrochemical industries are trying to discover new methods to control economically devastating pests, like insects, along with the diseases these organisms are capable of vectoring (Gross, 2014). There are numerous alternative methods such as, cultural, physical, genetic, biological controls and the use of biopesticides.
The contribution of biopesticides to the insecticide sector accounts for 15% of the total insecticide market (Rajamani & Negi, 2021). Unlike conventional insecticides which are based on a single active ingredient, plant-derived insecticides comprise an array of chemical compounds that act on both behavioural and physiological processes

(Hassan Adeyemi, 2010). The important number of plants possessing insecticidal substances have generated remarkable interest in recent years, as potential sources of natural insect control agents (Mckenna et al., 2013). Different types of botanical extracts may be used, including aqueous solutions and essential oils (EOs). These latter are secondary metabolites synthesized by plants and play very important roles in plant defence and signalling processes (Kumar et al., 2022).
In this context, the present study aims to analyze *in vitro* the efficacy of *Salvia microphylla* (Kunth) extracts (aqueous and EOs) against the aphids *Aphis craccivora* (Koch). To our knowledge, this is the first study to demonstrate the insecticidal activities of *S. microphylla* extracts against aphids.

MATERIALS AND METHODS

Insect and plant materials and extraction

The plant material used in this experiment consists of uninfected leaflets taken from broad beans (*Vicia faba* L.), in addition to the aerial part of *Salvia microphylla* (Lamiaceae), collected from the province of Batna (Eastern Algeria). It is among the medicinal species used in folk medicine to treat gastrointestinal upsets and spasms; it is a small shrub, with elliptic-oval leaves and pink to red flowers (Duarte & Siebenrock, 2016).
Regarding the extraction, two methods were adopted, the infusion method to obtain aqueous extracts and hydro-distillation for EOs. We prepared five concentrations for each extract type: 100, 500, 1000, 5000, and 10000 ppm for EOs and 1, 3, 5, 7, and 10% for aqueous extracts.
Whereas the animal material consisted of *A. craccivora* (Aphididae) individuals, taken from the same colony.

Toxicity test

We adopted the contact toxicity test method, which was carried out in May 2022. Faba bean leaflet was dipped in the corresponding concentration for 2-5 seconds. Ten treatments and 3 replications for each one was considered, in addition to the control (distilled water for the test of aqueous extracts and distilled water and 2% Tween 20 for EOs). We put in each Petri dish, one leaflet and ten apterous aphids. After 24 hours, we determine the number of dead aphids, and we calculate the corrected mortality rate according to Abbott formula (1925):
Corrected mortality rate = [(Tmp – Cmp)/(100 – Cmp)] × 100.
Knowing that: Tmp: Treatment mortality percentage, Cmp: Control mortality percentage.

Repellency test

The repellent effect of *S. microphylla* extracts (aqueous obtained by infusion and EOs) on the aphids *A. craccivora* was examined using two choice method. In total, 30 Petri dishes were prepared. Each dish was divided into two equal zones: one occupied by the untreated bean leaflet (control) and the other with the treated leaflet. Ten insects were placed in the center of each dish. The procedure is repeated three times for the ten considered treatments. After 24 hours, the number of insects found on each part was noticed, then the repellent index (RI) for each treatment was calculated as follows: RI = [(C – T)/(C + T)] × 100 (Pascual-Villalobos & Robledo, 1999).
Being C = the number of aphids on the control and T = the number of aphids on the treated leaflet.

Statistical analysis

To compare the average aphid mortalities between different concentrations of each plant extract type, an analysis of variance with one factor (ANOVA) was carried out. When there is a significant difference, a test Student-Newman-Keuls is used to highlight the homogeneous groups. These analyzes were performed using SPSS version 10.0.5 for Windows (SPSS, Inc., USA). In addition, the Lethal Concentration 50 (LC 50) was determined using *Probit* analysis of same software.

RESULTS AND DISCUSSIONS

Toxicity test

In general, the highest mortality of *Aphis craccivora* individuals was recorded on leaflets of *Vicia faba* treated with the EO concentrations 1000, 5000, and 10000 ppm of *Salvia microphylla* (the corrected mortality rate was 92, 97, and 100% after 24 hours, respectively) (Table 1). Furthermore, the LC50 value was determined by a probabilistic log analysis. The LC50 of the considered treatment is 814 ppm.

Whereas for the aqueous extracts, the statistical analysis revealed also significant differences in aphid mortality rates between the different concentrations. We note that the mortality rate of aphids was low (not exceeding 50%) for treatments 1 and 5%, while the percentage was greater than 50% for treatments 5, 7, and 10% (Table 2). In addition, the estimated LC 50 of the studied treatment was 4.62%.

Table 1. Corrected mortality percentages of aphids on different essential oil concentrations of *Salvia microphylla*

Treatments	Corrected mortality percentages after 24 h (Mean ± Standard-Error)
100 ppm	0.00 ± 19.09 a
500 ppm	20.83 ± 15.02 a
1000 ppm	91.67 ± 8.33 b
5000 ppm	97.17 ± 15.02 b
10000 ppm	100.00 ± 0.00 b
Signification	0.001*

*Values indicated with different letters are significantly different at P<0.05

Table 2. Corrected mortality percentages of aphids on different aqueous extract concentrations of *Salvia microphylla*

Treatments	Corrected mortality percentages after 24 h (Mean ± Standard-Error)
1%	19.10 ± 3.30 a
3%	34.70 ± 6.55 b
5%	57.73 ± 2.38 c
7%	57.83 ± 6.82 c
10%	74.00 ± 3.70 c
Signification	0.000*

*Values indicated with different letters are significantly different at P<0.05

The findings of the present investigation revealed a significant aphicidal potential of *S. microphilla* extracts, particularly for higher concentrations. As *A. craccivora* provokes considerable damage to crops, many studies were conducted to test the insecticidal effect of many plant extracts, and they revealed promising results. Thus, Abdel-Aziz et al. (2015) mentioned that spray treatment of three essential oil formulations (rosacide, sagix and cura) was most effective than the systemic one against *A. craccivora*. The same authors found significantly lower activities of two aphid enzymes (Glutathione S-transferase and acetylcholinesterase) than the control, due to the three tested compounds. Besides, the aqueous extract obtained from *Santolina africana* were the most effective against the cowpea aphid, among four tested plant species, with a mortality rate higher than 80% after 24 h of artificial infestation (Lebbal et al., 2017).

Concerning the other types of extracts, Dolma et al. (2021) confirmed that parent extract/ fractions of *Trillium govanianum* showed promising efficacy against *A. craccivora*. While among stem extract/fractions from *Cissampelos pareira*, the *n*-hexane fraction was more effective than the water and *n*-butanol fractions (Kumari et al., 2022). In addition, datura and henbane extracted by petroleum ether showed high potency against the same pest in the laboratory (Abdu-Allah, 2012), and the dichloromethane extract of *Acorus calamus* rhizome was found to have fumigative toxicity to aphids (Bandara et al., 1990).

Furthermore, studies carried out against other aphid species showed important aphicidal effect of different plant extracts, such as the crude ethanol extract of *Artemisia judaica* (Acheuk et al., 2017) on *Aphis fabae* and the combine used of *Moringa olerifera*, *Azadirachta indica* and *Eucalyptus globules* leave extract against *Diurophous noxia* (Ali et al., 2015).

On the other hand, our study revealed a proportional relation between the concentration of the extract (aqueous or EOs) and the mortality rate. Similarly, Osman and Elsobki (2019) found that the reduction percentage in the population of aphid insect (*A. craccivora*) increases with increasing concentration of *Moringa oleifera* ethanolic extract (1-8%).

In the present study, the EOs were globally more efficient than the aqueous extracts. Likewise, Amamri et al. (2021) found that the mortality rate of *A. fabae* was less important for *Hertia cheirifolia* aqueous extracts in comparison with the EOs of the same plant species. Additionally, the seed oil of *Triadica sebifera* (LC50 = 850.94 mg/L) was more effective against *A. craccivora* compared to extracts/fractions/compounds (Dolma et al., 2022).

The calculated LC50 in our case (814 ppm), showed that the EOs of *S. microphylla* were highly toxic for aphids. In contrast, Ateyyat et al. (2012) indicated that among the oil extracts of five medicinal plants, *Artemisia seiberi* oil extract was the most toxic to woolly apple aphid *Eriosoma lanigerum* with LC 50 after 24 h post-treatment of 6161 ppm. While El-Rokh et al. (2018) found that the butanol extract of *Nitraria retusa* was very highly toxic to *A. craccivora* after one day of treatment, with a calculated LC50 of 347.52 ppm.

Concerning *S. microphylla*, previous studies revealed that it expresses different biological activities such as antioxidant (Lima et al., 2012), and insecticidal activities against the fall armyworm *Spodoptera frugiperda* (Romo-Asunción et al., 2016). The plants of families such as Lamiaceae are highly targeted for anti-insect activities against insect orders of Lepidoptera, Coleoptera, Diptera, Isoptera, and Hemiptera (Kumar et al., 2022).

It seems that the studied plant contains secondary metabolites that affect the biotic traits of aphids, leading to their mortality. Secondary plant metabolites are important factors influencing the interactions between insects and plants, and many of them, including phenolics are known as protective agents against various species of aphids (Wójcicka, 2010). In addition, plant saponins possessing broad-spectrum insecticidal activities have gained attention in sustainable pest management practices (Singh & Kaur, 2018). Many researchers highlighted the role of these plant compounds in the reduction of aphid populations (Goławska et al., 2012; Dolma et al., 2018). As the *S. microphylla* extract tested positive for saponins (Romo-Asunción et al., 2016), the efficacy of the extracts tested in our case may be attributed partially to saponins.

Repellency test

Host selection by aphids is not a random process, because these insects employ a variety of sensory and behavioral mechanisms to locate and recognize their host plants (Powell et al., 2006). The results presented in the Figure show that the aqueous extract 10% of *S. microphylla* recorded the highest repellent effect (RI = 71.8%), followed by the EO 100 and 10000 ppm, then the aqueous extracts 5 and 7% (Figure 1).

Many plant species produce substances that protect them by repelling the insects that feed on them (Naboulsi et al., 2018). In the present study, results revealed that some concentrations of both EOs and aqueous extracts of *S. microphylla* showed some degree of repulsion effect on *A. craccivora*. Also, the aqueous extracts belonging to four plant species (*Santolina africana, Juniperus thurifera, Artemisia herba-alba* and *Pituranthos scoparius*) did not attract massively the larvae of the cowpea aphid (Lebbal et al., 2017). In addition, the essential oils of *H. cheirifolia* at a concentration of 10000 ppm and its aqueous extract had an important repellency rate against *A. fabae* superior to 70% (Amamri et al., 2021). On their side, Singh et al. (2012) affirmed that after 24 h of release of aphids *A. gossypii*, the highest repellency was recorded in *Azadirachta indica* leaf extract which gives 99% followed by *Eucalyptus globules* and *Ocimum basilicum* leaf extracts (96 and 91%, respectively). Similarly, among evaporated methanolic extracts of *Impatiens* spp., the most active one on green peach aphid *Myzus persicae*, was *I. parviflora* with highly percent repellency (90-100%) at different times (Pavela et al., 2009).

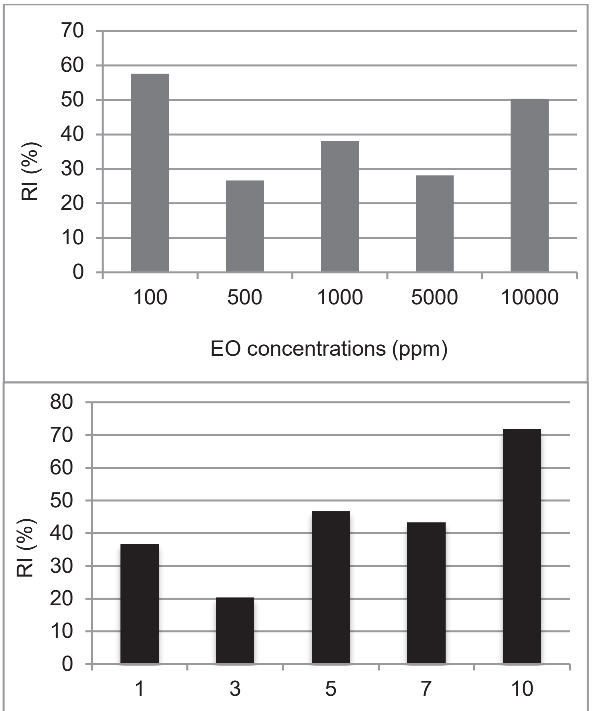

Figure 1. Repellency of the essential oils (EOs) and the aqueous extracts of *Salvia microphylla* against the cowpea aphid

On the other hand, previous studies showed important repellent activity of plant extracts against pests belonging to different insect orders. Volatiles from essential oils of coriander, lavender, rose, thyme and tea tree oil repelled adults of the citrus psylla *Diaphorina citri* compared with clean air in a two-port divided T-olfactometer (Mann et al., 2012). Besides, the comparative study conducted by Schultz et al. (2004) found both the catnip *Nepeta cataria* steam distillate and elemol, a major constituent of osage orange essential oil, to have good repellent properties to house flies *Musca domestica*, and American cockroaches *Periplaneta americana*.

CONCLUSIONS

Laboratory screening of the potential insecticidal and repulsive effect of aqueous extracts and essential oils of *Salvia microphylla* on *Aphis craccivora* was carried out.

Higher concentrations of extracts (10% for aqueous and 10000 ppm for essential oils) demonstrated an important aphicidal and repellent activities. The use of these extracts may constitute an important component of integrated pest management and thus reduction of chemical insecticide spraying. Moreover, the understanding of the mechanism involved by the studied extracts in affecting the biology and behaviour of aphids is highly recommended to use these natural products efficiently.

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