

## APHICIDAL ACTIVITY OF PLANT EXTRACTS AGAINST LARVAE OF *APHIS FABAE* (SCOPOLI, 1763) (HEMIPTERA: APHIDIDAE)

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### Abstract

The excessive use of pesticides has created many problems for both human health and the environment. Therefore, the search for alternative methods has become a necessity. This study aims to test the effect of aqueous extracts of three plant species (*Pistacia atlantica*, *Marrubium vulgare* and *Thymus algeriensis*) on the larvae of the black bean aphid (*Aphis fabae*) found on the faba bean (*Vicia faba*) under laboratory conditions.

With regard to extraction, two methods were adopted for the three plants: maceration and infusion. Each mixture of plant powder and distilled water was filtered and then diluted to obtain three concentrations (5, 15 and 25%). Three replications of 18 aqueous extracts and a control (distilled water) were tested for their insecticidal effect on the aphid mortalities by counting the dead larvae 6, 12 and 24 h after their placement on treated bean leaflets. In addition, phytochemical screening was carried out to detect the presence of saponins and polyphenols in the studied extracts.

Through our study, the maceration extract from *T. algeriensis* at a concentration of 25% was the most effective, with a 70% of larval mortality rate after 24 h. With respect to phytochemical screening, differences in chemical composition were observed between the analyzed extracts (such as the absence of saponins in *P. atlantica* and its presence in *T. algeriensis*), which may have a role in the efficacy of the examined treatments.

*T. algeriensis* extracts may be considered among the tools of integrated pest management (IPM) to minimize the use of synthetic pesticides.

KEY WORDS: aqueous extracts, *Thymus algeriensis*, aphid mortalities, phytochemical screening

### Introduction

In Algeria, faba bean (*Vicia faba* L.) takes first place among pulses with an annual area of about 65,000 ha (Zaghouane, 1991). In the Mediterranean region, the main aphid pests of faba bean are two green (*Acyrtosiphon pisum* Harris and *Myzus persicae* Sulz) and two black (*Aphis fabae* Scopoli and

*Aphis craccivora* Koch) species, of which the most important are the black bean aphid, *A. fabae* Scopoli and the cowpea aphid, *A. craccivora* due to their high frequency and strong deleterious effects on the plants (Weigand & Bishara, 1991). Beside the direct damage inflicted by probing and sucking plant juices, *A. fabae* is an important vector of many plant viruses, such as beet yellows virus (BYV), which causes a yellowing disease in *Beta vulgaris* and some other plants, but also brome mosaic virus (BMV), which primarily infects plants from the Poaceae family and causes damage to wheat plants (Stanković *et al.*, 2015). The excessive use of chemical pesticides to control aphids has contributed to the emergence of many problems, including pollution, poisoning and the selection of resistant strains of pests. Some aphid species that have been reported as having developed insecticide resistance are ranked among the most problematic pests worldwide (Foster *et al.*, 2007). Thus, there is a widespread effort to find new pesticides, and currently this effort is focused on natural compounds, such as flavonoids, terpenoids and phenolics from diverse botanical families from arid and semi-arid areas (Acheuk *et al.*, 2017). Developing natural pesticides could relieve African farming and governments from the expensive importation of synthetic pesticides (Belmain *et al.*, 2013). In this context, the aim of this manuscript was the study of the insecticidal activity of three medicinal plants, including one species endemic to North Africa, against the black bean aphid larvae.

## Materials and methods

### Plant collection and extraction

To carry out our study, we chose three plant species to obtain aqueous extracts: one species belongs to the family Anacardiaceae (*Pistacia atlantica* Desf.) and two belonging to the Lamiaceae family (*Marrubium vulgare* L. and *Thymus algeriensis* Boiss). *T. algeriensis* is the most pervasive North African species, endemic to Morocco, Tunisia, Algeria and Libya (Salhi *et al.*, 2016).

The leaves of the *P. atlantica* tree, harvested in the region of Arris (Batna province, northeastern Algeria) on 04 September 2016, and the whole aerial part of *M. vulgare*, collected in the area of Babar (Khenchela province, northeastern Algeria), on 16 April 2017, was used for the extraction. As for *T. algeriensis*, the whole plant was used (the aerial and underground parts). The harvest of this plant was made in the region of Ouled Fadhel (Batna province, northeastern Algeria) on 16 April 2017.

The various parts of the selected plants used were air-dried and then cleared of dust. Afterwards, they were finely crushed using a hand crusher.

With regard to extraction, two methods were adopted: maceration and infusion.

For maceration, a quantity of plant powder was diluted in cold distilled water at a ratio of 1/10 (w/v). Each of the three mixtures obtained was agitated for a few minutes and left for 24 h. The mixtures were then filtered using filter paper and subsequently diluted with distilled water to obtain three concentrations (5, 15 and 25%). As regards infusion, a quantity of plant powder was diluted in hot distilled water at a ratio of 1/10 (w/v). Each of the three mixtures obtained was agitated for a few minutes and then left for 2 h. The mixtures were then filtered and subsequently diluted with distilled water to obtain three concentrations (5, 15 and 25%).

The solutions obtained by maceration and infusion were kept in the refrigerator until use. The test of insecticidal effect was performed under laboratory conditions. Third and fourth instar larvae of *A. fabae* were selected as target pests.

### Test of insecticidal activity

We prepared 57 Petri dishes, with three repetitions for each treatment. A total of 19 treatments were tested: 9 obtained by maceration, 9 by infusion and 1 with distilled water (control).

Each dish contained one leaflet of the faba bean treated with a different solution. Each leaflet was dipped into the corresponding treatment for a few seconds. Then, ten aphid larvae (L3 and L4) were placed on the treated leaflets. Aphid larvae were taken from the outside from the same faba bean plants to avoid genetic dissimilarity among collected individuals. Dead larvae were counted at 6, 12 and 24 h after the artificial infestation for each repetition. Results are expressed as the percentage of dead larvae in relation to the total number of placed individuals.

### Phytochemical screening

Qualitative phytochemical screening for the presence or absence of polyphenols and saponins was carried out according to the methods described by N'guessan *et al.* (2009) and Soro *et al.* (2009).

### Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the mean aphid mortalities for each type of solution. When there is a significant difference, a Student-Newman-Keuls test was used to highlight the homogeneous groups. Analyses were performed using the SPSS software for Windows 10.0.5 (SPSS, Inc.).

## Results

ANOVA statistical analysis revealed significant differences in aphid mortality rates on the leaflets treated by different solutions (Table I). It was noted that the highest aphid mortality rate was recorded on the leaflets treated with the aqueous extract of *T. algeriensis* obtained by maceration (concentration of 25%). It achieved 70% of dead larvae, 24 h after treatment.

On the other hand, phytochemical screening revealed the presence of polyphenols in all three plants and for both extraction methods, with the exception of *T. algeriensis* obtained by infusion. Saponins were present in extracts of *T. algeriensis* made by both methods and in extracts of *M. vulgare* obtained by maceration (Table II).

Table I. Comparison of aphid mortality rates (%) between the tested extracts. PI- *Pistacia atlantica* by infusion, PM- *Pistacia atlantica* by maceration, MI- *Marrubium vulgare* by infusion. MM- *Marrubium vulgare* by maceration, TI- *Thymus algeriensis* by infusion, TM- *Thymus algeriensis* by maceration, DW- distilled water.

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

Solution	After 6 H	After 12 H	After 24 H
PM 5 %	0 a	6.67 a	10 abc
PM 15 %	0 a	0 a	13.33 abc
PM 25 %	0 a	0 a	0 a
PI 5 %	0 a	3.33 a	10 abc
PI 15%	0 a	0 a	0 a
PI 25 %	3.33 a	3.33 a	10 abc
MM 5 %	3.33 a	3.33 a	3.33 ab
MM 15 %	0 a	0 a	0 a
MM 25 %	0 a	0 a	0 a
MI 5 %	3.33 a	3.33 a	3.33 ab
MI 15 %	0 a	0 a	6.67 abc
MI 25 %	0 a	3.33 a	10 abc
TM 5 %	10 a	30 abc	46.67 cd
TM 15 %	23.33 ab	50 c	56.67 d
TM 25 %	36.67 b	56.67 c	70 d
TI 5 %	20 ab	40 bc	43.33 bcd
TI 15 %	20 ab	36.67 abc	56.67 d
TI 25 %	23.33 ab	26.67 abc	40 abcd
DW	10 a	16.67 ab	20 abc
<b>Signification</b>	<b>0.001</b>	<b>0.000</b>	<b>0.000</b>

Table II. Phytochemical screening of the tested plant extracts.

Plant	Extraction method	Saponins	Polyphenols
<i>Pistacia atlantica</i>	Maceration	-	+
<i>Pistacia atlantica</i>	Infusion	-	+
<i>Marrubium vulgare</i>	Maceration	+	+
<i>Marrubium vulgare</i>	Infusion	-	+
<i>Thymus algeriensis</i>	Maceration	+	+
<i>Thymus algeriensis</i>	Infusion	+	-

## Discussion

More than 2,000 species of plants are known to produce chemicals that have insecticidal activity (Hill, 1997). In the present study, we tested aqueous extracts from three plants against black bean aphid. In general, the endemic plant *T. algeriensis* showed remarkable insecticidal effect. According to Salhi *et al.* (2016), results of

the antioxidant activity have shown that the aqueous extract of this plant species possesses a moderate reducing power compared to the positive controls.

The extracts of *P. atlantica* and *M. vulgare* had no significant aphicidal activity, even though other studies proved biological activities of these plants, such as antibacterial activities (Masoodi *et al.*, 2008; Tohidi *et al.*, 2011).

On the other hand, some plant extracts, such as *Artemisia judaica* L. (Acheuk *et al.*, 2017) and *Allium sativum* L. bulb (Kulimushi, 2014), caused high mortality of *A. fabae*.

The phytochemical screening showed that the extracts of *T. algeriensis* were characterized by the presence of saponins. It seems that these chemicals had a role in the effectiveness of *T. algeriensis* against the black bean aphid. Plants with differing levels of secondary metabolites show differences in their defense responses to insect pests (Hu *et al.*, 1993). For instance, pea aphid fed with a high-saponin line of alfalfa showed a reduction in reproduction and survival, and disturbances in the development of its population (Golawska *et al.*, 2006). The results obtained by Szynekarczyk *et al.* (2001) suggested that the level of saponins might be an important factor in the protection of alfalfa against the pea aphid, and therefore attempts by plant breeders to reduce saponin content in order to increase alfalfa digestibility for livestock might make the plants more susceptible to aphids and other pests (Golawska *et al.*, 2012). Moreover, the study of Golawska (2007) mentioned that some saponins are toxic for the pea aphid. In addition, tea saponin was found to be more effective against *A. craccivora* after 96 h as compared to positive control (Dolma *et al.*, 2018).

## Conclusion

It was noticed that the extract obtained by maceration from *T. algeriensis* with a concentration of 25% was the most effective, with a larvae mortality rate of 70% after 24 h. Phytochemical screening revealed that the extracts of *T. algeriensis* were characterized by the presence of saponins, unlike the extracts of *P. atlantica* and the infusion extract of *M. vulgare*.

It is recommended to test these extracts on different stages of other pests, increasing the number of treated individuals.

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## АФИЦИДНА АКТИВНОСТ БИЉНИХ ЕКСТРАКАТА ПРОТИВ *APHIS FABAE* (SCOPOLI, 1763) (HEMIPTERA: ARHIDIDAE)

САЛИМ ЛЕБАЛ, НАБИЛА ХЕДЈАЗИ, ИКРАМ ТАБТИ, ХАМЗА УАРГИ И АЦЕДИНЕ ЗЕРАИБ

### Извод

Прекомерна употреба пестицида је проузроковала многе проблеме по здравље човека и околину. Стога је потрага за алтернативним методама постала потреба. Овај рад има за циљ да тестира водене екстракте три биљне врсте (*Pistacia atlantica*, *Marrubium vulgare* и *Thymus algeriensis*) на нимфе црне репине ваши (*Aphis fabae*) које су узорковане са боба (*Vicia faba*) у лабораторијским условима. Две методе су коришћене за обраду биљног материјала: мацерација и инфузија. Све смеше биљног праха и дестиловане воде су филтриране а затим разблажене да би се добиле три концентрације (5, 15 и 25%). Тестирано је 18 екстраката и контрола (дестилована вода) са три понављања да би се утврдио инсектицидни ефекат на биљне ваши пребројавањем мртвих јединки након 6, 12, и 24 часа излагања сапонинима и полифенолима у испитиваним екстрактима.

У овој студији екстракт биљке *T. algeriensis* у концентрацији 25% је био најефикаснији, са 70% морталитета након 24 часа. У складу са фитохемијским скринингом, разматране су разлике у хемијском саставу између испитиваних екстраката (као што је одсуство сапонина код *P. atlantica* и одсуство код *T. algeriensis*), што може утицати на ефикасност анализираних третмана.

Екстракти *T. algeriensis* се могу разматрати даље у интегрисаној контроли штеточина како би се смањило коришћење синтетичких пестицида.

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