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IN VITRO SCREENING OF INSECTICIDAL EFFECT OF PLANT AQUEOUS EXTRACTS ON THE COWPEA APHID *APHIS CRACCIVORA*

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ABSTRACT

The research of new control methods that have low negative effects represent an alternative aiming to limit disadvantages of chemical control of pests. This study aimed to test the effect of 11 aqueous plant extracts belonging to four species (*Santolina africana*, *Juniperus thurifera*, *Artemisia herba-alba* and *Pituranthos scoparius*), on the larvae of the aphid *Aphis craccivora* installed on the leaves of *Robinia pseudoacacia* under laboratory conditions. The insecticidal effect of aqueous extracts on the mortality of aphids was evaluated by the introduction of acacia leaflet into an extract and infestation of them by 10 larvae of the aphid. Later, a counting of dead larvae after 3, 6, 12 and 24 h was performed. In addition, we investigated the effect of the aqueous extracts on the orientation of the aphids by putting the latter in a position of choice between six leaflets, each treated with a different extract. In addition, a phytochemical screening was carried out to detect the presence of saponins and polyphenols in the extracts studied. Through our study, the extract obtained from *S. africana* at a concentration of 15% was the most effective with a mortality rate higher than 80% after 24 h of artificial infestation. As for the effect of the four extracts on the orientation of the cowpea aphid, they did not attract the larvae massively. Regarding phytochemical screening, differences in the chemical composition of the analyzed extracts were observed. These differences may explain in part the variation of aphicidal effects of tested plant extracts.

Keywords: *Santolina africana, orientation, mortality rate, polyphenols, saponins.*

INTRODUCTION

Chemical control is the most common way to limit aphid infestations. In addition to its high cost, this method presents a potential danger to the environment (Bhatia *et al*., 2011). Some pesticides pollute surface and groundwater (Horrigan *et al*., 2002). On the other hand, Sullivan (2008) mentioned that non-selective pesticides

can destroy auxiliaries. In addition, massive use of insecticides resulted in the development of resistant strains of aphids (Harmel *et al*., 2008) and other pest species (Brévault *et al*., 2003). As a result, scientists have begun to look for other ways to control these insects (Jordan, 2013). Plants represent a vast storehouse of potentially useful natural products, and indeed, many laboratories worldwide have screened thousands of species of higher plants not only in search of pharmaceuticals, but also for pest control products (Isman, 1997).

Aphis craccivora (Koch) colonizes the young shoots of many plants, in particular those of Leguminosae (Blackman and Eastop, 2006). Attacks on shrubs and trees are rarely of economic importance, except on *Robinia pseudoacacia* (L.), where outbreaks can sometimes affect its ornamental value (Fraval *et al*., 1997). Furthermore, this aphid is involved in the transmission of about 30 persistent and semi-persistent phytoviruses (Blackman and Eastop, 2007).

The aim of this work is to study the effect of some aqueous extracts of plants on the larvae of the cowpea aphid (*Aphis craccivora*) found on acacia locust (*Robinia pseudoacacia*) under laboratory conditions.

MATERIALS AND METHODS

Aqueous extraction and preparation of treatments

The aerial part (particularly the leaves) of four plant species (*Santolina africana*, *Artemisia herba-alba*, *Juniperus thurifera* female and *Pituranthos scoparius*) were dried in the open air and then freed from dust. Then, they are finely ground using an electric grinder. With regard to extraction, two methods have been adopted: maceration and infusion.

In our case, a quantity of 10 g of powder from the aerial part of each of the 4 plants was diluted in 100 mL of cold distilled water. Each of the four mixtures obtained was agitated for a few minutes and then left for 24 hours. Each mixture was then filtered and diluted with distilled water to obtain two concentrations (5 and 15%).

As for the thuriferous juniper, an infusion extract was made. In our case, an amount of 10 g of *J. thurifera* powder was diluted in 100 mL of hot distilled water. The mixture obtained was agitated for a few minutes and then left for 2 hours. It was filtered and diluted with distilled water to obtain two concentrations (5 and 15%).

Collection of aphids

The animal material consisted of larvae of the aphid *Aphis craccivora* taken from a tree of *Robinia pseudoacacia* in the Batna region (eastern Algeria).

Evaluation of the insecticidal effect of aqueous extracts on the mortality of aphids

The various bioassays were carried out under laboratory conditions. The uninfested leaves of *R. pseudoacacia* and larvae (L3 and L4) of *A. craccivora* located in the same tree were used. 36 Petri dishes were prepared. Each one contains 2 leaflets treated with false acacia. A total of 12 treatments were tested with 3 repetitions for each: distilled water (control), 8 solutions obtained by maceration, 2 solutions

obtained by infusion and treatment by mixing the 4 solutions 5% obtained by maceration. Each leaflet is inserted into the container containing the corresponding treatment in such a way that the foliage is well imbibed. 10 larvae / box were placed at a rate of 5 individuals / leaflet. Dead larvae were counted 3, 6, 12 and 24h after the artificial infestation.

Evaluation of the repellent effect of aqueous extracts on the orientation of aphids

Three repetitions were prepared. Each repetition consisted of a plate and six leaflets arranged at its periphery. One leaflet was submerged in distilled water, another in the extract 5% of *S. africana*, another in the extract 5% of *A. herba-alba*, another in the extract 5% of *J. thurifera*, another in the extract 5% of *P. scoparius*, and the last one in a mixture of the four extracts 5%. At the center of each plate 20 larvae of *A. craccivora* were introduced.

Larvae installed on each treated leaflet were counted 12 and 24 h after deposition of the aphids.

Phytochemical screening

For the characterization of compounds belonging to the group of polyphenols, the reaction with ferric chloride was used. A drop of alcoholic solution of ferric chloride ($FeCl_3$) at 2% was added to 2 mL of each solution. The appearance of a blackish or greenish coloration more or less dark indicates the presence of polyphenolic derivatives (Soro *et al*., 2009).

To search the saponosides, we poured 10 mL of the aqueous extract into a test tube. The tube was agitated for 15 seconds, then allowed to stand for 15 min. A persistent foam superior than 1 cm, indicates the presence of saponins (N'guessan *et al*., 2009).

Statistical analysis

In order to compare the aphid mortality averages of each treatment and the orientation of the aphids to the treated leaves, ANOVA one way was used. When there is a significant difference, a Student-Newman-Keuls test is used to show the homogeneous groups. These analyzes were performed using SPSS software for Windows 10.0.5 (SPSS, Inc.)

RESULTS AND DISCUSSION

The ANOVA analysis revealed a highly significant difference between treatments during the 4 inspections (Table 1). The extract of *J. thurifera* 15% obtained by maceration was the most effective during the counts of 3, 6 and 12 h, with a mortality rate of 50%. While the *S. africana* 15% extract recorded the highest mortality rate after 24 h. The concentration 15% gave generally the best results for the 4 plants.

Table 1. Comparaison of mortality rates of aphids on treated leaflets.

Extract	After 3h	After 6 h	After 12 h	After 24 h
Distilled water	3,33 a	6.66 a	10 a	30 ab
Mixture	6.66 ab	6.66 a	20 ab	20 a
Artemisia 5 %	10 ab	10 a	13.33 ab	33.33 ab
Artemisia 15 %	16.66 ab	20 ab	20 ab	46.66 b
Pituranthos 5 %	13.33 ab	23.33 b	30 ab	60 bc
Pituranthos 15 %	33.33 b	33.33 b	43.33 ab	53.33 b
Juniperus 5 %	46.66 c	46.66 bc	46.66 b	60 bc
Juniperus 15 %	50 c	50 c	50 b	60 bc
Juniperus 5 % infusion	36.66 bc	46.66 bc	50 b	56.66 bc
Juniperus 15 % infusion	23.33 b	23.33 b	26.66 ab	50 b
Santolina 5 %	23.33 b	30 b	46.66 b	76.66 c
Santolina 15 %	33.33 b	43.33 bc	50 b	83.33 c
Signification	0.000	0.000	0.001	0.000

* Values indicated with different letters are significantly different at P<0,05

In general, the aqueous extract of *S. africana* and *J. thurifera* showed a remarkable insecticidal effect on aphids *A. craccivora*. On their part, Attia *et al*. (2011 and 2012) revealed that *S. africana* oils can provide valuable acaricidal activity. These oils cause high mortality of *Tetranychus urticae*. In addition, *J. Phoenicea* essential oils showed a mortality of *Myzus persicae* aphids that exceeded 50 % (Hakimi *et al*., 2015).

As for the aqueous extracts of *A. herba-alba*, they gave less insecticidal effects. Similarly, Nia *et al*. (2015) found that the aqueous extract of desert wormwood did not show a significant insecticidal effect against *Myzus persicae* compared to ethanolic and etheric extracts. Furthermore, Chermenskaya *et al*. (2010) mentioned that extracts from many species belonging to the genera Artemisia (not including *A. herba-alba*) were effective against aphids.

Concerning the mixing of the extracts, it was noticed that it is less effective in comparison with the effect of the extracts of the plants alone. Also, a study conducted by Ali *et al*. (2015) found that some plant mixtures tested had lower mortality rates of the aphid *Diuraphis noxia* compared to these plants alone.

Evaluation of the effect of aqueous extracts on the orientation of aphids

Statistical analysis of the number of aphids installed on each treated leaflet showed that there was a significant difference during the 2 observations ($P < 0.05$). The mixture of the 4 plants studied was the most attractive followed by the control. While other plant extracts did not attract any individual (Table 2). This can be explained by the absence of attractants to the aphids in the composition of these extracts.

Table 2. Mean percentage of aphids installed on each treatment.

	After 12 h	After 24 h
Distilled water	8,35 b	13,35 b
Mixture	20 a	21,65 a
Artemisia herba-alba	0 b	0 c
Pituranthos scoparius	0 b	0 c
Juniperus thurifera	0 b	0 c
Santolina africana	0 b	0 c

* Values indicated with different letters are significantly different at P<0,05

Phytochemical screening

Phytochemical screening revealed the presence of saponosides in desert wormwood only (Table 3). Likewise, Nia *et al*. (2015) reported the presence of saponins besides the terpenoids in the aqueous extract of *A. herba-alba*. Saponins can be useful as natural aphicides and deterrents. Furthermore, the insect midgut epithelium is suggested to be a primary target of saponin activity (De Geyter *et al*., 2012).

Table 3. Phytochemical screening of the aqueous extracts

Extract	Saponins	Polyphenols
Artemisia herba-alba	+	+
Pituranthos scoparius	-	-
Juniperus thurifera	-	+
Santolina africana	-	+

On the other hand, polyphenols were present in the three analysed extracts, and they were absent only in *P. scoparius* (Table 3). Allelochemics which affect the behavior or population biology of insects can be extremely important factors in host plant resistance (Berlinger, 2008). Phenols are the principal class of secondary metabolites (Lattanzio *et al.*, 2006). It is likely that the quantity of some molecules belonging to these chemical families have the greatest effect on aphids, rather than the total amounts of polyphenols and saponosides. According to Dreyer and Jones (1981), most flavonoids, which represent a group of phenols, are strong deterrents.

CONCLUSION

In this study, the insecticidal and repellent effect of plant extracts of four plant species on the aphid *Aphis craccivora* was tested.

In general, the extract obtained from *Santolina africana* at a concentration of 15% was the most effective with a mortality rate higher than 80% after 24 h. As for the effect of these extracts on the orientation of the cowpea aphid, these extracts alone did not induce larval attraction.

As regards phytochemical screening, differences in the chemical composition (saponosides and polyphenols) were observed between the analyzed extracts, which may explain differences in the efficacy of each extract.

These preliminary results pave the way for further research to confirm the results obtained under field conditions on one side; and to know by what mechanism the plant extracts influence the biology and ecology of pests on the other side.

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