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Aphicidal and Behavioral Effects of *Vicia faba* L. (Fabales: Fabaceae) Leaf Extracts against *Aphis fabae Scopoli* (Hom., Aphididae)

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ABSTRACT

The effects of methanolic and aqueous leaf extracts of the aphid resistant cultivar, CV-12 of broad bean (*Vicia faba*) on the behavior and mortality of the black bean aphid, *Aphis fabae* were compared. The main objective of the study was to assess the effects of extracts of *V. faba* leaves an available insecticide against *A. fabae*. The results showed that the aqueous leaf extracts of the cultivar CV-12 had a repulsive effect against *A. fabae* (aver. 1.3 adults / leaf) compared to methanolic leaf extracts of the same cultivar (aver. 3.7 adults / leaf). The results revealed that the methanolic extracts had a toxic effect induced a high *A. fabae* mortality that reached up to 65.71% compared to the aqueous leaf extracts (mortality rate = 1.43%). The present study suggests that extracts from leaves of resistant cultivar CV-12 have insecticidal and repellent properties against *A. fabae* and could be used as an alternative of chemical insecticides in an integrated pest management program to *A. fabae*.

Keywords: *Aphis fabae*, biopesticide, flavonoids, leaf extracts, phenols, *Vicia faba.*

INTRODUCTION

Broad bean, *Vicia faba* L. (Fabales: Fabaceae) is a Mediterranean legume that is among the most cultivated vegetable plants in Algeria, where it covers about 37,668 ha and generating production 42,386 tones of dry matter (FAO, 2013).

The black bean aphid, *Aphis fabae* Scopoli (Hom., Aphididae) is the most destructive insect pest of broad bean in Algeria. It is one of the 14 aphid species of most agricultural importance. It is a polyphagous species, because there are a number of bewildering complexes of species, at least some of which also have wide host ranges (Blackman and Eastop, 2007). *A. fabae* has a wide distributional range. It occurs in Europe, Western Asia, Arab countries particularly Jordan (Mustafa and Qasem, 1984), Africa, and South America. It is a vector of more than 30 plant viruses, including non-persistent viruses of bean and peas, beets, crucifers, cucurbits, *Dahlia*, potato, tomato, and tulip, and the persistent beet yellow net virus and potato leaf roll virus (Blackman and Eastop, 2007). *A. fabae* is usually controlled using insecticides. However, the high costs of synthetic pesticides and associated toxicity risks (Mihale *et al.,* 2009), the increasing development of insect resistance to pesticides, (Ogendo *et al.,* 2003), the destruction of beneficial insects (pollinators, parasitoids and predators), pesticide residue magnification in humans and wildlife and disruption of ecosystem (Ruchika and Kumar, 2012), have increased the need to search for alternative insect control methods.

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In recent years, several researches investigated new bioactive compounds from plants for the development of ecologically safe plant protectants (Chermenskaya *et al.*, 2010). Indeed, many studies demonstrated that botanical biopesticides have been reported to have a wide range of biological activities against insects. These include repellence and anti-feedant activities (Viglianco *et al.,* 2008), oviposition deterrence, toxicity, sterility, growth regulatory and fecundity reduction, molting and respiration inhibition, and cuticle disruption (Tinzaara *et al.,* 2006). 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). These secondary metabolites are released in the form of plant volatiles. Plant extracts are a complex mixture of general leaf volatiles, found in most plant species with more specific components that are shared by some plant species groups (Van Tol *et al*., 2007). Essential oils generally consist of several constituents produced as secondary plant metabolites, the majority of which are terpene hydrocarbons, polyphenolic compounds and alkaloids (Agostini-Costa *et al*., 2012).

Insecticidal activity of plant extracts against aphid species is largely studied in the literature. Among them, *A. fabae* (Salari *et al*., 2010, 2012; Habou *et al*., 2011; Mmbone *et al*., 2014), *Aphis citricola* (Larif *et al*., 2013) *Aphis craccivora* (Baidoo *et al*., 2012), *Aphis gossypii* (Bagavan *et al*., 2009; Salari *et al*., 2010; 2012), *Aphis nerii* (Salari *et al*., 2012), *Brevicoryne brassicae* (Phoofolo *et al*., 2013; Wubie *et al*., 2014), *Lipaphis erysimi* (Arya *et al*., 2014; Sable and Kushwaha, 2014), *Melanocallis caryaefoliae* (Marin-Dominguez *et al*., 2014), *Myzus persicae* (Pavela *et al*., 2009; Salari *et al*., 2010, 2012; Ikeura *et al*., 2012; Ben Hamouda *et al*., 2015; Nia *et al*., 2015), *Rhopalosiphum padi* (Bushra *et al*., 2014), *Schizaphis graminum* (Chermenskaya et al., 2010), and *Sitobion avenae* (Bushra *et al*., 2014). Also, the repellent property of plant extracts against aphids was largely also studied, among the aphid species, *Brevicoryne brassicae* (Wubie *et al*., 2014), and *Myzus persicae* (Pavela *et al*., 2009; Ikeura *et al*., 2012; Salari *et al*., 2012).

The aim of this study was to investigate the aphicidal and/or repellent effect of methanolic and aqueous leaf extracts of aphid resistant cultivar, CV-12 of *V. faba* as source of sustainable alternatives to synthetic insecticides for controlling the black bean aphid without affecting the environment, beneficial organisms or men.

2. MATERIALS AND METHODS

2.1. Plant treatment

The plant material consisted of leaves of the aphid resistant cultivar, CV-12 and the aphid susceptible cultivar, CV-4 of *V. faba*. The seeds of the two cultivars were individually grown in plastic pots (13 cm diameter × 14 cm) under greenhouse conditions of 17±5°C, 70±10% RH and 14: 10 (L: D) h photoperiod. When the plants reached the 13th growth stage (three leaves fully expanded) (Mier, 2001), all leaves of the two cultivars were cut. The leaves of the cultivar, CV-4 considered as a biological material used in this study, whereas CV-12 leaves were used for the preparation of the methanolic and aqueous extracts.

2.2. Insect rearing

The aphids used in this study were obtained from a single apterous adult of *A. fabae*. The latter was collected in early February, 2014 from broad bean plants in a field situated at the region of Biskra (in the east of Algeria). The aphids were reared on broad bean seedlings (*V. faba*) under greenhouse conditions of 17±5°C, 70±10% RH and 14: 10 (L: D) h photoperiod.

2.3. Methanolic and the aqueous extracts preparation

To obtain the methanolic extracts from leaves of CV-

12, 1 g of dried leaves, collected at the growth stage 13 was put with two mixtures. The first one contained 60 mL of distilled water and 140 mL of 70% methanol. The second one included 10 mL of distilled water and 0.02 g of $Na_2O_5S_2$. 40 mL were extracted from the mixture and were placed in a covered flask with agitation for 20 min. After that, the mixture was filtered through Whatman filter paper n° 1 and then the second extraction was done by adding 40 mL from the mixture, and then was filtered again.

For the aqueous extracts, 1 g of dried leaves was harvested at the 13th growth stage in 1 L of distilled water and kept in a covered flask and it was agitated during 20 min. The extract was filtrated through Whatman filter paper n° 1. After that, the extract stored until use.

2.4. Effect of treated leaves on behavior of *A. fabae*

Preference was determined in a multi-choice experiment by offering simultaneously three freshly leaves of the cultivar CV-4. The first leaf was treated by the distilled water only (control), the second leaf was treated by aqueous extracts from CV-12 leaves and the third leaf was treated by methanolic extracts from CV-12 leaves. The three types of leaves were submerged during three seconds in each solution.

The three leaves were randomly placed in circle (Petri dish; 9 cm × 1.3 cm), and directed towards the center (Castro *et al.*, 2005). Twelve aphid adults at rate of four aphids per leaf were placed in the middle of each Petri dish (Budak *et al.*, 1999). The number of replications was ten. After six hr, the number of adults on each treatment leaf was recorded. The same experiment was repeated under the dark (Castro *et al.*, 1999; Hesler and Tharp, 2005) with five replications. After six hr, the number of adults on each treatment leaf was recorded.

2.5. Aphicidal activity of treated leaves against *Aphis fabae*

The last treated leaves were placed individually in Petri dishes. The experiment was replicated seven times (leaves) for each treatment (solution). One apterous adult was placed on each treatment leaf. After 24 h, the number of dead adults was counted to compute mortality rate. When no leg or antennal movements were observed, insects were considered dead (Salari *et al.*, 2010).

2.6. Total phenolics and total flavonoids contents

To estimate the amount of total phenols and total flavanoids of the two cultivars, CV-12 and CV-4, the same steps were followed as for obtaining the methanolic extract. Finally, the quantity of total phenols and total flavanoids was measured using the photometric method after preparation of samples consisting of 1 mL of the extract added to 1 mL of $AlCl_3$ to determine the amount of total flavanoids; or 0.5 mL of the extract added to 1 mL of Na_2CO_3, 5 ml of distillated water and 1 mL of Folin-Ciocalteu reagent to analyze the total phenols content.

The absorbance reading was measured at 725 nm against a reagent blank (Gallic acid) in spectrometer for the total phenols and at 430 nm against a reagent blank (Quercetin) for the total flavonoids. Total phenols were expressed as mg gallic acid equivalents per g of dried leaves. Total flavonoids contents were calculated from a calibration curve using quercetin as a standard, and expressed as μg quercetin equivalents per g of dried leaves.

2.7. Statistical analysis

The data concerning multi-choice test (in light and in dark), total phenols and total flavonoids contents were compared using one-way analysis of variance (ANOVA). If the ANOVA demonstrated significant differences, the means were separated using the Duncan's test at $P \leq 0.05$ (Gomez and Gomez, 1984). The rate mortality of *A. fabae* was subjected to the Chi-square test (X^2) at 5% level. All statistical analyses were performed with SPSS statistical software (Version 10.0.5) (SPSS, 1999). All Experiments were designed in a randomized complete block design (RCBD).

3. RESULTS

3.1. Effect of treated leaves on behavior of *Aphis fabae*

In the first test (in the light), the results indicated significant difference ($P \leq 0.01$; $F_{5.49} = 7.226$) among the three treated leaves. The mean number of aphids was greatest on leaves treated by methanolic extracts (3.7 adults/leaf) and lowest on the control leaves and aqueous extracts (0.8 and 1.3 adults/leaf, respectively). In the second test (in the dark), the results showed that the atractivity of the three types of leaves for aphids did not differ significantly ($P > 0.05$; $F_{3.88} = 2.667$). The mean number of aphids was ranged from 0 to 0.4 adults/leaf (Table 1).

Table 1. Effect of methanolic and aqueous leaf extracts of the resistant cultivar CV-12 extracted from *Vicia faba* on behavior of *Aphis fabae* adults (Means ± SE).

Treatment type	Number of *Aphis fabae* adults	
	Test in light (n = 10)	Test in dark (n = 5)
Control	0.8 ± 0.36a	0.40 ± 0.24a
Aqueous leaf extracts	1.3 ± 0.62a	0.00 ± 0.00a
Methanolic leaf extracts	3.7 ± 0.70b	0.00 ± 0.00a
F	7.226	2.667
P	0.003	0.110 (ns)

n: number of replications, ns: not significant, means within a column followed by different letters are significantly different following Duncan's test.

3.2. Aphicidal activity of treated leaves against *Aphis fabae*

The results showed a highly significant difference ($P < 0.001$; $X^2_{13.82} = 109.37$) among the different treatments. The methanolic extracts caused the highest mortality (65.71%), while the aqueous extracts and the control had a very low rate of mortality of *A. fabae* adults (1.43%) (Table 2).

Table 2. Effect of methanolic and aqueous leaf extracts of the resistant cultivar CV-12 as compared to the untreated control on mortality rate of *Aphis fabae* adults.

	Control	Aqueous extracts	Methanolic extracts	Total adults	Chi-square test (X^2)
Survival adults	69	69	24	162	X^2 = 109.37 df = 2 P = 0.000
Dead adults	1	1	46	48	
Total adults	70	70	70	210	
Mortality rate (%)	1.43	1.43	65.71	22.85	

3.3. The biochemical analysis

The data analysis demonstrated that the content of total phenols did not differ significantly between the resistant CV-12 and the susceptible CV-4 cultivars ($P > 0.05$; $F_{7.71} = 3.302$). Nevertheless, a significant difference in flavonoids content between the two cultivars was recorded ($P \leq 0.01$; $F_{21.20} = 21.715$). The resistant cultivar CV-12 had the lowest content (43.15 µg/g dried leaves) than the susceptible cultivar CV-4 (69.44 µg/g dried leaves) (Table 3).

Table 3. Total phenols and flavonoids content of two broad bean cultivars (Means ± SE).

Cultivars	Total phenols (mg g^{-1} dried leaves) (n = 3)	Total flavonoids (µg g^{-1} dried leaves) (n = 3)
CV-4	10.70 ± 0.03a	69.44 ± 1.17b
CV-12	10.82 ± 0.05a	43.15 ± 5.52a
F	3.302	21.715
P	0.143 (ns)	0.010

n: number of replications, ns: not significant, values followed by different letters within a column are significantly different (ANOVA: P ≤ 0.05).

4. DISCUSSION

4.1. Effect of treated leaves on behavior of *Aphis fabae* adults

Our results showed that *A. fabae* was not repelled by the methanolic leaf extracts in the light when compared to the aqueous leaf extracts. On the other hand, *A. fabae* had a same behavior against the two treated leaves (methanolic and aqueous leaf extracts) in the dark. The most important finding of the present study is the demonstrated repellent property of the aqueous leaf extracts of the resistant aphid cultivar, CV-12 in light and dark tests. The comparison of both tests showed that the color perhaps had a high part in the selection of *A. fabae* for treated leaves by methanolic extracts, because the attraction of this last was high in light (3.7 adults/plant) and hopeless in dark. Thus, the light was an important role in the process of the choice of the ideal leaves. In the dark, *A. fabae* only used the feel olfactory to choose the favorite leaves, what explains the difference of attraction between light and dark tests. The leaves treated by aqueous extracts were less preferred by adults of *A. fabae* in both tests; probably they had a high content of repulsive substances. Mmbone *et al.* (2014) who stated that *A. fabae* was not repelled by the crude leaf extracts of *Tagetes minuta* and *Tephrosia vogelii*.

Several factors were responsible for the selection of the host plant. Among these factors, the essential oils from different plant species are an important source of repellents (Abtew *et al.*, 2015), also, the volatile substances, Webster *et al.* (2008) identified 15 electrophysiologically active compounds of broad bean (var. Sutton Dwarf) to winged *A. fabae*. The biochemical analysis of the two methanol extracts of leaves of resistant CV-12 and susceptible CV-4 cultivars did not show any significant difference ($P<0.05$) in the phenolics content. However, the resistant cultivar CV-12 had the lowest level of flavonoids (43.15 µg/g dry leaves) than the susceptible cultivar CV-4 (69.44 µg/g dry leaves). Similarly, Goławska *et al.* (2008) indicated that the resistant cultivar of *Medicago sativa* had the highest level of saponins and the lowest level of flavonoids than the susceptible cultivar against *Acyrthosiphon pisum*. Other studies showed that the secondary metabolites had an aphicidal activity against aphids. For instance, Kordan *et al.* (2008) noted that the resistance cultivar of *Lupinus luteus* contained a high concentration of lupanine (0.59µg/g dry matter) than the susceptible cultivar (0.51µg/g dry matter) against *A. pisum*. Also, Cai *et al.* (2004) reported that the resistant cultivar of wheat to the grain aphid, *S. avenae* had high indole alkaloids content during vegetative growth.

4.2. Aphicidal activity of treated leaves against *Aphis fabae*

The aphicidal activity against black bean aphid was observed with methanolic leaf extracts of the aphid resistant cultivar CV-12 (65.71%). It should be noted that the aqueous extracts with the control had a very low aphid's mortality rate (1.43%). The high difference in the mortality rate of *A. fabae* in leaves treated by methanolic leaf extracts of CV-12 comparing to the leaves treated by aqueous leaf extracts of CV-12 gives a good insight about its bioactivity. The aqueous leaf extracts of CV-12 had a knockdown effect, which resulted in low death of aphids. This difference in mortality of aphids perhaps resulted by the toxic effect of the methanol alone or with other molecules of CV-12. In contrast, this effect was totally absent in the case of aqueous leaf extracts. Moreover, the difference in black bean aphid mortality to the methanolic leaf extracts is probably related to its penetration and detoxification mechanisms.

Other many studies have been reported the aphicidal effect of plant extracts on black bean aphid, such as the crude leaf extracts of *T. minuta* at concentration of 150g/L soaked for 48 h (Mmbone *et al*., 2014), the acetonic seed extracts of *Peganum harmala* with a concentration of 60 mg/mL (Salari *et al*., 2012), the *Jatropha curcas* oil at concentration of 15% after 96 h (Habou *et al*., 2011), the acetonic leaf extracts of *Otostegia persica* with a concentration of 80 µL/mL (Salari *et al*., 2010).

Our results showed that the methanolic leaf extracts of aphid resistant cultivar CV-12 had a flavonoids low level (43.15 µg/g dry leaves). Indeed, Frah *et al.* (2013) indicated that the increase of flavonoids concentration extracted from *V. faba* did not affect the mortality rate of *A. craccivora*. However, Frah (2009) and Ateyyat *et al.* (2012) demonstrated that the increase of these molecules concentration affected nymph's mortality of *Dysaphis plantaginea and Eriosoma lanigerum*, respectively. Nevertheless, rutin hydrate is more toxic to *E. lanigerum* than quercetin dehydrate and naringin (Ateyyat *et al.*, 2012).

The comparison of the two experiments (aphicidal and repellency effects) showed that the leaves treated by aqueous leaf extracts of CV-12 had a high level of repulsive volatile substances, efficient in both tests (light and dark), but in the same time contains non toxic substances against *A. fabae*, which explains the very low mortality rate (1.43%). On the other hand, the leaves treated by methanolic extracts had a high content of toxic substances responsible for the high mortality rate of *A. fabae* (65.71%), and had a low repellence property in light.

Through this study, the good choice was probably the application of the aqueous leaf extracts of CV-12 because it has a good repellence property, it was avoided the totally infestation of the *V. faba* plants by *A. fabae*. But, in the case of high infestation of plants by *A. fabae*, the uses of the methanolic leaf extracts of CV-12 was priority for their high toxicity against *A. fabae* comparing to the aqueous leaf extracts.

The *V. faba* leaf extract is potent and could be useful for *A. fabae* because it was able to kill the treated pest through contact and oval toxicity, and it also as a strong insect repellent. This study is a preliminary investigation and its purpose was to compare the effect of a two types of *V. faba* leaf extracts (methanolic and aqueous) against *A. fabae*. More studies are needed to bioassay the activity of other plants and each identified compound against *A. fabae*.

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تأثير مستخلصات أوراق نبات الفول واستعمالها كمبيد حشري وطارد لمن الفول الأسود

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ملخص

تمت دراسة مقارنة مستخلصي الكحول الميثانولي والمائي لأوراق النوع المقاوم رقم 12 من نبتة الفول على سلوك ونسبة موت من الفول الأسود البالغ *Aphis fabae* في هذا البحث. الهدف الرئيس من هذا البحث هو معرفة مدى تأثير مستخلصات أوراق نبات الفول وإمكانية استعماله كمبيد حشري ضد الآفة الأولى للنبتة ألا وهي من الفول الأسود. أظهرت التحاليل الإحصائية أن المستخلص المائي لأوراق الفول لديه خاصية الطرد للحشرة بمعدل (1.3 حشرة بالغة) مقارنة بالمستخلص الميثانولي (3.7 حشرة بالغة). كما بينت النتائج أن المستخلص الميثانولي لأوراق الفول لديه خاصية سمية عالية ضد من الفول الأسود وصلت إلى 65.71% مقارنة بالمستخلص المائي والتي لم تتجاوزبسببه نسبة الموت 1.43%. تشير هذه الدراسة إلى أن مستخلصات أوراق الفول لديها خاصية الإبادة الحشرية والطرد لمن الفول الأسود بحيث يمكن استعمالها في برامج المكافحة المتكاملة ضد الآفات الحشرية كبديل عن مكافحة الآفات الكيميائية.

الكلمات الدالة: مَنّ الفول الأسود، مبيد حيوي، فلافونويدس، مستخلصات الأوراق، فينولس، الفول.

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