Aphidical and Behavioral Effects of Vicia faba L. (Fabales: Fabaceae) Leaf Extracts against Aphis fabae Scopoli (Hom., Aphididae) Fouad Meradsi*, Malik Laamari* 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. Aphidical and Behavioral... Fouad Meradsi, Malik Laamari –1086– 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. 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 CVJordan Journal of Agricultural Sciences, Volume 12, No.4 2016 -1087- 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 Na2OSS2. 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 x 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 AlCl3 to determine the amount of total flavanoids; or 0.5 mL of the extract added to 1 mL of Na2CO3, 5 ml of distilled 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 flavanoids. 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 ≤ 0.05 (Gomez and Gomez, 1984).

The rate mortality of A. fabae was subjected to the Chisquare test (X2) at 5% level. All statistical analyses were performed with SPSS statistical software (Version 10.0.5) (SPSS, 1999). All Experiments were designed in Aphicidal and Behavioral… Fouad Meradsi, Malik Laamari –1088– 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 ≤ 0.01; F5.49 = 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 attractivity of the three types of leaves for aphids did not differ significantly (P0.05; F3.88 = 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 0.00 ± 0.00a 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; X2 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 Chisquare test (X2) Survival adults 69 69 24 162 X2 = 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; F7.71 = 3.302). Nevertheless, a significant difference in flavonoids content between the two cultivars was recorded (P ≤ 0.01; F21.20 = 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). Jordan Journal of Agricultural Sciences, Volume 12, No.4 2016 –1089– 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