DOI: 10.17707/AgricultForest.63.1.11

Fatma ACHEUK, Wassima LAKHDARI, Khemais ABDELLAOUI, Messaouda BELAID, Rabea ALLOUANE, Fatma HALOUANE[1]

PHYTOCHEMICAL STUDY AND BIOINSECTICIDAL EFFECT OF THE CRUDE ETHONOLIC EXTRACT OF THE ALGERIAN PLANT *ARTEMISIA JUDAICA L.* (ASTERACEAE) AGAINST THE BLACK BEAN APHID, *APHIS FABAE* SCOP.

SUMMARY

Plants are the nature's biochemical factories. They bio-synthesize a diverse array of different natural products, such as alkaloids, terpenes and terpenoids, phenolic compounds, flavonoids and coumarins through their structural mechanisms to reduce insect attacks, both constitutive and inducible, while insects have evolved strategies to overcome these plant defenses.

There is a widespread effort to find new pesticides, and currently it is focused on natural compounds such as flavonoids, coumarins, terpenoids, and phenolics from diverse botanical families from arid and semi-arid lands. Algeria by the diversity of its habitats has a very diverse flora. Some of these plants have very interesting insecticidal properties. The aim of this study is to evaluate the insecticidal effect of the plant *Artemisia judaica* L. (Asteraceae). The crude ethanol extract of the plant *A. judaica* was tested on the black bean aphid *Aphis fabae* Scop. Four doses (12.5, 6.25, 3.12 and 1.56 mg mL^{-1}) were tested on contact wingless adults. The results have showed that the tested extract has been very powerful to aphids. At the highest dose 12.5 $mgmL^{-1}$, the 100% of mortality were recorded 2 hours after treatment, and for the lowest dose (1.56 $mgmL^{-1}$) it was after 96 hours. The LD50 calculated 2 hours after treatment from the regression lines Probit = *f* (doses) shows that it is 2.75 $mgmL^{-1}$. This powerful insecticidal activity of the tested crude extract could be due to the richness of the plant on phenolics compounds known for their bio-insecticide action.

Keywords: *Artemisia judaica, Crude extract, Aphis fabae, Insecticidal activity, Phytochemical study*

INTRODUCTION

In Algeria the bean, *Vicia faba* L., is the crop that is part of farming systems since a long time in different agro-ecological areas of the country. It's the most important food legume with 58000 hectares or 44.3% of the total area

[1]Fatma Acheuk, (corresponding author: fatma.acheuk@yahoo.fr), Messaouda Belaid, Rabea Allouane, Fatma Halouane, Valcore Laboratory, Department of Biology, Faculty of Sciences, University of Boumerdes, Boumerdes, 35000 ALGERIA, Wassima Lakhdari, National Institute of Agronomic Research, Station of Sidi Mehdi, Touggourt, ALGERIA, Khemais Abdellaoui, Department of Biological Sciences and Plants Protection, High Institute of agriculture, Chott-Mariem, Sousse University, TUNISIA

Paper presented at the 7th International Scientific Agricultural Symposium "AGROSYM 2016".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

reserved for this crop category (Bouznad *et al.*, 2001). In addition to abiotic stresses (cold, frost, heat and salinity), beans crop are exposed to the harmful effects of weeds, fungal and viral diseases, nematodes and insects (Maatougui, 1996). Among subordinate insects, aphids have a special place. The black bean aphid, *Aphis fabae*, can severely damage bean plants by checking growth, decreasing yield and impairing the quality of the seed (Banks and Macaulay,1967). Honey dew excretion and growth of sooty molds create also an indirect damage through impending some physiological processes in plant (Hurej and Van Der Werf, 1993). Beside the mechanical damage caused, they also serve as the largest group of vectors of plant viruses (Blackman and Eastop, 2006).

However the yields and quality of agricultural products depend largely on the use of synthetic chemical insecticides needed to control the populations of these pests. These compounds are often found as residues in food and pose significant risks to human health and the environment. In the interests of environmental respect and in the context of sustainable development, it should greatly to reduce the amount of synthetic pesticides and develop alternative control strategies. These new approaches should be based on the combined use of biomolecules provided with bioinsecticidal properties. Many molecules that exhibit toxic and defensive action against plant pests have been identified and more than 2000 plant species with insecticidal properties have been identified (Benayad, 2008).

The genus *Artemisia* is one of the largest and most widely distributed genera of the family Astraceae (Compositae). It is a heterogenous genus, consisting over 500 diverse species distributed mainly in the temperate zones of Europe, Africa, Asia and North America (Kundan and Anupam, 2011).

Artemisia juadaica L. is a perennial fragrant shrub, with pubescent leaves, which grows widely in the deserts (Abd-Elhady, 2012) and is a very common anthelmintic drug in most North African and Middle-Eastern countries where it is known by the Arabic name of "*Shih*" (Van Wyk and Wink, 2004). The plant has been used also to treat gastro-intestinal disorders, poor eyesight, cardiovascular disease, skin disorders, and weak immune systems as well as to decrease the risk of atherosclerosis, cancer, and arthritis (Liu *et al.*, 2004; Abd-Elhady, 2012).

The aim of this study was to investigate the insecticidal effect of methanolic extract of the aerial parts of *A. judaica* against the black bean aphid, *A. fabae* Scop. under laboratory conditions.

MATERIALS AND METHODS

Plant collection and preparation of crude ethanolic extract Aerial parts of *A. judaica*were collected during spring seasons 2012 in the Tamnraset region (South of Algeria). The plant was taxonomical identified and confirmed by PrAbdelkrim from the National High College of Agriculture, Algiers, Algeria. The crude ethanolic extract of the above-ground portion of the plant was prepared from leaves, flowers and stems dried in the shade and ground into a fine powder using electrical blender. The extraction was carried out by macerating the

powder for 3 days in ethanol, followed by filtration and evaporation at 40°C. The percentage of yield was calculated and the dried extract was kept at 4 °C until further use.

Phytochemical screening

The ethanolic extract was tested for plant secondary metabolites, alkaloids, sugar, phenolic compounds, flavonoids, saponins, tannins, iridois and coumarins. Phytochemical screening of the extract was carried out according to the standard method (Dohou *et al.*, 2003). Visible color change or precipitate formation was taken into consideration for presence (+) or absence (-) of particular active constituents.

Test organisms and bioassays

Stock of adults wingless aphids used in this study was collected randomly from infested bean filed in Ain Taya, Algiers area. The bioassays were conducted on Petri dishes under laboratory conditions at temperature of 22 ± 2 °C, 40-80% relative humidity and 16L:8D light regime. Forty aphids were transferred to Petri dishes on fresh leaves of *V.fabae* serving as a support for the aphids. Wet cotton discs were placed under the bean leaves to keep them fresh during the test period. Four doses of crude ethanolic extract were prepared (12.5, 6.25, 3.12 and 1.56 $mgmL^{-1}$). An appropriate quantity of *A. judaica* extract was dissolved in ethanol to obtain each test solution. The insecticide solution was applied by topic application (contact) to adult aphids using micropipette. Controls were treated with only absolute ethanol. Mortalities percentages were determined for each treatment after 2, 4, 24, 48, 72 and 96h. The LD50, the concentration that produces 50% mortality, was determined by log probit analysis.

Enzymatic assays

The AChE activity was carried out following the method of Ellman *et al.* (1961) using acetylthiocholine as a substrate. Aphids were sampled from control and treated groups (at low dose 1.56 $mgmL^{-1}$). Pools of twenty adults aphid were homogenized in the solution containing 38.03 mg of ethylene glycol tetraacetic (EGTA), 1mL Triton X-100, 5.845 g NaCl and 80 mL Tris buffer (10Mm, pH 7). The homogenate was centrifuged (5000 g for 5 min at 4°C), and the resulting supernatant was used for enzymatic assay. The AChE activity was measured in aliquots (100µL) of resulting supernatants added to 100 µLof 5-5' dithiobis-(2-nitrobenzoic acid) (DNTB) in Tris buffer (0.01 M, pH 8)and 1 mL Tris (0.1 M, pH 8).After 5 min, 100µLof acetylthiocholine was added. Measurements were conducted at a wavelength of 412 nm with a run time of 20 minutes. GST activities were determined with the soluble fraction as enzyme source. GST activities toward 1-chloro-2, 4-dinitrobenzene (CDNB) were measured according to Habig *et al.* (1974). Treated (at low dose 1.56 $mgmL^{-1}$) and control aphids were homogenized in sodium phosphate buffer (0.1 M, pH 6) and centrifuged (14000 g, 30 min). Two hundred microliter of the resulting supernatant was added to 1.2 mL of reaction mixture containing 1Mm of CDNB and 5 Mm of reduced glutathione (GST) in the homogenization buffer. Changes in absorbance were recorded at 340 nm. Total protein content was determined according to

method of Bradford (1976) using bovine serum albuminasa standard. Enzyme activities were expressed as µmolmin^{-1}mg^{-1}proteins.

Statistical analysis

Results are expressed as means ± standard deviation (SD). To identify significant effects of the treatments on the variables measured. Data were submitted to a monofactorial ANOVA using XLSTAT 7.5.2. Means were compared using Tukey's HSD test ($P< 0.05$).

RESULTS AND DISCUSSION

Phytochemical screening

The crude ethanolic extract of *A. judaica* was subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using classical methods of analysis. The results of phytochemical study (Table 1) revealed the presence of phenolic compounds, flavonoids, alkaloids, tannins, saponins and comarins. Antocyans and iridoiswere absents.

Table 1.Qualitative phytochemical screening of crude ethanolic extract of *A. judaica*

Alkaloids	Antocyans	Comarins	Tannins	Saponins	Iridois	Flavonoids
+	-	+	+++	+	-	+++

Insecticidal activity

The results of the toxicity assay against of the black bean aphid, *A.fabae*, are given in the figure 1 and the table 2. The test compound showed high insecticidal activity for all tested concentrations.

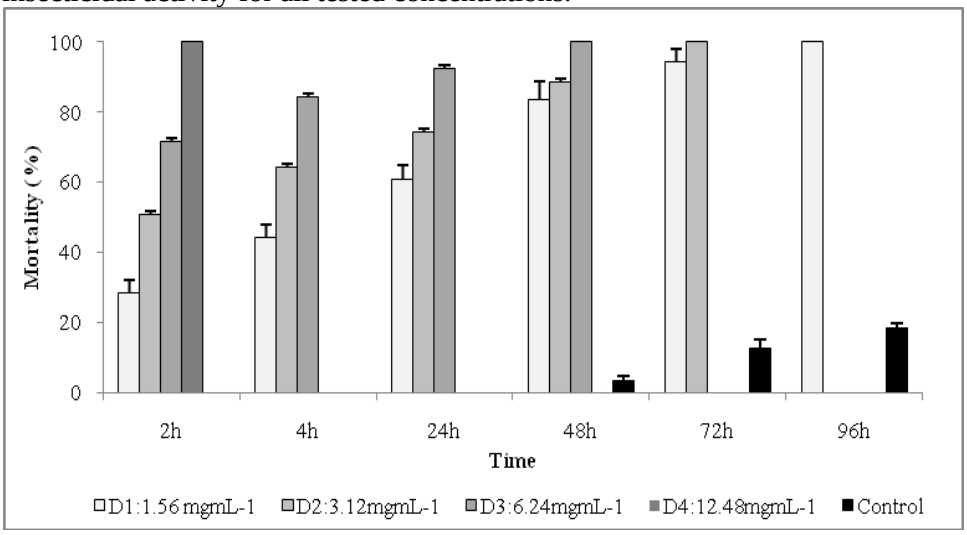

Figure 1. Effect of crude ethanolic extract of *A. judaica* on mortality of the the black bean aphid, *A.fabae* (Maen ± SD). N=60 aphids /replicate, ($p <0.05$).

At the highest dose 12.5 $mgmL^{-1}$, the 100% of mortality were recorded 2 hours after treatment, and for the lowest dose (1.56 $mgmL^{-1}$) it was after 96 hours. Mortality rates ranged from 50.83 to 71.66% at 2 h after treatment for the average concentration 3.12 and 6.24 $mgmL^{-1}$ respectively. Total mortality (100%) was achieved 48h and 72h after treatment respectively for the concentrations 6.24 and 3.12 $mgmL^{-1}$.

Table 2. LD50 values of *A. judaica* crude methanolic extract against the black bean aphid, *A.fabae*

Time (h)	LD50 values ($mgmL^{-1}$)
2	2.79
4	1.91
24	1.24
48	1.04
72	0.82

The results of probit analysis showed that *A. fabae* was susceptible to the crude ethanolic extract of *A.judaica*. The LD50 was obtained 2h after treatment.

Enzymathic effects

The effect of the crude ethanolic extract of *A. judaica* on enzymatic activities (GST and AchE) is presented in figures 2 and 3. The results showed an inhibition of AchE activity in treated aphids at high concentration of the crude extract of *A. judaica*. However, an activation of GST activity was observed on treated aphids.

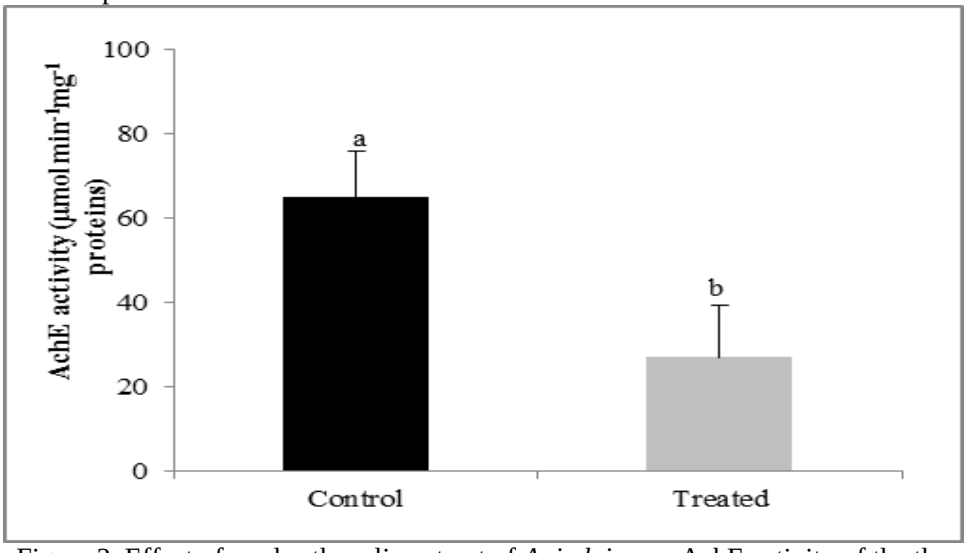

Figure 2. Effect of crude ethanolic extract of *A. judaica* on AchE activity of the the black bean aphid, *A. fabae* (Maen ± SD), N=60 aphids. Different letters denote significant differences (Tukey's test, $p < 0.05$).

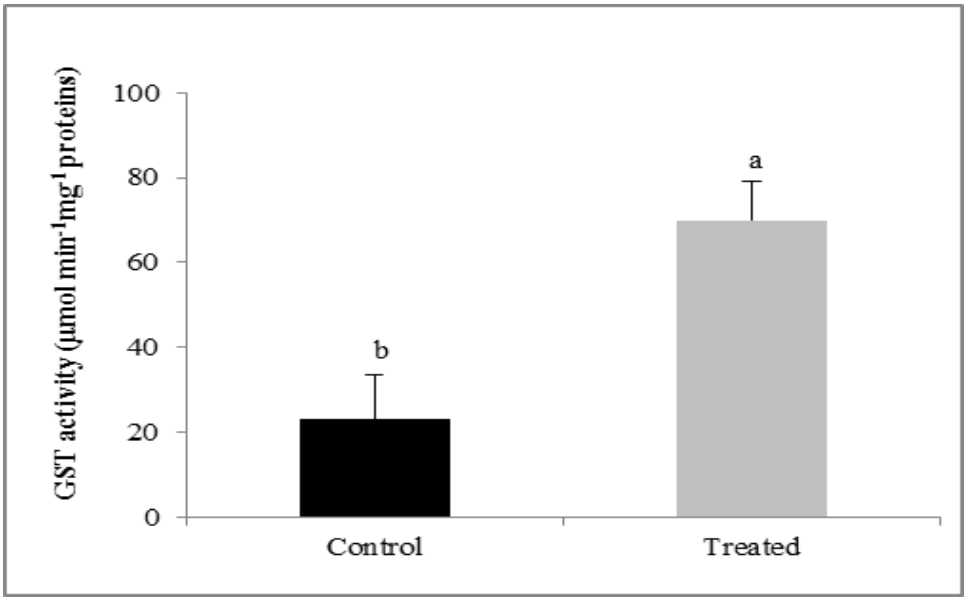

Figure 3. Effect of crude ethanolic extract of *A. judaica* on GST activity of the the black bean aphid, *A.fabae* (Maen± SD), N=60 aphids. Different letters denote significant differences (Tukey's test, $p < 0.05$).

Certain natural products can be suitable alternatives to synthetic pesticides owing to their generally reduced negative impacts on humans, beneficial insects and the environment. Higher plants constitute a diverse source of highly bioactive agents that include some that have contributed significantly to the successful use of natural products and analogues for crop protection (Akhtar and Isman, 2013). In this study, phytochemical screening and the insecticidal activity of the crude ethanolic extract of the Algerian plant *A. judaica* were studied. The results of phytochemical screening revealed the presence of phenolic compounds, flavonoids, alkaloids, tannins, saponins and comarins. These results are in agreement with knows compositions of many *Artemisia* species (Masotti *et al*., 2012). Kundan and Anupam (2011) reported that the *Artemisia* species comprise mainly terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes.

Many researchers have reported on the effectiveness of plant extracts against insects (Acheuk *et al.*, 2012; Abdellaoui *et al.*, 2013, 2016; Pavela,2004 and Nathan *et al.*, 2006). In the present work, the crude ethanolic extract of *A. judaica* showed potent insecticidal effects against the black aphid, *A.fabae*. Total mortality (100%) was achieved 2h after treatment with the higher concentration. This toxic effect of the extract might be due to the various bioactives compounds that exist in the aerial part of the plant. Indeed, the crude extract of plant is a mixture of potentially bioactive substances which may act synergically (Acheuk *et al.*, 2012) or independently (Kabir, 2013). Previous studies demonstrated the insecticidal effects of *Artemisia* plant extracts against other insects pest. Abd-

Elhady (2012) reported that volatile oils from the aerial parts of *A. judaica*, were found to have an insecticidal effect against *Callosobruchus maculatus* (Fab.). A concentrated extraction of 50% was shown to have the highest mortality. As our results, Masotti *et al.* (2012) reported that ethanolic extracts of *A. molinieri* and *A. campestris* Varglutinosa showed larvicidal activity against mosquito *Culex pipiens*. However, extracts of *A. molinieri* revealed a higher larvicidal activity than those of *A. campestris*. The biocide differences found for the tested extracts can be explained by their different chemical compositions, with aromatic polyacetylene dominant fraction for *A.molinieri* ethanolic extracts. In the same way, Dane *et al.* (2016) reported that the methanolic extract from *A. absinthium* showed potent toxicity for *Sitophilus oryzae* and causing 100% in 24h at a dose of 60 $mgcm^{-2}$.

Many natural plant compounds used in the control of insect pest are known to exhibit effects in the enzymatic profiles (Smirle *et al.*, 1996; Zhang *et al.*, 2013). The finding of our study showed that, the crude ethanolic extract of *A. judaica* inhibited AchE activity on treated aphids. It's known that AchE regulate nerve impulse transmission across cholinergic synaps. Monoterpenoids tested by López and Pascual-Villalobos (2010), showed inhibitor effect on acethylcholinesterase enzyme. From the all compounds tested by these authors, fenchone, S-carvone and linalool produced the highest inhibition. Similarly, the study conducted by Abdel-Aziz *et al.* (2015), demonstrated that, rosacide treatment showed the highest AchEpercent of inhibition (72.06%) on *Aphis craccivora*. This inhibition is due to the presence of high amount of the monoterpenoid 1, 8-cineolein rosacide, which is known for its insecticidal, feeding, deterrent and repellent properties. It inhibits acetylcholinesterase by occupying the hydrophobic site of enzyme's active centre. In agreement, Nathan *et al.*(2008) found that Azadirachtin significantly inhibits the activity of AchE in *Nilaparvata lugens*.

In insects, allelochemical defense system include P450, gluthathione S-transferase and esterases which are typically concentrated in midgut allowing rapid elimination of ingested toxic substances (Rattan, 2010). Elevated detoxification enzymes activity in insects tissues are often associated with enhanced detoxification of allelochemicals (Valles *et al.*, 1999). GST play a pivotal role in detoxification and antioxidant defense of insects against natural and synthetic exogenous xenobiotics including insecticides, allelochemicals and endogenously activated compounds (Papadopoulos *et al.*, 2004). GSTs catalyse the addition of a tripeptide glutathione to a wide variety of electrophilic substrates including host plant secondary compounds (Yu and Hsu, 1993). In our study, the GST activity in *A. fabae* was significantly stimulated by the low dose of the crude ethanolic extract of *A. judaica*. Similarly, different plant secondary compounds; catechol (phenolics), gramine (alkaloid) and L-ornithine-HCl (non protein amino acid), tested in the artificial diet activated the GST activity in the English grain aphid *Sitobion avenae* and a significant correlation was also observed between the concentration of each compound in diet and GST activity

in aphids (Zhang *et al*., 2013). Our findings are also in agreement with those of Abdel-Aziz *et al.* (2015) who reported that the green insecticide Cura (curcuma oil mixed with mineral and vegetable oils) increased the GST activity with (10.16%) than control in *Aphis craccivora*.

CONCLUSIONS

The results of our study demonstrate the potent aphicidal activity of the crude ethanolic extract of the Algerian Asteraceae *A. judaica*. However, further studies for guided isolation of the insecticidal compounds and their effects on the non target organisms are strongly needed.

REFERENCES

Abdel-Aziz N.F., Abdou W.L., Abdel-Hakim E.A., El-Hawarya F.M., El-Bakry A.M., Sammour E.A. (2015). The effect of some green insecticides from essential oils on *Aphis craccivora* and their side effects. Journal of Entomological Research, 39 (4), 275-286.

Abd-Elhady H.K. (2012). Insecticidal activity and chemical composition of essential oil from *Artemisia judaica* l. against *Callosobruchus maculatus* (f.) (Coleoptera: Bruchidae). Journal of Plant Protection Research, 52 (3), 347-352.

Abdellaoui K., Miladi M., Ben Marzouk I., Bahloul N., Acheuk F., Chaira N., Ben Halima-Kamel M. (2016). Antifeedant and antigonadotropic effects of *Ruta chalepensis* methanolic extract against *Locusta migratoria*. Tunisian Journal of Plant Protection, 11, 91-104.

Abdellaoui K., Ben Halima-Kamel M., Acheuk F., Soltani N., Aribi N., Ben Hamouda M.H. (2013). Biochemical and histological effects of gibberellic acid on *Locusta migratoria migratoria* fifth instar larvae. Pesticide Biochemstry and Physiology, 107, 32-37.

Acheuk F., Cusson M., Doumandji-Mitiche B. (2012). Effects of a methanolic extract of the plant *Haplophyllum tuberculatum* and of teflubenzuron on female reproduction in the migratory locust, *Locusta migratoria* (Orthoptera: Oedipodinae), Journal of Insect Physiology, 58, 335-341.

Akhtar Y., Isman M.B. (2013). In IshaayaI., Palli S. R.,Horowitz, A. R. Plant Natural Products for Pest Management: The Magic of Mixtures. Ed Spring, 231-247.

Banks C.J., Macaulay E.D.M. (1967). Effects of *Aphis fabae* Scop.and of its attendant ants and insect predators on yields of field beans (*Vicia faba* L.). Annals of Applied Biology, 60, 445-453.

Benayad N. (2008). Les huiles essentielles extraites des plantes médicinales marocaines: moyen efficace de lutte contre les ravageurs des denrées alimentaires stockées (Essential oils extracted from Moroccan medicinal plants: Effectiveness against fight against pests of stored foods). Projet de recherche. UNISCO. Université Mohamed V. Faculté des Sciences de Rabat, 1-63.

Blackman R.L., Eastop V.F. (2006). Aphids on the world's her because plants and shrubs: Vol. 2. Chichester: John Wiley & Sons.

Bouznad Z., Louanchi M., Allala L., Mérabti N. (2001). Les maladies de la fèveen Algérie cas de maladie à tache chocolat causée par *Bortrytis* spp. (Diseases of bean in Algeria: case of chocolat spot disease caused by *Botrytis* spp.) Quatrièmes journées scientifiques et techniques phytosanitaires, INPV, 12-13 Novembre 2001, 2 p.

Bradford M.M. (1976).A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 72, 248-254.

Dane Y., Mouhouche F., Canela-Garayoa R., Delpino-Rius A. (2016). Phytochemical analysis of methanolic extracts of *Artemisia absinthium* L. 1753 (Asteraceae), *Juniperus phoenicea* L., and *Tetraclinis articulata* (Vahl) Mast, 1892 (Cupressaceae) and evaluation of their biological activity for stored grain protection. Arabian Journal for Science and Engineering, 41(6), 2147-2158.

Dohou W., Yamni K., Tahrouch S., Idrissi Hassani L.M., Bado A. Guira N. (2003). Screening phytochimiques d'une endemique ibére-macrowave, *Thymelaea lythroides* (Phytochemical screening of the endemic Ibero-macrowave, *Thymelaea lythroides*). Bulletin de la Société de Pharmacie de Bordeaux**,** 142**,** 61-78.

Ellman G.L., Courtney K.D., Andres V., Featherstone A. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7, 88-95.

Habig W.H., Pabst M.J., Jakoby W.B. (1974). Glutathione S-transferases, the first enzymatic step in mercapturic acid. Journal of Biological Chemistry, 249, 7130-7139.

Hurej M., Van Der Werf W. (1993). The influence of the black bean aphid, *Aphis fabae* Scop and its honeydew on the photosynthesis of sugar beet. Annals of Applied Biology, 122,189-200.

Kabir K.E., Choudhary M.I., Ahmed S., Tariq R.M. (2013). Growth-distruping, larvicidal and neurobehavioral toxicity effects of seed extract of *Seseli diffusum* against (L.) (Diptera: Culicidae). Ecotoxicology and environmental Safety, 90, 52-60.

Kundan S.B. Anupam S. (2011). The Genus *Artemisia*: A Comprehensive Review. Pharmaceutical Biology, 49(1), 101-109.

Liu C.Z, Murch S.J, El-Demerdash M., Saxena P.K. (2004).*Artemisia judaica* L., micropropagation and antioxidant activity. Journal of Biotechnology, 110, 63-71.

López M.D., Pascual-Villalobos M.J. (2010). Mode of inhibition of acetylcholinesterase by monoterpenoids and implications for pest control. Industrial Crops and Products, 31, 284-288.

Maatougui M.E. (1996). Situation de la culture des fèvesen Algérie et perspectives de relance (Bean crop situation in Algeria and recovery prospects), In rehabilitation of fababean. Ed. Actes, Rabat, 17-30.

Masotti V., De Jong L., Moreau X., Rabier J., Laffont Schwob I., Thiery A. (2012). Larvicidal activity of extracts from *Artemisia* species against *Culex pipiens*L. mosquito: Comparing endemic versus ubiquist species for effectiveness. Comptes Rendus Biologies, 335, 19-25.

Nathan S.S., Chung P.G.Murugan K. (2006). Effect of biopesticides on the digestive enzymatic profiles of *Cnaphalocrocismedinalis* (Guenée) (the rice leaffolder) (Insecta: Lepidoptera: Pyralidae). Ecotoxicology and Enviromrental Safety, 64,382-389.

Nathan S.S, Choi Y.M, Seo YH, Paik H.C, Kalaivani K., Kim D.J. (2008). Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stål). Ecotoxicology and Environmental Safety, 70, 244-250.

Papadopoulos, I.A., Polemitou, I., Laifi, P., Yiangou, A. and Tananaki, C. (2004).Glutathione S-transferase in the developmental stages of the insect *Apis mellifera macedonica*. Comparative Biochemistry and Physiology, Part C, 139, 87-92.

Pavela, R., (2004). The effect of ethanol extract from plants of family Lamiaceae on colorado potato beetle adults (*Leptinotarsa decemlineata* Say). National Academy Science Letters, 27, 195-203.

Rattan R.S. (2010). Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Protection, 29, 913-920.

Smirle M.J., Lowery D.T., Zurowski C.L. (1996). Influence of Neem Oil on Detoxication Enzyme Activity in the Oblique banded Leafroller, *Choristoneura rosaceana*. Pesticide Biochemistry and Physiology 56, 220-230.

Valles, S.M., Koehler, P.G. Brenner, R.J. (1999). Comparative insecticide susceptibility and detoxification enzyme activities among pestiferous blattodea. Comparative Biochemistry and Physiology pat C, 124, 227-232.

Van Wyk B.E. Wink M. (2004). Medicinal Plants of the World; Briza Publications: Pretoria, South Africa, 54-56.

Yu S.T., Hsu E.L. (1993). Induction of detoxification enzymes in phytophagous insects: role of insecticide synergists, larval age and species. Archive of Insect Biochemistry and Physiology, 24, 21-32.

Zhang M., Fang T., Pu G., Sun X., Zhou X. Cai Q. (2013). Xenobiotic metabolism of plant secondary compounds in the English grain aphid, *Sitobion avanae* (F.) (Hemiptera: Aphididae). Pesticide Biochemistry and Physiology, 107, 44-49.