The Journal of Animal & Plant Sciences, 25(2): 2015, Page: 456-462
ISSN: 1018-7081

APHIDICIDAL ACTIVITY OF DIFFERENT AQUEOUS EXTRACTS OF BITTER APPLE *CITRULLUS COLOCYNTHIS* (L.) AGAINST THE BIRD CHERRY-OAT APHID, *RHOPALOSIPHUM PADI* (L.) (HOMOPTERA: APHIDIDAE) UNDER LABORATORY CONDITIONS

Khalid A. Asiry*

Department of Biology, Faculty of Science, University of Hail, Saudi Arabia.
*Corresponding Author Email address: Prof_1974@hotmail.com.

ABSTRACT

The impacts of aqueous extracts from different parts (root, stem, leaf and fruit) of bitter apple *Citrullus colocynthis* (Cucurbitales: Cucurbitaceae), with five different concentrations, on controlling the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae) were investigated under laboratory conditions. Results showed that the effects of the aqueous extracts from different parts of *C. colocynthis* significantly differed in reducing *R. padi* numbers. The stem caused a higher mortality of *R. padi*, compared to other parts, in which the mortality percentages of *R. padi* were 75%, 71.25%, 60%, 43.75% and 40% respectively, when the aphids were treated with the aqueous extract of stem at 100000, 1000, 10, 0.1, 0.001 ppm, respectively. Also, toxicological parameters including LC_{50}, LC_{90}, toxicological index and slope of the toxicity lines showed that stems of *C. colocynthis* were more potent in controlling *R. padi*, compared to other parts. LC_{50} values of stem, root, leaf and fruit of *C. colocynthis* were 0.0002, 0.024, 0.043 and 0.055 ppm, respectively. Stem was revealed to be more effective than root by 0.83%, leaf by 0.47% and fruit by 0.36% for controlling *R. padi*. This could be justified to a higher amount of some toxicants in the stem such as terpenoids, cucurbitacin, glycosides, flavonoids and other compounds that may have a positive effect in controlling aphid pests. This study suggests that more detailed, long-term studies on both the chemistry of *C. colocynthis* and its effects on the pests' natural enemies are required before recommending its use as an alternative to synthetic pesticides.

Keywords: Bitter apple, *Citrullus colocynthis*, Botanical pesticides, Aqueous extracts, *Rhopalosiphum padi*, Aphid control.

INTRODUCTION

Cereal crops are heavily attacked by aphid pests that congregate on plant stems and leaves, sucking plant nutrients as well as transmuting plant diseases which weaken plant growth and development and result in yield losses (DeBach and Rosen, 1974; Dixon, 1987; Shah *et al.*, 2007; Davoodi Dehkordi *et al.*, 2013). The bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera: Aphididae) is considered to be one of the most destructive pests of cereal crops in different parts of the world (Taheri *et al.*, 2010; Descamps and Chopa, 2011). For instance, the estimated damage to cereal crops caused by *R. padi* in some parts of Europe can result in yield losses of up to 15% (Ostman *et al.*, 2003). This has led to the extensive application of synthetic pesticides annually in cereal fields in order to guarantee sufficient levels of food production. Indeed, the worldwide pesticide market in 2000 was estimated to be worth approximately $29 billion (Marshall *et al.*, 2003). However, the application of synthetic pesticides has adverse effects on humans and the environment.

Any adverse effects of synthetic pesticides on humans are caused by pesticide residues left on crops when they are eaten. These residues can negatively affect human health through chronic illnesses such as cancer and sterility (DeBach and Rosen, 1974; Bale *et al.*, 2008). Adverse effects of the application of pesticides on the environment can be summarized as follows: biodiversity loss, water contamination, disruption of food chains and soil degradation (Bale *et al.*, 2008; Altieri, 1991). This has led policy makers and scientists to seek safer methods for controlling insect pests (Coll and Bottrell, 1995; Pimentel, 2005; Fetoh and Asiry, 2012).

In recent years, there has been a growing interest in the use of botanical pesticides as an alternative to synthetic pesticides, although botanical pesticides have been used for thousands of years against insect pests (Koul and Walia, 2009; Fetoh and Asiry, 2012). Botanical pesticides can be defined as the extraction of toxicant chemicals from plants by using a suitable solvent in which these chemicals occur naturally (Koul and Walia, 2009). Plants provide a valuable source of active chemicals such as alkaloids, terpenoids, cucurbitacin, glycosides, flavonoids and other compounds that have been used as toxins against several insect pests which affect commercial crops (Koul and Walia, 2009). It has been suggested that leaf and flower extracts of the Sweet Orange *Citrus sinensis,* Sweet Basil *Ocimum canum,* Holy Basil *Ocimum sanctum* and Snak Jasmine

Rhinacanthus nasutus in different solvents including acetone, chloroform, ethyl acetate, hexane and methanol, can be used as an eco-friendly method to control lepidopteran pests in the field setting (Koul and Walia, 2009).

Bitter apple plants *Citrullus colocynthis*, also known as bitter cucumber and colocynth or desert gourd, belong to the family of Cucurbitaceae and have a wide distribution, being commonly found in the sandy lands of India, Arabia, West Asia, and Tropical Africa and in the Mediterranean region (Pravin *et al.*, 2013). Recently, an increased attention has been paid to the use of *C. colocynthis* as a natural insecticide and the biological activity of this plant has been investigated against many insect pests (Soam *et al.*, 2013). This plant is known to have a range of compounds, which show insecticidal, antibacterial, larvicidal, deterrent, antifeedant, growth-regulating and antifertility effects (Pravin *et al.*, 2013; Soam *et al.*, 2013). Furthermore, this plant can be used medically as an abortifacient, cathartic, purgative and vermifuse, as well as for the treatment of fever, cancer, amenorrhea, jaundice, leukemia, rheumatism, tumour and as an insect repellant (Soam *et al.*, 2013).

The current study aimed to investigate the toxicity effect of aqueous extracts from different parts of *C. colocynthis* (root, stem, leaf and fruit) against the bird cherry-oat aphid, *R. padi* under laboratory conditions. This study expects that there will be no significant differences between the different extracts of *C. colocynthis* parts against *R. padi.*

MATERIALS AND METHODS

Preparation of *Citrullus colocynthis* extracts: Different parts (root, stem, leaf and fruit) of bitter apple *C. colocynthis* were collected from Hail desert located in the north central part of Saudi Arabia (27° 31′ 0″ N, 41° 41′ 0″ E), in spring 2013. The collected plants and their parts were carefully isolated from combined impurities such as weeds, soil particles and other inessential matter. The root, stem, leaf of *C. colocynthis* were washed and dried at room temperature in dark conditions for three weeks, while the fruit was left to dry for three months. The root, stem, leaf and fruit of *C. colocynthis* were separately powdered using an electric blender. Then, 10 grams of grinded powder from each part of *C. colocynthis* were dissolved in 100 ml distilled water in a conical flask, exhaustively shaken manually and left to stand for 24 hrs, then filtered through filter papers to acquire the stock solution (10% w/v). Five dilutions were prepared for each plant part used, as follows: 100000, 1000, 10, 0.1, 0.001 ppm. Thus, five serial concentrations of aqueous extracts from four different parts of *C. colocynthis* were investigated.

Collection of the bird cherry-oat aphid, *Rhopalosiphum padi:* Wingless adults of the bird cherry-oat aphid *R. padi* were collected from pesticides-free barley crop (*Hordeum vulgare* L.) which was grown in a private farm in Al-Homimah district, Hail region. Aphids were collected from host plants, kept in plastic bags, and then transmitted to the laboratory where they were kept in the fridge for twenty-four hours prior to commencing the experiment. The design of the experiment included dividing the collected aphids randomly into four groups (one for each plant part). Each group was tested with the five extracts' dilutions respectively, where each dilution was tested on 20 aphids. Moreover, each dilution was replicated four times.

Bioassay and aphid mortality: In order to assess the effect of aqueous extracts from the four parts (root, stem, leaf and fruit) of *C. colocynthis* on the mortality of *R. padi*, pieces measuring 7 cm in length were cut out from the center of barley leaves. Each leaf piece was separately dipped into aqueous extracts from root, stem, leaf and fruit of *C. colocynthis* with five different concentrations for 30 seconds and then air dried. Then, each leaf piece was placed in a Petri dish (90mm diameter × 15mm deep) and 20 adults of *R. padi* were individually transmitted to the middle of each leaf piece by using a soft hair brush. Each Petri dish was covered with a wax Parafilm to ensure that no aphids could escape from the Petri dish, and then the Petri dishes were kept in an incubator at 25 ± 1^{0}C and 40-50% humidity. Aphid mortality was assessed by counting dead aphids in the Petri dishes after twenty-four hours.

Data analyses and Statistics: Prior to performing analyses of the response variable (the mortality of *R. padi*), data were transformed to $\log_e$ (N+1) to improve normality and homogeneity of variance. To assess the effect of *C. colocynthis* on the mortality of *R. padi*, transformed data were subjected to analysis using a general linear model (GLM) within the Statistical Analysis Software (SAS) package 9.2 (SAS, 2009). The response variable of the mortality of *R. padi* numbers ($\log_e$ (N+1) transformed) was tested against the explanatory variables of aqueous extracts of the different four parts of *C. colocynthis* and their concentrations (with 5 levels) and the interaction between these two factors. This model included replicate blocking as a covariate factor. Differences among means were considered significant at a probability level of five percent ($P \leq 0.05$). Where significant treatment effects were found, *post hoc* Tukey's test was performed to identify differences in treatment means. In addition, calculation of the percentage of mean mortality of *R. padi* was preformed; results were adjusted using Abbot's formula and statistically computed according to Finney (1971). To achieve the corresponding log-concentration probit lines, the calculated percentage mortality of *R. padi* was plotted with consistent concentrations on logarithmic probability paper. The regression lines were created to determine the lethal concentrations of 50% and 90% (LC_{50} and LC_{90}) on *R. padi*. Additionally, the toxicity index was calculated according to the equation of Sun (1951).

RESULTS AND DISCUSSION

Data revealed higher significant variations between aqueous extracts from the four parts (stem, root, leaf and fruit) of *C. colocynthis* on the mortality of *R. padi* ($F_{3.57}$ =53.71, P < 0.001), showing higher effects of stem extract to decrease the survivorship of *R. padi* (Table 1). Moreover, there were significantly higher differences between the five concentrations of the aqueous extracts from all parts of *C. colocynthis*, as more *R. padi* adults were killed under these concentrations, ($F_{4.57}$ =94.76, P < 0.001), showing a higher influence of the 100000 ppm and 1000 ppm on the mortality of *R. padi* from all four parts of *C. colocynthis* (Table 1). However, there was no a significant effect of the arrangement of the Petri dishes (block) on the mortality of *R. padi* ($F_{3.57}$ =1.14, P = 0.34). Interestingly, the interaction between *C. colocynthis* parts and their concentrations showed a higher significant impact upon the mortality of *R. padi* ($F_{12.57}$ =4.20, P < 0.001). In a general trend, all five concentrations of stem extract had higher significant effects on the *R. padi* mortality compared to other parts of *C. colocynthis* (t < 0.05), although in some cases there were no significant differences between the concentrations of stem, root, leaf and fruit on the *R. padi* mortality (t > 0.05), according to *post hoc* Tukey's test (Table 1).

The percentage of aphid mortality fluctuated between 75% and 40% when the aphids were treated with the highest (100000 ppm) and lowest (0.001 ppm) concentrations of stem extract, respectively (Table 2). This effect also was greater when the aphids were treated with the highest concentration (100000 ppm) of other *C. colocynthis* parts in which root exerted 60% of aphid mortality, while leaf and fruit showed 62.5% (Table 2). However, the percentages of aphid mortality declined when the aphids were treated with other concentrations of other *C. colocynthis* parts (root, leaf and fruit) compared with stem (Table 2). Similarly, toxicological index in (Table 3) also showed a higher toxicity of the aqueous extract from stem against *R. padi* compared with other parts of *C. colocynthis*. In this table, LC_{50} values of aqueous extracts from stem, root, leaf and fruit of *C. colocynthis* were 0.0002, 0.024, 0.043 and 0.055 ppm, respectively. The LC_{90} values also showed the same trend: 14.378, 21.637, 35.617 and 128.08 ppm of the stem, root, leaf and fruit, respectively. Stem extract of *C. colocynthis* showed the maximum toxicity, as this part has been revealed to be more effective than root by 0.83%, leaf by 0.47% and fruit by 0.36% (Table 3). In addition, Probit lines of predicted percentages of *R. padi* death showed a similar effect for the stem extract in reducing *R. padi* numbers compared to other plant parts (Figure 1). The figure shows that with increasing concentrations of the aqueous stem extract, more death of *R. padi* occurred, compared to the others parts of *C. colocynthis*. Moreover, slope measures of the toxicity lines of stem, fruit, root and leaf of *C. colocynthis* were 0.27, 0.38, 0.43 and 0.44, respectively. In summary, all data analyses indicated that the aqueous stem extract of *C. colocynthis* is the most effective for controlling the *R. padi* aphids. The bird cherry-oat aphid (*R. padi*) is one of the most destructive insect pests that cause yield losses of cereal productions on a world scale (Ostman *et al.*, 2003). This, in turn, has led to extensive application of synthetic insecticides for controlling this aphid species within agro-ecosystems. The extensive use of synthesized insecticides has adverse effects on humans and the environment (Marshall *et al.*, 2003; Pimentel, 2005; Palis *et al.*, 2006; Schou *et al.*, 2006; Winqvist *et al.*, 2011). This has led both policymakers and scientists to try to find other sustainable methods such as natural products from wildlife plants (Fetoh and Asiry, 2012). In this study, the impact of aqueous extracts of *C. colocynthis* on the mortality of the bird cherry-oat aphid *R. padi* under laboratory conditions was investigated, revealing the higher toxicity of *C. colocynthis* on aphids. In this study, the toxicity of *C. colocynthis* against *R. padi* was very obvious when aphids treated with the higher concentration (100000 pm) of all *C. colocynthis* parts showed a higher mortality of aphids after twenty-four hours.

The bitter apple plant *C. colocynthis* has antibacterial, larvicidal, deterrent, antifeedant, growth-regulating and antifertility components affecting the survivorship of insect pests (Pravin *et al.*, 2013; Soam *et al.*, 2013). Such alkaloids, terpenoids, cucurbitacin, glycosides and flavonoids have insecticidal attributes of *C. colocynthis* (Gurudeeban *et al.*, 2010). This may explain the higher aphid mortality in the results of the current study. This finding is in accordance with some other studies, although studies of aphid control by using the extract of *C. colocynthis* are rare. Sayeda *et al.* (2009) found that formulated extract of *C. colocynthis* was extremely toxic against adults of the cowpea aphid (*Aphis craccivora* Koch.) with LC_{50} 123.68 ppm. Similarly, Soliman *et al.* (2005) reported a higher toxicity of hexane, diethyl ether, ethyl acetate, acetone and ethanol extracts of *C. colocynthis* against adults of the cotton aphid (*Aphis gossypii* Glover.). Torkey *et al.* (2009) noted that the ethanolic extract of *C. colocynthis* fruit exerted the highest toxicity (LC_{50} 11003ppm) against *A. craccivora* compared with n-hexane, methylenechloride and chloroform extracts. More recently, Soam *et al.* (2013) documented that the application of aerial parts of *C. colocynthis* at 250µg/ml and 500µg/ml concentrations led to 100% mortality of mustard aphid (*Lipaphis erysimi* Kalt) after 24hrs.

Several studies on the activity of insecticides of *C. colocynthis* against other insect pests have been conducted, supporting the toxicity of *C. colocynthis* against *R. padi*, which was noted in the current study. Mullai and Jebanesan (2007) observed 100% mortality of the larval stage of the filarial vector *Culex quinquefasciatus* (Say) (Diptera : Culicidae) when this pest was treated with the leaves extract of *C. colocynthis* at 450 ppm, suggesting that the plant *C. colocynthis* exhibits larvicidal, ovicidal and repellent activities against *Cx. quinquefasciatus* and can be used as a natural insecticidal product against mosquitoes. In addition, Rahuman *et al.* (2008) found out that the leaves extract of *C. colocynthis* had the oleic and linoleic acids, which showed toxic effects against mosquito larvae. Rawi *et al.* (2011) revealed that the methylene chloride fruits extract of *C. colocynthis* was effective for reducing larval stage of the Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd.). Ramzi *et al.* (2013) reported the pesticidal activity of the lectin of *C. colocynthis* against *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) larvae, showing the toxicity effect of *C. colocynthis* lectin on the survivorship of *E. ceratoniae* larvae. Bashir *et al.* (2013) showed the acaricidal activity of crude aqueous leaves extract of *C. colocynthis* against the stored grain mite, *Rhizoglyphus tritici* (Acari: Acaridae) in which the activity of *C. colocynthis* for reducing this pest fluctuated from 22% to 80%, 30% to 82%, 41% to 88%, and 47% to 92% after exposure periods of 7 days, 14 days, 21 days, and 28 days, respectively.

Table (1): The effect of the five different concentrations of aqueous extracts (ppm) across four parts of *Citrullus colocynthis* on the mortality of *Rhopalosiphum padi* adults. The mortality value of each concentration from each part of *C. colocynthis* is the transformed mean of four replications and standard errors (±SE) of mean after twenty-four hours.

Concentration (ppm)	Plant part			
	Stem	Root	Leaf	Fruit
100000	2.76±0.12 a	2.55±0.11 a	2.59±0.12 a	2.59±0.10 a
1000	2.71±0.10 a	2.27±0.05 b	2.13±0.09 c	2.08±0.06 c
10	2.56± 0.07 a	2.10±0.07 c	1.75±0.05 ab	1.75±0.05 ab
0.10	2.27±0.10 b	1.56±0.22 ab	1.37±0.12 ac	1.54±0.11 ab
0.001	2.19±0.07 b	0.90±0.14 abc	1.17±0.10 abc	1.25±0.03 ac

Means with the same letter are not significantly different (t > 0.05), according to *post hoc* Tukey's test of means.

Table (2): the percentage of mean mortality and standard errors (±SE) of mean of *Rhopalosiphum padi* affected by different concentrations (ppm) of aqueous extracts from different parts of *Citrullus colocynthis*.

Concentration (ppm)	Plant part			
	Stem	Root	Leaf	Fruit
100000	75±1.18	60±1.56	62.5±1.53	62.5±1.37
	(90-55)	(80-50)	(75-45)	(75-50)
1000	71.25±1.44	43.75±0.55	37.5±0.75	35±0.47
	(85-55)	(50-40)	(40-30)	(40-30)
10	60± 0.94	36.25±0.55	23.75±0.29	22.5±0.29
	(70-50)	(40-30)	(25-20)	(25-20)
0.10	43.75±0.87	20±1.05	15±0.47	18.75±0.55
	(50-35)	(30-10)	(20-10)	(25-15)
0.001	40±0.67	7.50±0.33	11.25±0.29	13.75±0.87
	(45-35)	(10-5)	(15-10)	(20-5)

Note: Numbers in parentheses refer to the mortality range.

In most cases, the insecticidal activity of *C. colocynthis* extracts might be justified to the presence of several compounds including: saponin, alkaloids and glycosides (Gurudeeban *et al.*, 2010; Ali *et al.*, 2013). However, no studies have been yet conducted to analyse and estimate the amount of these compounds from all *C. colocynthis* parts. The current study reports for the first time the higher toxicity effect of *C. colocynthis* stems on aphid control, suggesting that this part of the plant of *C. colocynthis* could have an important chemical compounds, which can be used as an alternative and sustainable method to synthetic pesticides for controlling aphid pests. This finding is in agreement with the study of (Gutierrez, Jr. *et al.*, 2014) who found out that the methanolic extracts of stem part of the following plants: *Jatropha curcas*, *Citrus grandis* and *Tinospora rumphii* have larvicidal activities against the larval stages of *Aedes aegypti* mosquito. Thus, chemical composition and long-term studies are needed to understand the underlining mechanism of how *C. colocynthis* extracts affect aphid suppression. Large-scale field studies are also required to test the stability of *C. colocynthis* as an insecticide for controlling aphid pests. It would be also worth if considering investigating whether there are any side effects of *C. colocynthis* extracts on the natural enemies of aphids. This, in turn, could provide a decision for advising farmers to use it as a sustainable mean of controlling aphid pests.

Table (3): Toxicological parameters of aqueous extracts from the different parts of *Citrullus colocynthis* after twenty-four hours on *Rhopalosiphum padi* adults.

Toxicological parameters	Plant part			
	Stem	Root	Leaf	Fruit
LC_{50}	0.0002	0.024	0.043	0.055
LC_{90}	14.378	21.637	35.617	128.08
95% FL (Lower)	0.000054	0.01	0.018	0.02
95% FL (Upper)	0.0007	0.073	0.15	0.257
Index of toxicity	100	0.833	0.465	0.364
Resistance Ratio	1	120	215	275
Slope	0.267	0.433	0.439	0.381

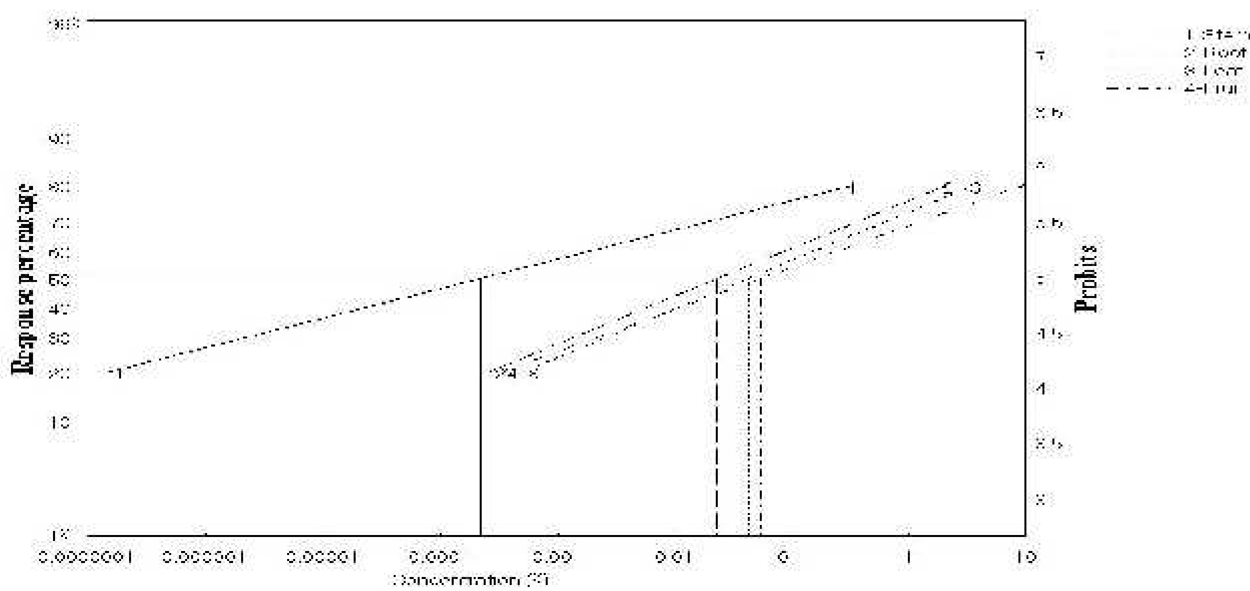

Figure (1). Log- Probity curve of four aqueous extracts of *Citrullus colocynthis* on *Rhopalosiphum padi* adults after twenty-four hours.

Acknowledgements: The author would like to thank the Al-Jomaiah's Chair of Sustainable Development in Agricultural Communities at the University of Hail for providing financial support. Also, I would like to thank Nasir Adam for helping with data collection. I am also grateful to Prof. Sondos Abdel-Tawab Mohamed, Plant Protection Research Institute, for the assistance she provided in statistic analyses of this study.

REFERENCES

Ali, A.A., A.A. Mohamed, and H.A. Elmahi (2013). Phytochemical analysis of some chemical metabolites of Colocynth plant (*Citrullus colocynths* L.) and its activities as antimicrobial and antiplasmodial. J. Basic. Appl. Sci. Res. 3(5): 228-236.

Altieri, M.A. (1991). Increasing biodiversity to improve insect pest management in agro-ecosystems. In Biodiversity of Microorganisms and Invertebrates: Its Role in Sustainable Agriculture. 1st Ed. Howrth Press (USA). 165-182 p

Bale, J.S., J.C. van Lenteren, and F. Bigler (2008). Biological control and sustainable food production. Philos. Trans. R. Soc. B-Biol. Sci. 363(1492): 761-776.

Bashir, M.H., M.D. Gogi, M. Ashfaq, M. Afzal, M.A. Khan, and M. Ihsan (2013). The efficacy of crude aqueous extracts of some plants as grain protectants against the stored grain mite, *Rhizoglyphus tritici*. Turk. J. Agric. For. 37: 585-594.

Coll, M., and D.G. Bottrell (1995). Predator-prey association in mono and diculture: Effect of maize and bean vegetation. Agric. Ecosyst. Environ. 54: 115-125.

Davoodi Dehkordi, S., A. Sahragard, and J. Hajizadeh (2013). The effect of prey density on life table parameters of *Hippodamia variegata* (Coleoptera: Coccinellidae) fed on *Aphis gossypii* (Hemiptera: Aphididae) under Laboratory Conditions. I.S.R.N. Entomol.13:1-7.

DeBach, P.,and D. Rosen (1974). Biological control by natural enemies. 2nd Ed. the Press Syndicate of the University of Cambridge; New York. 323p

Descamps, L. R., and C.S. Chopa (2011). Population growth of *Rhopalosiphum padi* L. (Homoptera: Aphididae) on different cereal crops from the semiarid Pampas of Argentina under laboratory conditions. Chil. J. Agri. Res. 71(3): 390-394.

Dixon, A.F.G. (1987). Cereal aphids as an applied problem. Agric. Zool. Rev. 2: 1-57.

Fetoh, B. E.-S.A., and K.A. Asiry (2012). Toxicological and larvicidal activities of Alzanzalakhet, *Melia azedarach* against cucurbit fly, *Dacus ciliatus* at Hail Province in Saudi Arabia. Toxicol. Environ. Chem. 94(7): 1350-1356.

Finney, D. J.(1971). Probit analysis, 3rd ed. Cambridge University Press, London (UK).318 p

Gurudeeban, S., K. Satyavani, and T. Ramanathan (2010). Bitter Apple (*Citrullus colocynthis*): An overview of chemical composition and biomedical potentials. Asian J. Plant Sci. 9: 394-401.

Gutierrez, Jr., P. M., A.N. Antepuesto, B.A.L. Eugenio, and M.F.L. Santos (2014). Larvicidal activity of selected plant extracts against the Dengue vector *Aedes aegypti* mosquito. Int. Res. J. Biological Sci. 3(4): 23-32.

Koul, O., and S. Walia (2009). Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. CAB Reviews. 4: 1-30.

Marshall, E.J.P., V.K. Brown, N.D. Boatman, P.J.W. Lutman, G.R. Squire, and L.K. Ward (2003). The role of weeds in supporting biological diversity within crop fields. Weed Res. 43(2): 77-89.

Mullai, K., and A. Jebanesan (2007). Larvicidal, ovicidal and repellent activities of the leaf extract of two cucurbitacious plants against filarial vector *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Trop. Biomed. 24(1): 1-6.

Ostman, O., B. Ekbom, and J. Bengtsson (2003). Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. Ecol. Econ. 45: 149-158.

Palis, F.G., R.J. Flor, H. Warburthon, and M. Hossain (2006). Our farmers at risk: behavior and belief system in pesticide safety. AM. J. Public Health. 28(1): 43-48.

Pimentel, D. (2005). Environmental and economic costs of the application of pesticides primarily in the United States. Environ. Dev. Sustain. 7: 52-229.

Pravin, B., D. Tushar, P. Vijay, and K. Kishanchnad (2013). Review on *Citrullus colocynthis*. IJRPC. 3(1): 46-53.

Rahuman, A. A., P. Venkatesan, and G. Gopalakrishnan (2008). Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.). Schrad. Parasitol. Res. 103(6): 1383-1390.

Ramzi, S., A. Sahragard, J.J. Sendi, and A. Aalami (2013). Effects of an extracted lectin from *Citrullus colocynthis* L. (Cucurbitaceae) on survival, digestion and energy reserves of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae). Front. Physiol. 4: 1-8.

Rawi, S.M., F.A Bakry, M.A. Al-Hazmi (2011). Biochemical and histopathological effect of crude extracts on *Spodoptera littoralis* larvae. J. Evolution. Biol. 3(5): 67-78.

SAS., 2009. SAS, version 9.2. SAS Institute, Cary, NC (USA).

Sayeda, F. F., H.M. Torkey, and H.M. Abou-Yousef (2009). Natural extracts and their chemical constituents in relation to toxicity against whitefly (*Bemisia tabaci*) and aphid (*Aphis craccivora*). Aust. J. Basic Appl. Sci. 3(4): 3217-3223.

Schou, J., B. Hasler, and B. Nahrstedt (2006). Valuation of biodiversity effects from reduced pesticide use. Integrated Environ. Assess. Manag. 2(2): 174–181.

Shah, S. I. A., I.A. Khan, Z. Hussain, M. Ahmad, and S. Ahmad (2007). Comparing the effectiveness of a biopesticide with three synthetic pesticides for aphid control in wheat. Sarhad J. Agric. 23(3): 723-728.

Soam, P. S., T. Singh, and R. Vijayvergia (2013). *Citrullus colocynthis* (LINN.) and *luffa acutangula* (L.) roxb, schrad.source of bioinsecticides and their contribution in managing climate change. IJABPT. 4(4): 7-9.

Soliman, M. M. M., A.A. Hassanein, and H. Abou-Yousef (2005). Efficiency of various wild plant extracts against the cotton Aphid *Aphis gossypii* Glov. (Aphdidae:Homoptera). Acta.

phytopathol. Et. Entomol. Hung. 40(1-2): 185-196.

Sun, Y. P. (1951). Toxicity index and improved method of comparing the relative toxicity of insecticides. J. Econ. Entomol. 43: 45-53.

Taheri, S., J. Razmjou, and N. RaSTegari (2010). Fecundity and development rate of the bird cherry-oat Aphid, *Rhopalosiphum padi* (L) (Hom.: Aphididae) on Six Wheat Cultivars. Plant. Prot. Sci. 46: 72-78.

Torkey, H.M., H.M. Abou-Yousef, A.Z. Abdel A zeiz, and E.A.F. Hoda (2009). Insecticidal effect of Cucurbitacin E Glycoside isolated from *Citrullus colocynthis* Against *Aphis craccivora*. Aust. J. Basic Appl. Sci. 3(4): 4060-4066.

Winqvist, C., J. Bengtsson, T. Aavik, F. Berendse, L.W. Clement, S.N. Eggers, C. Fischer, A. Flohre, F. Geiger, J. Liira, T.P.R.C. Thies, T. Tscharntke, W.W. Weisser, and R. Bommarco (2011). Mixed effects of organic farming and landscape complexity on farmland biodiversity and biological control potential across Europe. J. Appl. Ecol. 48: 570–579.