Insecticidal Activity of Diverse Extracts of the *Citrullus colocynthis* (L.) Against the Blue Green Aphid *Acyrtosiphon Kondoi* Shinji

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Abstract. The efficiency of three different extracts from stem and leaf of *Citrullus colocynthis* against the blue green aphid *Acyrtosiphon kondoi* Shinji was studied. Results showed that the ethanolic extracts from the two parts of *C.colocynthis* caused higher mortalities of aphids after 48 hours (96 % and 93 % for the stem and leaf, respectively). Toxicological parameters demonstrated that ethanolic extract from the stem of *C.colocynthis* was more toxic to aphids than leaf extract. This study suggests that detailed chemistry on the stem of *C. colocynthis* is required before applying it in the integrated pest management of aphids.

Keywords: Aphid Control, Bitter apple, Bioassay, Botanical insecticides, Extracts.

1. Introduction

Aphid is a major insect pest that damages crops by congregating on foliage parts of plant and sucking plant nutrients and transmit a wide range of plant viruses which cause huge yield losses (Shah *et al.*, 2007; Davoodi Dehkordi *et al.*, 2013; Asiry, 2015). The limited damage to tissues occurs during primary feeding and the prolonged interactions of aphid mouthparts with the plant cells cause plant responses to the distinctive feeding media of the chewing insects (Walling, 2000). In addition, many aphid species acquire resistance to pesticides, and some aphids develop their virulence profile to overcome plant-resistance genes. Environmental concerns associated with the use of insecticides have led to the development and cultivation of many aphid-resistant crops in the past century (Smith, 2005). Pesticides have played an important role in enhancing crop yields. However, their extensive and wasteful uses have been caused a serious hazard to human and the environment. Sometime, aphid

control with synthetic insecticides is not acceptable and has adverse effects such as pesticide residues, biodiversity loss, environmental pollution, destabilization of ecosystem and enhanced resistance to insecticides (Asiry, 2015). Therefore, there is a need to use eco-friendly and cost effective control methods such as plant botanicals. Plant botanicals are the secondary plant metabolites synthesized by the plant itself (Koul and Walia, 2009). For example, extract from hot pepper found to be more effective against pea aphid, *Acyrtosiphon pisum* Harris (Iqbal *et al.*, 2011). Aphid incidence could managed by using 3% Neem Kernel extract (in cow urine) alone as well as with combination of decreased amount of Dimethoate 0.03% (Gupta, 2005). Aphid mortality was up to 90% due to tobacco leaf extract followed by Neem and garlic (Bahar *et al.*, 2007). The highest concentration of botanical insecticides (from *Azadirachta indica*, *Pongamia glabra* and *Chrysanthemum cinerariifolium*) caused 100% mortality of the green peach aphid *Myzus persicae*, the red

spider mite *Tetranychus urticae* and the Egyptian cotton leafworm *Spodoptera littoralis* (Pavela, 2007). Wabale and Kharde (2010) found that *Ipomea fistulosa* (99.41%) and *Pongamia pinnata* L. (99.41%) were the best plant extracts in managing the population of aphid. Asiry (2015) concluded that aqueous extracts from stems of bitter apple *Citrullus colocynthis* caused a higher mortality of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.).

The objectives of this study is to investigate the effect of different solvent (aqueous, ethanolic and chloroform) for extract from stem and leaf of the bitter apple *C. colocynthis* by using several concentrations on controlling the blue green aphid (*A. kondoi* Shinji and Kondo) which is described as the most critical pests of alfalfa crops.

2. Materials and Methods

2.1 Preparation of *Citrullus colocynthis* Extracts

The preparation of plant extracts was carried out according to (Asiry, 2015) Bitter apple *C. colocynthis* plants were collected from Hail desert which is located in the north central part of Saudi Arabia. Combined impurities such as weeds, soil particles and other inessential matter were carefully removed from the gathered *C. colocynthis* plants. The collected samples of *C. colocynthis* were washed and shade-dried at room temperature. Dried plant materials were powdered using an electrical blender; 10 g of the powder was macerated separately in 100 ml of distilled water, ethanol and chloroform for 72 h. The shaker was used to mix the crude preparation for 24 hours at room temperature and then centrifuged at 4000 rpm for 20 minutes. This mixture was then relocated to a 250 ml beaker and the solvent was evaporated at 60°C to concentrate the plant extract. Four

concentrations were used 100, 200, 400 and 800 ppm by diluted it in different solvent.

2.2 Collection of Aphids

The blue green aphids *A. kondoi* were collected from pesticides-free alfalfa crop (*Medicago sativa* L.), Hail region, Saudi Arabia. The blue green aphid *A. kondoi* on their host plant were kept in plastic bags and were transferred and kept in the fridge in the laboratory for twenty-four hours before running the experiment.

2.3 Toxicity Bioassay

To evaluate the toxicity effect of different extracts (aqueous, ethanolic and chloroform) from the stem and leaf of the bitter apple *C. colocynthis* against the blue green aphid (*A. kondoi* Shinji and Kondo), leaves of alfalfa measuring 3 cm in length were separately dipped into the different extracts (aqueous, ethanolic and chloroform) of *C. colocynthis* with four different concentrations for 30 seconds and then air dried. Each leaf piece was placed in a Petri dish (90mm diameter × 15mm deep) and 20 adults of *A. kondoi* were individually transmitted to the middle each leaf piece by using a soft hair brush. In this experiment, a completely randomized block design was used where collected aphids divided into 24 groups with regards to the plant (2 treatments (stem and leaf), solvents (3 solvents: aqueous, ethanolic and chloroform) and their concentrations (4 dilutions)). Each group replicated five times and each replicate had 20 adults of *A. kondoi*. Also, this experiment had controls in which aphids were placed in Petri dishes that contained non treated leaves of alfalfa. The Petri dishes were kept in an incubator at 25°±1 and 40-50% humidity. After 24 and 48 hours, dead aphids were counted in the Petri dishes (Asiry, 2015; Nia *et al.*, 2015; Alghamdi, 2018).

The percentage of mean mortality of *A. kondoi* was performed using the following formula:

$$\% \text{ aphid mortality} = \frac{\text{mean of aphid mortality}}{\text{total of introduced aphids}} \times 100$$

Values of LC_{50} were calculated according to Finney 1971. Data were corrected for control mortality using Abbott's formula 1925.

2.4 Data Analysis

Results were adjusted using Abbot's formula and statistically computed according to Finney (1971). All statistical analyses were applied in the statistical package SPSS® 14.0 for Windows.

3. Results and Discussion

The treatment of the stem extract from the bitter apple *C. colocynthis* against the blue green aphid *A. kondoi* caused a higher mortality as shown in (Table 1). In the conducted experiment, toxicity effect of the stem part of the bitter apple *C. colocynthis* by different solvent: aqueous, ethanol and chloroform showed a significant effect on controlling the blue green aphid *A. kondoi* after 24 and 48 hours. All tested solvents from the stem of the bitter apple *C. colocynthis* were toxic to the blue green aphid *A. kondoi* in a dose dependent manner, although the toxicity effect was relatively low for aqueous extract. The stem extract of the bitter apple *C. colocynthis* was the most effective, where its 800 ppm ethanolic and chloroform extracts caused 96 and 91% mortalities after 48 hours of exposure respectively, while the aqueous extract produced 88% mortality after the same period of exposure (Table 1). The ethanolic extracts of the stem of the bitter apple *C. colocynthis* was generally more toxic than the aqueous or chloroform extracts. Data showed that the highest percentage of aphids was killed consistently across the time after application by ethanolic leaf extracts

compared with other solvents (Table 2). The mortality of *A. kondoi* was shown by the ethanolic leaf extract and followed by the chloroform and aqueous extracts which they showed 93, 89 and 85 % mortality after 48 hours, respectively. The stem extract of the bitter apple *C. colocynthis* with all solvents demonstrated a higher mortality than leaf extract with the same solvents. The toxicity mechanism of aphids with the tested plant extracts can be attributed to the feeding deterrent (Schumutterer, 1990; Saxena *et al.*, 1988). Nia *et al.*, (2015) declared that Etheric extract of leaves of *Artemisia herbaalba* Asso, *Eucalyptus camaldulensis* Dehnh and *Rosmarinus officinalis* L. was effective and caused mortalities (100 %, 53 % and 60 % respectively) at the highest concentration. Also, phytochemical screening showed the richness of Terpenes in Etheric extract. The results of this study are in agreement with Rojht *et al.*, (2012) which found out that the ethanol extract of *Rosmarinus officinalis*, *Lavandula angustifolia* and *Ruta graveolens* showed a higher repellent activity on the adult of *Acanthoscelides obtectus* and *Leptinotarsa decemlineata*. In addition, Asiry (2015) concluded that the aqueous extract from stem part of *C. colocynthis* is more effective in reducing aphid numbers compared with other parts.

In this study, CL_{50} calculated as the lethal concentrations causing 50% of mortality in the aphids treated with the stem and leaf extracts of the bitter apple *C. colocynthis* (The LC_{50} values and 95% confidence limits (CL) are given in Tables 3 and 4).

The range of acute (24 hr) and chronic (48 hr) LC_{50} values were 122.6-921.4, 59.4-511.4 and 89.5-755.1 ppm for the aqueous, ethanolic and chloroform of leaf extracts, respectively (Table 3). Both the ethanolic and chloroform extracts of the bitter apple *C. colocynthis* were significantly lower LC_{50} s (48

hr) than the aqueous extracts (24 and 48 hrs). The ethanolic extract of the bitter apple *C. colocynthis* was generally more toxic than the aqueous or chloroform extracts. Table 4 demonstrated the LC₅₀ for the effect of the stem extract with the three solvents against the blue green aphid *A. kondoi*. From this table, the ethanolic extract from the stem part of the bitter apple *C. colocynthis* was more toxic to aphid than the aqueous and chloroform extracts with 44.1, 101.5 and 70.2 ppm, respectively. These data confirmed that the ethanolic extract of the stem of the bitter apple *C. colocynthis* is more toxic to aphids than the other two solvents. Also, the stem extract of the bitter apple *C. colocynthis* with the three solvents is more toxic to aphids than the leaf extract. Soliman (2007) showed that *Artemisia herbaalba* oil gave a higher toxicity with LC₅₀ 0.023 % and caused 90.44 % reduction in the population on *Aphis gossypii* (Glover). Mullai and Jebanesan (2007) examined the effect of

bitter melon extract against the mosquito of *Culex quinquefasciatus* (Dip: Culicidae). Their results suggested that bitter melon extract has toxic and lethal effects on the studied insect and mortality enhanced with increasing concentration. The results of Rawi *et al.* (2011) indicated that the methylene chloride extract of the bitter melon has insecticidal effect on larvae of *Spodoptera littoralis*. According to the results of this study, it can be concluded that the blue green aphid *A. kondoi* has reasonable sensitivity to the bitter apple *C. colocynthis* extract specially with the stem extract. This study recommends that more detailed chemical analyses of the stem of the bitter apple *C. colocynthis* are required to understand the underlining mechanism of how the bitter apple *C. colocynthis* extracts control aphids. Thus, the stem extract of this plant could be introduced as an alternative to conventional synthetic pesticides aphid pests.

Table 1. Percentage of mortalities of the blue green aphid *Acyrtosiphon kondoi* after treatment with stem extract of the bitter apple *Citrullus colocynthis*.

Plant Materials	Conc. Ppm	Aqueous Extract		Ethanolic Extract		Chloroform Extract	

		% Mortality after (hrs)					
		24	48	24	48	24	48
Stem extract	800	45	88	60	96	54	91
	400	41	80	52	88	47	85
	200	35	73	45	84	42	80
	100	30	70	40	80	37	76
	Cont.	0.0	0.0	2.0	0.0	0.0	0.0

Table 2. Percentage of mortalities of the blue green aphid *Acyrtosiphon kondoi* after treatment with leaf extract of the bitter apple *Citrullus colocynthis*.

Plant Materials	Conc. Ppm	Aqueous Extract		Ethanol Extract		Chloroform Extract	

		% Mortality after (hrs)					
		24	48	24	48	24	48
Leaf extract	800	45	85	55	93	50	89
	400	36	77	47	85	45	82
	200	32	70	40	80	40	75
	100	28	55	35	68	35	60
	Cont.	0.0	0.0	0.0	0.0	2.0	0.0

Table 3. Insecticidal activity of the bitter apple *Citrullus colocynthis* leaf extract against the blue green aphid *Acyrtosiphon kondoi*.

Leaf extracts	Bioassay Time	Regression of NED response(Y) on close (X)	LC ₅₀ (Conf. limits) ppm	Slope	P
Water	24h	Y=-1.60+0.50X	921.4(393.12-1008.42)	0.50	0.878
Ethanol	48h	Y=-1.81+0.98X	122.6(99.81-324.33)	0.81	0.821
	24h	Y=-1.54+0.57X	511.4(274.12-769.14)	0.57	0.96
Chloroform	48h	Y=-1.63+1.05X	59.4(48.22-88.20)	1.06	0.81
	24h	Y=-1.31+4.51X	755.12(299.74-1066.32)	0.4	0.99
	48h	Y=-1.84+1.06X	89.51(56.33-104.89)	1.035	0.84

LC₅₀ = lethal concentration killing 50 % of the insects.

Table 4. Insecticidal activity of the bitter apple *Citrullus colocynthis* stem extract against the blue green aphid *Acyrtosiphon kondoi*.

Stem extracts	Bioassay Time	Regression of NED response(Y) on close (X)	LC ₅₀ (Conf. limits) ppm	Slope	P
Water	24h	Y=-1.42+0.45X	801.5(327.11-987.13)	0.44	0.98
Ethanol	48h	Y=-0.93+0.70X	101.5(88.71-321.11)	0.70	0.68
	24h	Y=-1.40+0.56X	281.4(106.91-409.31)	0.87	0.45
Chloroform	48h	Y=-0.96+0.87X	44.11(35.24-88.220)	0.88	0.44
	24h	Y=-1.29+0.47X	401.31(244.37-799.22)	0.47	0.89
	48h	Y=-0.68+0.68X	70.21(66.22-305.66)	0.68	0.86

LC₅₀ = lethal concentration killing 50 % of the insects.

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النشاط الإبادي لمستخلصات متنوعة من نبات الحنظل (سيترولوس كولوسينثس) ضد آفة المن الأخضر المزرق

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المستخلص. تمت دراسة فعالية مستخلصات مختلفة من ساق وأوراق الحنظل ضد آفة المن الأخضر المزرق. وقد أظهرت النتائج أن المستخلص الإيثانولي لساق وأوراق نبات الحنظل كان فعالاً وتسبب في نفوق عدد كبير من حشرات المن بعد ٤٨ ساعة (٩٦٪ و ٩٣٪ للساق والأوراق على التوالي). كما أظهرت معاملات السمية أن المستخلص الإيثانولي من الساق كان أكثر سمية من مستخلص الورقة. في الختام، توصي هذه الدراسة بإجراء التحليل الكيميائي لمستخلص ساق الحنظل قبل استخدامه كمبيد في الإدارة المتكاملة لأفات المن.

كلمات مفتاحية: نبات الحنظل، تقييم حيوي، مبيدات نباتية، مستخلصات، مكافحة المن.