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Research Article

Evaluation of some phenolic extracts against aphids (*Aphis craccivora*) Koch under laboratory conditions

Aly M. Aly*
Department of Natural Resources, Faculty of African Postgraduate Studies, Cairo University, Giza 12613, Egypt
Eman M. Ismail
Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
Ashraf M. A. El-Kady
Formulation Research Department, Central Agricultural Pesticides Lab. (CAPL), Agriculture Research Center (ARC), Dokki, Giza 12618, Egypt
Mohamed M. I. Hefnawy
Formulation Research Department, Central Agricultural Pesticides Lab. (CAPL), Agriculture Research Center (ARC), Dokki, Giza 12618, Egypt

*Corresponding author. Email: saad_capl@yahoo.com

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Abstract
Local farmers worldwide have complained in recent years that insect pests have become resistant to the majority of insecticides, owing to pesticide abuse. In addition, highly poisonous and harmful substances may cause health and environmental dangers. Friendly alternatives such as plant extracts are the main targets as substituents to synthetic pesticides. The present study aimed to extract total phenols from some plants and evaluate their efficacy on aphids, *Aphis craccivora,* under laboratory conditions. Four methanolic plant extracts from *Punica granatum, Lantana camara, Portulaca oleracea* and *Ziziphus jujuba,* containing phenolic components were evaluated against *A. craccivora* through: slide dipping, spraying, and leaf dipping techniques. Generally, positive relationships between the concentrations of the tested phenolic extracts and their mortality percentages were noticed in the case of slide dipping and spraying techniques. Conversely, no biological efficacy was found using the leaf dipping technique. The descending order of effectiveness of the tested extracts depending on their EC_{50} values was 0.017, 0.321, 1.142 and 16.114 ppm for *Z. jujuba, P. oleraceae P. granatum* and *L. comara,* respectively, in the case of the slide dipping technique. In contrast, *P. granatum, L. camara, P. oleraceae* and *Z. jujuba* had EC_{50} values of 0.0023, 0.017, 0.321 and 2.3409 ppm, respectively, in the case of the spraying technique. Additionally, a direct proportion was found between mortality percentages and treatment period for plant extracts under study with both techniques. After formulation and completion of additional essential field research, phenols isolated from the plants under study could be employed to combat *A. craccivora.*

Keywords: Aphids, Plant Extraction, Phenolic Components

INTRODUCTION

Aphids come in over 4,000 different species, 250 of which are plant pests. They infest a wide range of ornamental and vegetable plants. Although most aphid species are plant-specific, some aphid species attack a wide range of hosts (Boukria, 2020). Aphids feed on plant sap, curling and distorting the leaves, especially when there is a large population. Honeydew, a sugary substrate excreted by aphids, encourages the growth of sooty mould (*Capnodium* spp.) on harvestable plant parts and leaves, lowering their quality (Liburd *et al.,* 2015).

Aphis craccivora Koch is a polyphagous bug that prefers Leguminosae family plants. It can reduce the production of diverse legume species by up to 100% (Das, 2002). It was identified in fifty host plants belonging to nineteen different families. (Mehrparvar *et al.*, 2012). *A. craccivora* may infest many plant parts, including flowers and pods (Berberet *et al.* 2009). Feeding induces leaf shrivelling and bud deformation, and as the population grows, the infested plants become weak due to

sap loss and toxin injection (Silva *et al.,* 2005).

Phenols probably constitute the largest group of plant secondary metabolites widespread in nature and are found in most classes of compounds having aromatic moieties. They range from a simple structure with one aromatic ring to highly complex polymeric substances such as tannins and lignins. Phenols are compounds synthesized by plants via the pentose phosphate shikimate and phenylpropanoid pathways (Randhir *et al.*, 2004).

The bioactive features of phenolics are free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes, and anti-inflammatory activity (Lima *et al.,* 2014). The toxicity of phenol and its derivatives is always linked to their ability to disrupt the structure of membrane permeability and barrier, resulting in a cascade effect that leads to an imbalance in the intracellular cell environment, and the disruption in its function can eventually lead to cell death (Wasi *et al.,* 2013)**.** In addition, natural phenols' antioxidant activity has been linked to their ability to scavenge free radicals (Mathew *et al.,* 2015).

Insecticides are commonly used to keep aphid populations under control. (Boulogne *et al.,* 2012). On the other hand, synthetic pesticides have a variety of disadvantages when used frequently, such as increased pest resistance, food residues, negative environmental effects, and negative effects on nontarget species, such as humans (Isman 2006, Sayeda *et al.,* 2009). As a result, research regarding alternative aphid control methods has become more relevant in recent years (Smaili *et al.,* 2014; Kichaoui *et al.,* 2016; Aziz *et al.,* 2018). Plant-derived extracts are an alternative strategy to chemical insecticides, which are usually more selective and biodegradable and have little to no negative effects on nontarget species.

Furthermore, since botanical insecticides are made up of a variety of biologically active compounds, pest resistance is less likely to evolve (Pavela 2009, Dang *et al.,* 2010; Dang *et al.,* 2012). Plant-derived pesticides may be a green alternative to synthetic pesticides to improve agricultural production efficiency and sustainably reduce food crises while protecting consumer health. They are inexpensive, environmentally benign, and function through a variety of processes in a more targeted manner, implying that they pose less of a threat to humans and the environment (Souto *et al.,* 2021).

This research aimed to extract phenolic compounds from natural sources and test their efficacy on *Aphis craccivora* in the lab as a first step toward discovering new botanicals with biological activity and low risks to humans and the environment.

MATERIALS AND METHODS

Tested plants

The Latin names, local names, used parts, families and the source of the plants used in this study are listed in Table 1. The used part of each plant was air-dried, ground in a Wiley mill, sieved through a 400 u sieve, and stored in dark glass bottles for further investigation.

Preliminary qualitative screening for the phytochemical constituents of the tested plants

a) Extraction of crude extracts

Twenty-five grams from the respective parts of all tested plants after drying and grounding were extracted with 125 ml methanol by soaking for 72 hrs. Then, the resulting extracts were filtered through pieces of cloth (0.016 mesh) and filter paper (.009 mesh). The filtered extracts were concentrated by using a rotary evaporator under reduced pressure at 40 C^o. Finally, the crude extract was kept in the refrigerator at 3-4 C^o. for phytochemical screening.

b) Phytochemical analysis

All crude extracts were examined for the presence of diverse classes of chemical constituents, alkaloids, tannins, flavonoids, sterols and saponins, with suitable chemical reagents according to Harborne (1973).

c) Phenol extraction

Phenol extraction was carried out according to the method of EL-khayaat (1986).

Insect bioassay

The biological efficacy of the extracted crudes was determined according to the following biological techniques:

a) Slide dipping technique

The FAO (Food and Agriculture Organization) recom-

Table 1. General information about plants and parts used for extraction

Scientific name	Family name	Vernacular name	Part used	Phytogeograpical Regions
Punica granatum	Lytherceae	Pomegranate	Peel shell	Egypt & Sudan
Lantana comara	Verbenace	Shrub verbena	Leaf	Egypt & Sudan
Portulca oleracea	Portuaceae	Mexicean	All plant	Egypt & Sudan
Zizphus jujube	Myrtaceae	Jambdan	Leaf	Egypt & Sudan

mended the slide dipping technique as the standard method for the detection of insecticide resistance in aphids (FAO, 1979). Four serial concentrations for each phenolic extract were used to draw the concentration mortality regression line (LCP, i.e., log concentration probit), three replicates from a petrous adult (1-2 days) were made for each concentration. A group of ten aphids were gently taken using a fine camel hair brush (No. 0.00) and placed in their backs on double-sided adhesive tape fixed on glass slides, so their legs and antennae were free. Then, the slides with aphids were immersed vertically in the aqueous solution of tested extracts for 10 seconds. The slides were left to dry and then transferred to a clean container. Four concentrations (10, 100, 1000 and 10000 ppm) were used for each extract with three replicates. Control for these bioassays included slides with aphids dipped in tap water. Mortality rates were recorded after 2, 8, 16 and 24 hrs. from treatment and compared to control assays. Aphids responding to touch with a brush were considered live. Mortality data were corrected according to Abbott (1925) as follows:

Mortality = a – b x 100/a ……(1)

where a = % of living insects in the control and b = % of living insects in the treatment.

b) Spraying technique

Serial concentrations of 10, 100, 1000 and 10000 ppm were prepared in distilled water from the crude phenolic extracts. Plants of faba bean (*Vicia faba*) were taken 10 days after germination with roots covered by a piece of wet cotton and placed in a Petri dish (15 cm diameter). Ten *A. craccivora* adults were transferred into the dish as replicates (3 replicates), and a total of 30 aphids were used for each concentration of each extract. Ten milliliters of each concentration was sprayed on each replicate within the treatment. Replicates sprayed with water were used as controls. The Petri dishes were maintained in an incubator at 25±1 °C and 75±5% RH. The mortality of aphids was recorded after 24, 48 and 72 hrs. from treatment.

C) Leaf dipping technique

Serial concentrations were prepared from phenolic crude extracts of *P. granatum, L. camara, P. oleracea and Z. jujube* in distilled water. Faba beans were taken 10 days after germination with roots covered by pieces of wet cotton. Two plants were dipped in each concentration, and plants dipped in distilled water were used as controls. After air-drying, the treated plants were placed in Petri dishes (15 cm diameter). Ten *A. craccivora* adults were transferred into the dish. Three replicates were used, and a total of 30 aphids were placed in three dishes that were maintained in an incubator at 25±1.0 C^{o} and 75±5.0% RH. Mortality of aphids was recorded after 24 hrs. from treatment.

RESULTS

The data presented in Table 2 show that all tested phenolic extracts of plants *(P. granatum, L. camara, P. oleracea and Z. jujube*) possessed pyrogallol tannins, except *L. camera*, which revealed the presence of catechol tannins only. Similarly, all tested plants contained saponine except *P. granatum*. On the other hand, alkaloids were recorded in the case of *L. camara* and *P. oleraceae* only, whereas flavonoids were recorded with *P. granatum* and *L. camera.* All tested plants except *P. granatum* were free from sterols or triterpenes, all of which were free from phenol.

In addition to many other active components, phytochemical screening of methanol extracts of the plants under study revealed the presence of catechol and pyrogallol phenols, terpenoids in *P. granatum* (Sabbah *et al.,* 2017), flavonoids and saponines in *L. camara* (AL-Snafi, 2019), saponines, and alkaloids in *P. oleraceae* (Wang *et al,* 2014).

The results in Table 3 show that two hours after treatment, phenol extracts of *P. granatum, L. camara, P. oleraceae* and *A. cepa* recorded a highly significant (calculated f = f from table) insecticidal effect between the control and tested concentrations. Additionally, the same indication was found between most tested concentrations, and their percentages of mortality were between 66.6 to 86.7, 63.3 to 83.3 and 33.3 to 76, respectively, resulting in treatment with 10 to 10000 ppm. In contrast, phenols of *Z. jujube* showed a nonsignificant (calculated f > f from table) insecticidal effect be-

Table 2. Phytochemical screening of methanol crude extracts of tested plants

Tested plants	Phytochemical analysis						
	Catechol	**Pyrogallol**	**Flavonoids**	**Phenol**	**Saponine**	**Alkaloids**	**Sterols or triterpenes**
P. granatum	-	+++	+++	-	-	-	++
L. camara	+++	-	+	-	+	+	-
P. oleraceae	-	+++	-	-	+	+	-
Z. jujube	-	+++	-	-	+++	-	-

(+++): high amount; (++): moderate amount; (+): low amount; (-): absent

Table 3. Insecticidal effect of tested phenol extracts on *A. craccivor* after 2-hour interval exposure under the slide dipping technique under laboratory conditions

Source of phenol	Concent-ration ppm	%Mortality after treatment ± SD				L. S. D		
		2 hrs.	8 hrs.	16 hrs.	24 hrs.	BC	BT	IBCT
P. granatum	0	3.3± .577	20 ±1.00	33.3±.577	43.3± .577	13.3	10.0	NS
	10	66.6±.577	60±1.00	100±0.00	100±0.00			
	100	70±2.00	86.7±.577	100±0.00	100±0.00			
	1000	76±.577	96.7±.577	100±0.00	100±0.00			
	10000	86.6±.577	100±0.00	100±0.00	100±0.00			
L. camara	0	3.3±0.577	20±1.00	33.3±.577	43.3±0.577	6.7	4.4	NS
	10	63.3±0.577	76.7±1.55	93.3±.577	100±0.00			
	100	76±0.577	86.7±.577	96.7±.577	100±0.00			
	1000	76.6±0.577	86.7±.577	96.7±.577	100±0.00			
	10000	83.3±0.577	90±0.00	100±0.00	100±0.00			
P. olerceae	0	3.3±0.577	20±1.00	33.3±.577	43.3±0.577	7.3	6	13.4
	10	33.3±0.577	76.7±1.55	93.3±.577	100±0.00			
	100	60±0.577	86.7±.577	93.7±.577	100±0.00			
	1000	73±0.577	90±1.732	93.7±.577	100±0.00			
	10000	76±0.577	93±0.577	100±0.00	100±0.00			
Z. jujube	0	3.3±0.577	23±0.577	33±1.000	43.3±0.577	NS	4.9	11
	10	30±1.00	73.3±.577	80±0.577	100±0.000			
	100	33±0.577	70±1.000	90±0.000	100±0.000			
	1000	56±0.577	80±0.000	93±0.577	100±0.000			
	10000	70±0.577	90±0.000	93±0.000	100±0.000			

L.S.D.: Least Significant Differences; S.D.: standard deviation; BC: between concentration; B.T.: between after treatment; I.B.C.T.: interaction between concentration and time; ppm.: part per million N.S.:

Table 4. Insecticidal effect of tested phenol extracts on *A. craccivor* after 24 hrs. Interval exposure under spraying tests under laboratory conditions

Source of phenol	Concent-ration Ppm	%Mortality after treatment ± SD			L. S. D		
		24 hrs.	48 hrs.	72 hrs.	BC	BT	IBCT
P. granatum	0	3.3± 0.577	3.3 ±0.577	86 ± 0.577	8	N.S	N.S
	10	56±0.577	66.7±0.577	90±0.000			
	100	63.3±1.154	66.7±.0577	90±0.000			
	1000	63.3±1.528	76.7±0.577	93.3±0.577			
	10000	83.3±1.000	83.3±0.000				
L. camara	0	3.3±0.577	3.3±0.577	13.3±.0577	15.8	7.9	N.S
	10	30 ±1.000	50 ±1.000	83.3±0.577			
	100	46±0.577	60 ±1.000	86±0.577			
	1000	56.7±0.577	66 ±0.577	86 ±0.577			
	10000	70 ±0.000	56.7±1.159	90±0.000			
P. olerceae	0	3.3±1.000	3.3±1.155	13.3±0.577	11.1	N.S	N.S
	10	70 ±1.000	60 ±1.155	60 ±0.577			
	100	70 ±1.000	66 ±0.577	73.3±0.577			
	1000	73.3±1.000	76 ±0.577	80 ±.0.000			
	10000	76.7±1.000	70 ±1.000	86.7 ±0.00			
Z. jujube	0	3.3 ±0.00	3.3±0.577	13.3±0.577	12.2	6.2	13.93
	10	20 ±1.600	36 ±1.155	83.3±0.577			
	100	36.7±0.577	46.7±0.577	86.7±0.577			
	1000	53.3±0.577	63.3±0.557	90±0.000			
	10000	60±1.000	70±1.000	96.7±0.577			

LSD: least significant difference; SD: standard deviation; BC: between concentration; B.T.: between after treatment; I.B.C. T: interaction between concentration and time ppm. part per million; NS: nonsignificant

tween the tested concentrations and the control and between concentrations, and their percentages of mortality were between 30 and 70, resulting in 10 – 10000 ppm.

On the other hand, the insecticidal effect of the tested phenols against *A. craccivora* increased gradually with increasing time after treatment to record their highest effect 24 hours after treatment (100% mortality). From another point of view, the role of time after treatment on the insecticidal effectiveness of the tested phenols was highly significant with all tested phenols, while the effect of the interaction between the tested concentration and time after treatment was highly significant in the case of *P. olerceae* and *Z. jujube* only.

The data in Table 4 indicate that at 24 hours after treatment, a highly significant insecticidal effect was found between the tested control and concentrations under the spraying technique with all tested phenols. Additionally, the same indication was recorded between most tested concentrations. The mortality percentages were between 65 to 83.3, 30 to 70, 20 to 60 and 70 to 76, resulting treatment with 10-10000 ppm *P. granatum, L. camara, P. olerceae* and *Z. jujube,* respectively. Generally, the insecticidal effect of the tested phenols against *A. craccivora* increased gradually with increasing time after treatment to record the highest mortality after 72 hours of treatment. The percentage of mortality after 72 hours was between 86 and 93, 83 to 90, 60 to 86 and 83 to 96 as a result of treatment with 10 to 10000 ppm. in case of *P. granatum,*

L. camra, P. oleracae and *Z. jujube,* respectively. The mortality percentage at 10000 ppm in *L. camara* decreased from 70% after 24 hours to 56.7 after 48 hours, and the same indication was noticed in the case of *P. oleracedo* at 10, 100 and 10000 ppm. This indication may be due to the effect after 24 hours consisting of two modes of action (insecticide and insect static) that changed to become insecticide only after 48 hours.

According to LSD values, the differences between the percentage of mortality resulting from the tested concentration at all times after treatment were highly significant; in contrast, the effect of time after treatment on the mortality percentage was highly significant in the case of *L. camara* and *Z. jujube,* whereas the effect of the interaction between the tested concentration and time after treatment was highly significant in the case of *Z. jujube* only.

The slide dipping technique showed that the ascending order according to EC_{50} values was 0.017, 0.321, 1.142 and 16.114 ppm, corresponding to toxicity index of 100, 0.053, 1.48 and 0.103 for *Z. jujuba, P. oleraceae, p. granatum* and *L. camara,* respectively. When comparing slope values, the highest slope value was recorded with the extract of *P. granatum* (0.690), followed by *Z. jujube* (0.110), *P. portulca (*0.299) and *L. camara* (1.175). All slope values were steep Table 5.

The data in Table 6 show that, generally, all tested extracts were biologically effective against *A. craccivora* after 72 hrs. from treatment under spraying technique. The highest effect was recorded with *P.* granatum, with an EC_{50} value of 0.0023 ppm, followed by *L. camara, P. oleraceae* and *Z. jujuba*. On the other hand, extracts of *P. granatum* and *L. camara* recorded the nearest slope values of 0.244 and 0.212, respectively.

DISCUSSION

The obtained results concluded that the tested plant phenolic extracts may have bioactive properties as reported by Ibrahim *et al.* (2020), including inhibition of

Table 5. Toxicity of tested phenol extracts to *A. craccivora* under the slide dipping technique after 8 hrs. form treatment under laboratory conditions

Source of total phenol	EC_{50} ppm	Lower limit	Upper limit	Slope	Toxicity index
Z. jujuba	0.017	-	-	0.110	100.0
P. oleraceae	0.321	0.0016	2.786	0.299	0.053
P. granatum	1.142	0.092	3.612	0.690	1.48
L. camara	16.114	10.45	37.709	1.175	0.103

Table 6. Toxicity of phenol extracts against *Aphis craccivora* after 72 hrs. from treatment under spraying techniques under laboratory conditions.

Source of total phenol	EC_{50} ppm	Lower limit	Upper limit	Slope	Toxicity index
P. granatum	0.0023	-	-	0.244	100.0
L. camara	0.017	3.5909	0.743	0.212	013.5
P. oleraceae	0.321	0.0057	2.065	0.390	000.71
Z. jujuba	2.3409	-	-	0.101	000.098

hydrolytic and oxidative enzymes (Lima *et al.,* 2014). After penetration of the cell, phenols undergo active conversion mainly with the participation of oxidases within cytochrome P450, and the conversion processes lead to a fast increase in toxicity by forming electrophilic components that harm DNA and/or enzymes in the cell (Michałowicz and Duda, 2007). The cytotoxic effects of phenolic compounds depend on reactivity. Phenols exert higher reactivity, rapidly undergo radical reactions and provoke lipid peroxidation of cell membranes (Abdel Hady *et al.,* 2014). The mode of action of the tested plant phenolic extracts was poisoned through contact, so there was biological activity under both the slide dipping technique and spraying technique only (Czerniewicz *et al.,* 2016; Osman and Elsobki, 2019).

From another point of view, the polarity of phenolic compounds may prevent penetration from the plant leaf surface, consisting of wax and fat that mix with plant sap and is considered an aphid food. Therefore, no biological effect was observed for the tested plant phenolic extracts in the case of the leaf dipping technique (Lichiheb *et al.,* 2015).

Generally, except for *P. oleraceae,* the EC_{50} values in the spraying technique were less than those in the case of the slide dipping technique. This indication may be due to the application method because the exposure periods in the case of the slide dipping technique were less than those in the spraying technique (Paramasivam and Selvi, 2017). On the other hand, the slope values for both tested techniques were flat (less than 1). Except for *Z. jujuba*, which recorded slope values in the spraying technique were less than those in the slide dipping technique**.** The slope of the probit regression reflects the quality of the enzyme system detoxifying insecticides in an insect body (Torkamand *et al.,* 2013). The descending order of effectiveness of the tested plant phenolic extracts depending on EC_{50} values changed from *Z. jujuba, P. portulca, P. granatum* and *L. camara* (their EC_{50} values were 0.017, 0.321, and 2.482, respectively, in the case of the slide dipping technique) to *P. granatum, L. camara, P. portulca* and *Z. jujuba* (their EC_{50} values were 0.0023 and 0.017 0.321 and 2.3409) in case of spraying technique. This indication may be due to the method of application of each technique and exposure periods. The percentage mortality for each tested plant phenolic extract treatment increased substantially over time for both tested techniques (Paramasivam and Selvi, 2017). The same findings were achieved against *A. craccivora* Koch (Miller, 2003) and using citric acid and its soluble powder formulation on the same pest (El kady *et al.*, 2010). Any population even *Aphis craccivora* consisting of individuals varies in their tolerance; sensitive individuals die directly, while individuals who are more tolerant need more time to die (Mohamed and El kady, 2010).

Conclusion

Under laboratory conditions, phenolices extracted in methanol from *P. granatum, L. camara, P. oleracea,* and *Z. jujube* tested against *A. craccivora* using slide dipping, spraying, and leaf dipping techniques revealed that two of the techniques slide dipping and spraying techniques were effective, while the third one leaf dipping was found to be ineffective. The EC_{50} values for the spraying technique were lower than those for the slide dipping technique, and both spraying and slide dipping techniques yielded a direct relation between the percentage of mortality and the treatment period. After more research was completed, phenolic extracts could control *A. craccivora*.

Conflict of interest

The authors declare that they have no conflict of interest.

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