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Evaluation of Eruca Sativa and Capsicum annuum alcoholic extracts as pesticide against aphid and whitefly pests

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Abstract The aphid (Aphidoidea family) and the whitefly (Aleyrodidae family) are closely related, quickly breeding insects that, when infected, render plants susceptible to diseases since they suck the juices of these plants. They damage the aerial parts of plants and even their roots. Their control is of high concern to limit the damage and minimize the loss of the crops with the use of the safest pesticides. The present study was conducted to assess the perished effect of the *Eruca sativa* (local name: arugula) leaves and the *Capsicum annuum* (local name: hot pepper) fruits on aphids and on the eggs and nymphs of whitefly by spraying their alcoholic extracts on tested species. Three pesticide concentrations (0.5, 1 and 1.5%) of either extract were prepared and tested. Adhesives, propellants and other materials were added to the extracts to enhance their effectiveness. The results indicated that the pesticide prepared from these extracts has a concentration- and time-dependent impact on the growth of all tested species; the highest mortality rate was achieved at 1.5% concentrations. The time required for that highest effect was 72 hours for *Eruca sativa leaf* extract on aphids (100%); and on the eggs (89%) and nymphs (94.1%) of whiteflies. Meanwhile, the highest mortality rates achieved by 1.5% *Capsicum annuum* fruit extract were after 48 hours on aphids (100%), on the nymphs of whiteflies (100%), and 72 hours on whitefly eggs (96.4%). These effects may be related to the presence of many bioactive substances that were detected in the extracts of these two plants, including tannins, glycosides, phenols, resins, flavonoids, saponins, alkaloids, coumarin, steroids and terpenes.

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Keywords: *Aphid, Aphidoidea, whitefly, Aleyrodidae, Eruca sativa, arugula, Capsicum annuum, hot pepper, flavonoids, coumarin, alkaloids, saponins.*

1. INTRODUCTION

Whitefly has become a global pest, causing significant losses in crop and vegetable production in many parts of the world. Currently, many studies are being conducted to find out other ways to combat it instead of traditional pesticides, especially in places where the use of pesticides is highly unusual. In addition to the rapid construction of resistance to pesticides in this insect, the whitefly has developed resistance against many pesticides, including pyrothroids, organophosphorus, carbamate pesticides and even insect growth retardants. This has led to the need to seek alternative solutions to reduce the hazard and spread of this pest (1). Pesticides of plant origin have been known since ancient times, when many plants were characterized as sources of pesticides. An example of these plants is the neem trees (Azadirachta indica, Indian lilac), where these trees are a known source of insecticides, insect repellents and growth inhibitors, as well as being used to control the growth of nematodes and fungi. Although the materials extracted from these plants are highly toxic to insects, they are relatively safe for humans. Moreover, Chrysanthemum morifolium, which is a known source of natural pyrethrum, has been used for many years in combating household pets and in the recent preparation of tens of new pesticides for fighting many health and agricultural pests (2). Recent studies have demonstrated the properties of extracts derived from plants against insects and pests targeted effectively so that the extracts are biodegradable and non-toxic and can be suitable for use in integrated pest management programs. These materials are highly specialized in their mechanisms of action since they affect one type of insect with a low resistance rate among treated insects. Many researchers have studied the effects of natural pesticides from plant sources and tested their

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effectiveness on insects (3). Eruca sativa, known as arugula, is an important winter herb of the Brassicaceae family (4-6). Its cultivation succeeds in temperate regions all year round, except for very hot and cold months. The plant is described as standing, 30–60 cm long, with large, lobed leaves with large oval upper lobes and long necks. The flowers are light purple or yellow. It blooms from March to July (7). Capsicum annuum, known as red hot pepper, is the fruit of some annual varieties of bell pepper that belong to the solanaceae family. It is an interesting plant that is characterized by high levels of ascorbic acid, vitamin A and calcium (8–11)

2. MATERIALS AND METHODS

Preparation of extracts for phytochemical testing

The leaves of *Sativa Eruca* and the fruits of *Capsicum annuum* were obtained from Iraqi local markets. They were dried in a well-ventilated shade place at room temperature. The dried parts were grinded with electric grinder and placed in dark at room temperature until use. About 50g of each plant's powder was placed in 6500ml beaker to which 200ml of ethanol (90%) is added. The extractions system is placed in dark for 3days at room temperature and was filtered thereafter using Whatman No 2.4 filter paper, the solvent is evaporated using water bath and the semisolid yield was stored at 4 ºC until further use.

Phytochemical testing

About 1% ethanolic stock solution of each plant's extract was prepared for phytochemical detection of alkaloids, saponins, tannins, cardioactive glycosides, coumarin, resins, flavonoids, terpenes and steroids, carbohydrates and phenolic acids following standard methods below

1. **Alkaloids (**Dragendroffs test): About 1ml of Dragendroffs reagent was added to 2ml of the test solution (11).
2. **Saponins (**Foam test method): 5ml of the test solution was vigorously shacked for five minutes (12).
3. **Tannins (**Ferric test): few drops of 5% ferric chloride solution were added to 2ml of the test solution with gentile shaking (13).
4. **Cardioactive glycosides (**Keller Killiani test): 1ml of the test solution was gently added to a mixture of 1.5ml solution of glacial acetic acid and 2-3 drops of 5% ferric chloride followed by the addition of 1ml and observing the acetic acid layer (13).
5. **Coumarins (**Coumarin test): 2ml of the test solution was added to a mixture of 0.1ml of 10% sodium hydroxide and 1ml of chloroform (14).
6. **Resins (**Turbidity test): 10ml of distilled water were added to 1g of the dried extract. The mixture then ultrasonicated at 30ºC for 15 min and filtered (15). ultrasonicated for 15 min at 30 ºC. The mixture was filtered. Occurrence of turbidity showed the presence of rasins (Mir et al., 2013) ultrasonicated for 15 min at 30 ºC. The mixture was filtered. Occurrence of turbidity showed the presence of rasins (Mir et al., 2013)
7. **Flavonoids (**Ammonium test): few grams of the dried extract were dissolved in 2ml of chloroform and 1ml of 1% ammonium was then added and the mixture shaken vigorously (15)
8. **Terpenes and steroids (**Liebermann–Burchard test): 2gm of the dried extract were dissolved in 1ml of chloroform and filtered and placed in a test tube. About 1ml of glacial acetic acid and 1ml of concentrated sulfuric acid were added carefully (16).
9. **Carbohydrates (**Anthrone test): 1ml of Anthrone reagent was added to 2 ml of the test solution and the mixture was warmed and let to cooldown (17).
10. **Phenolic acids (**Ellagic acid test): to 1ml of the test solution few drops of 5% glacial acetic acid and 5% sodium nitrite solutions were added (18).

Preparation of alcoholic extracts for testing pesticide effect

About 50g of each of the dry powders of *Sativa eruca* leaves and *Capsicum annuum* fruits were put in a 2L conical flask, and about 1L of 70% alcohol was added. The macerate was left in a vibrating incubator for 48 hours and for another 24 hours in the refrigerator. Each macerate was filtered several times with medical gauze and then once by Buchner apparatus using Whatman No 2.4 filters paper. About 90% of the solvent was evaporating from the filtrate using a vacuum rotary evaporator at 40 ºC. The residue was placed in petri dishes and dried in a vacuum electric oven at 40 ºC. The dried extract was scraped and dried again in clean, tightly sealed glass containers (11). About 7g of the dried extract of *Sativa eruca* leaves and 5g of the dried extract of *Capsicum annuum* fruits were obtained by this cold extraction method. The estimated pH of these extracts was 5.5 and 6, respectively. Three concentrations for each extract were prepared (0.5%, 1.0%, and 1.5%) by dissolving 0.5, 1.0, and 1.5g in 100 ml of distilled water to serve as pesticides. Adhesives and propellants were added to the pesticide, according to the references. The resulting pesticides were translated into a spray tower. A blank solution containing distilled water and the additives was prepared to serve as the control solution.

Experimental design

The perishing effects of the prepared pesticides and control solution were evaluated on aphids and on the eggs and nymphs of whiteflies. For each concentration of the pesticide and the control solution, five petri dishes with a diameter of 9 cm were floor-mated with medical cotton and moistened with distilled water. In each petri dish, five discs of castor leaves, each infected with four aphids, were placed. This represents a total

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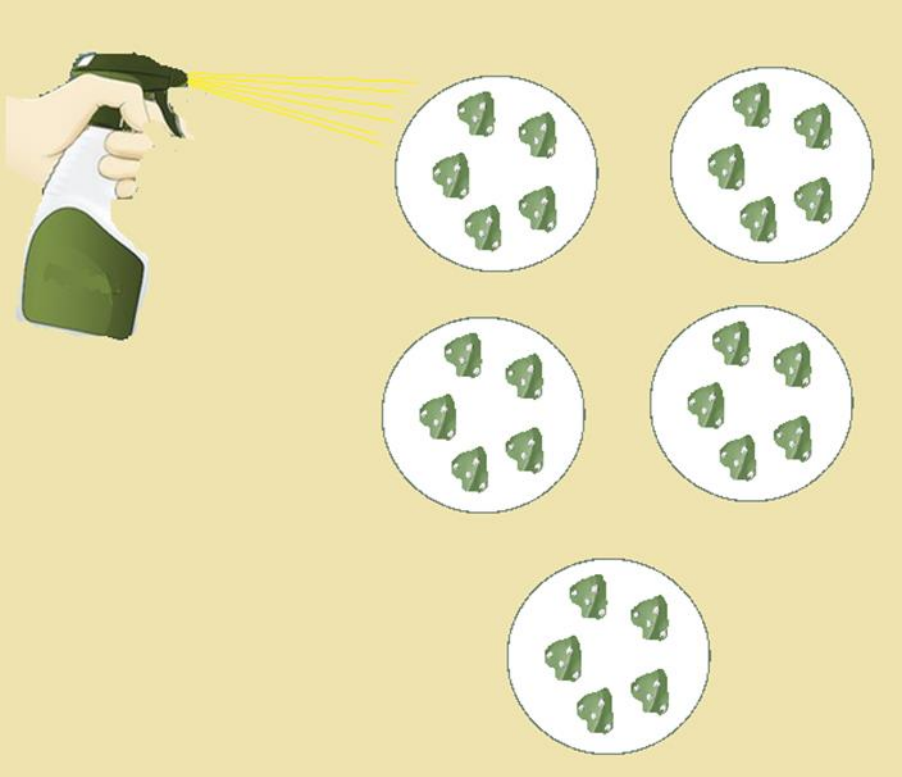
of 100 aphids to be sprayed with 2 ml of either the designated pesticide or the control solution (figure 1). The sprayed petri dishes were then incubated at 27±6 ºC. The percentage of perished species was counted after 24, 48 and 72 hours of spraying. The same procedure was repeated for evaluating the lethal effect on the eggs and nymphs of the whitefly. The statistical analysis was performed using IBM SPSS software.



3. RESULTS AND DISCUSSION

The phytochemical analysis of extracts from the leaves of *Eruca sativa* and the fruits of *Capsicum annuum* (Table 1) indicates the presence of alkaloids, tannins, saponins, flavonoids, carbohydrates and other active substances. Moreover, the alcoholic extract of *Capsicum annuum* fruits appears to also contain coumarnes. The relative perishing efficiency of 0.5%, 1.0%, and 1.5% pesticides prepared from *Eruca sativa* leaf extract on aphids and on whitefly eggs and nymphs is shown in Table 2. On these insect species, the pesticide showed a concentration- and time-dependent increase in perishing efficiency. The highest perishing effect was achieved by 1.5% on aphid (100%), whitefly eggs (89%), and whitefly nymphs (94.1%) after 72 hours of spraying. However, on each of the tested species, the perishing effect achieved by either concentration did not significantly differ with the time after spraying ($P > 0.05$). Meanwhile, although aphids were shown to be more sensitive to the lethal effect of the pesticide than the others at comparable time intervals, the difference was not significant ($P > 0.05$). In this context, whitefly eggs were the least sensitive to the pesticide among all tested species, showing a respective perishing percent of 66%, 76% and 89% after 24, 48 and 72 hours of spraying. However, such responses were not significantly different from those achieved by other species ($P > 0.05$). On the other hand, the pesticide prepared from *Capsicum annuum* fruit extract was tested in the same manner as that prepared from *Eruca sativa* leaf extract on the categorized group of insect species. The effects on aphids and on whitefly eggs and nymphs of 0.5%, 1.0%, and 1.5% of this pesticide are illustrated in Table 3. Interestingly, the pesticide showed a significant concentration- and time-dependent impact on the viability of aphids and whitefly nymphs ($P<0.05$). However, the highest perishing rate was achieved by the 1.5% concentration after 24 and 72 hours (89.5% and 100%, respectively). Similarly, the effect of this concentration was significantly increased with time on whitefly nymphs, with 92.3% and 100% perished rates after 24 and 72 hours, respectively. Meanwhile, the effect on whitefly eggs was not significantly changed by time ($P>0.05$). While aphids and whitefly nymphs are more sensitive to the pesticide effect, the effect on whitefly nymphs was significantly higher than that on aphids ($P<0.05$). The botanical pesticide activity of the prepared extracts is related to some of the detected phaytochemicals, including toxic alkaloids, terpenes, saponins, phenolic acids and coumarin (19–25). In conclusion, the results of the study showed a clear perishing effect of the pesticides prepared from *Eruca sativa* leaves and *Capsicum annuum* fruit extracts on aphids and on the nymphs of whiteflies. However, further studies have to be conducted to determine the mechanism by which these plants exert their pesticide effect.

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Table 1: *Phytochemical analysis of alcoholic extract of Eruca sativa leaves and Capsicum annuum fruits.*

Detection	Observation	Outcomes	
		Eruca sativa leaves	*Capsicum annuum* fruits
Alkaloids	Orange-to- brown precipitate	+	+
Carbohydrate	Bluish green	+	+
Coumarin	Yellow color	-	+
Flavonoids	Yellow color	+	+
Cardioactive glycosides	Blue color in acetic acid layer	+	+
Phenolic acids	Black precipitate	+	+
Resin	Turbidity	+	+
Saponins	Stable foam	+	+
Steroids	Dark blue ring (after 12hr)	+	+
Tannins	Dark blue color	+	+
Terpenes	Light-brown ring (immediate)	+	+

Table 2: *The percent perished achieved by prepared pesticide from Eruca sativa leaves extract on aphids, and on whitefly eggs and nymph.*

Concentration of tested pesticide	Percent of perished insect species								
	On aphids (n=100)			whitefly eggs (n=100)			whitefly nymphs (n=100)		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
0.5%	61	77	88.5	32	48	60.2	58.9	69.8	74.3
1%	70	88	93	45.2	61.3	77.6	72.1	80.3	87.8
1.5%	85	93	100	66	76	89	75.9	84.2	94.1
P value	0.89			0.76			0.99		

n= number of insects species before spraying.
Control unsprayed insect species expressed a null perished percent.

Table 3: *The percent perished achieved by prepared pesticide from Capsicum annuum fruits extract on aphids, and on whitefly eggs and nymph.*

Concentration of tested pesticide	Percent of perished insect species								
	On aphids (n=100)			whitefly eggs (n=100)			whitefly nymphs (n=100)		
	24hr	48hr	72hr	24hr	48hr	72hr	24hr	48hr	72hr
0.5%	60.3	71.8	82.6	49.9	59.6	72.3	59.6	71.3	82.3
1%	75.4	84.2	95.6	67.3	78.6	86.6	75.4	84.4	95.1
1.5%	89.5	100		80.5	87.9	96.4	92.3	100	
P value	<0.001			0.95			<0.001		

n= number of insects species before spraying.
Control unsprayed insect species expressed a null perished percent

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