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ORIGINAL ARTICLE

Study on the Effects of Acetonic Extract of *Otostegia Persica* (Labiatae) on Three Aphid Species and One Stored Product Pest

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ABSTRACT

Aphids are very important pest species of many greenhouse and field plants in the world. Much effort has been devoted to the discovery and development of plant extracts and phytochemicals as alternatives to synthetic insecticides for pest management. The experiments were directed to determine the effect of acetonic extract of *Otostegia persica* (Labiatae) on different pests. The insects were included 3-4 days-old individuals of *Aphis fabae* Scopoli, *Aphis gossypii* Glover and *Myzus persicae* (Sulzer) as well as 1-7 days-old adults of *Tribolium castaneum* (Herbst). *T. castaneum* was reared on wheat flour. Topical treated aphids with two concentrations of *O. persica* were placed on the broad bean leaf discs (4.5 cm diameter) in the round plastic Petri dishes (5.5 cm diameter), filled with a 0.5-cm-thick agar gel layer. The experiments were carried out at 25±1°C temperature, relative humidity of 60±10% and 16 hours of artificial light at an intensity of about 4000 lux. In control treatments only distilled water and DMSO (dimethyl sulfoxide) were applied. Mortality was determined after 24, 48 and 72 hours from commencement of exposure. 20 replicates for each concentration and in each replicate 10 insects were done. In concentrations of 60 and 80 µl/ml after 24 hours, the mortality of *A. gossypii* treatment was 63.0% and 87.4% respectively and it was significantly highest among different aphid species, while, it was less than 2% and 35% in the *T. castaneum* treatment, respectively. The mortality of *A. fabae* treatments were 42.1% and 64.9% in concentration 60 and 80 µl/ml, respectively. The mortality percentage was significant higher in *A. fabae* and *M. persicae* than in *T. castaneum* treatments. It seems that the destructive effects of synthetic pesticides on the human and the environment to study more on the botanical compounds and determining their proper dose, their recommendations could be a better substitute for conventional synthetic pesticides.

Key words: Aphid species, Botanical insecticide, *Otostegia persica*, *Tribolium castaneum*

Introduction

Aphids are serious pests and vectors of multitude of viral diseases of field crops, vegetables, ornamentals, and greenhouse crops [3]. They have displayed a remarkable ability to establish resistance to almost every insecticide with which it has been treated. *Tribolium castaneum* (Herbst) is considered as a major pest of stored grains [8]. Thus chemical control of these pests like other pests has three major disadvantages: pollution of the environment by insecticide residues, development of insect resistance and potential toxicity to non target organisms [14,2, 11]. To overcome these problems, it is necessary to search for alternative methods of pest control. The failure of chemical pesticides to control the pest and growing public concern for safe food and a healthy environment have catalyzed the search for more environmentally benign control methods for the management of these pests [16].

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Plants are rich sources of bioactive compounds that can be used to develop environmentally safe pest managing agents[5]. One alternative method to control aphids and other pest insects is the use of plant extracts and essential oils. These products are described as complex mixtures of natural substances made by plants. Such compounds of secondary metabolism as alkaloids, terpenoids, phenols, flavonoids, steroids, etc. confer some resistance against phytophagous animals[7]. They are used to repel or kill insect pests that feed on flowers, fruits, leaves and woods. They can act as attractive, repulsive, antifeedant, toxic as well as growth regulator affecting several physiological processes of insects [11,4].

Although many plant species have been tested in their capacity as anti-insect agents[6], most efforts have concentrated in species from families that include either the most traditionally used botanical pesticides[9], or species with high contents of essential oils. Herbs and medicinal plants with their constituents as a source of alternative fumigants and insecticide have been suggested by many contributors [17,12,13].

One of these plant extract is extract of *Otostegia persicae* (Labiatae) which has been one of the important medicinal plants in Iran. The purpose of the experiments reported here is to demonstrate the effectiveness of acetonic leaf extract of *O. persicae* on four pest species, that are themselves important agricultural pests, either in conventional or organic production.

Materials and methods

Insects rearing:

The insects in this study were included three aphid species and one stored product pest. The aphids were *Aphis fabae* Scopoli, *Aphis gossypii* Glover and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). The stored product pest used in this study was *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae).

The aphid species colony used in this study was derived from Shahid Bahonar University field infestation and after identification a stock culture was maintained on broad beans *Vicia faba* L. cv. Aquadulce. Aphids were placed on the freshly excised broad bean leaf discs (4.5 cm diameter) were placed in the round plastic Petri dishes (5.5 cm diameter). The round plastic Petri dishes were filled with 0.5 cm-thick-layer of 0.7% agar gel, and with a meshed hole in the lid to allow air exchange, that were reared in growth chamber at 25±1°C temperature, relative humidity of 60±10% and 16 hours of artificial light at an intensity of about 4000 lux. The relative susceptibility of 3-4-day-old individuals of the *A. fabae*, *A. gossypii* and *M. persicae* were used for experiments. *T. castaneum* was reared on wheat flour at home temperature. Adult insects, 1–7 days old, were used for experiments.

Plant material and extracts:

Dry leafs of *O. persica* were prepared. The dried leaf (700 g) was powdered mechanically using commercial electrical stainless steel blender for five minutes and then placed in the erlenmeyer flask (500 ml) extracted with acetone (purity 99.9%). Then the fixed extracts were filtered and concentrated in round Petri dishes (15 cm diameter) under laboratory hood for 24 hours to give a dried residue. Extracted substance was stored in refrigerator at 4 °C.

Bioassay test:

In order to determine the effects of the acetonic leaf extract of *O. persica*, on these pests, the relative susceptibility of 3-4-day-old individuals of the *A. fabae*, *A. gossypii* and *M. persicae* as well as 1-7 days-old adults of *T. castaneum* was used as biotest. The method of bioassay test for insecticidal effect was topical test, and two concentrations of 60 and 80 µl/ml were applied. All experiments were carried out under the same laboratory conditions, at 25±1°C temperature, relative humidity of 60±10% and 16:8 (L:D) photoperiod. In control treatments only distilled water and DMSO (dimethyl sulfoxide) were applied. 20 replicates for each concentration and in each replicate 10 pests were done. Mortality was determined after 24, 48 and 72 hours from commencement of exposure. When no leg or antennal movements were observed, insects were considered dead.

Statistical analysis:

The mortality data were adjusted for mortality in the water control using Abbott's correction [1]. The actual pesticide mortality was calculated as: M_a (%) = $[(M_t - M_c)/(100 - M_c)] \times 100$; with M_a = corrected mortality (%), M_t = mortality in treatment (%), and M_c = mortality in the water control (%).

For statistical comparison among several means, all the data from the laboratory studies on mortality of pests were subjected to a one-way analysis of variance (ANOVA) followed by a Tukey Test (StatPlus 4.9, 2007).

Results and discussion

The results showed a significant differences in mortality of insects to acetonic extract of *O. persica* with two concentrations after 1-3 days ($p \geq 0.05$)

(figure 1 and table 1). The mortality of two concentration of acetonic leaf extract of *O. persica* on three aphid species and *Tribolium castaneum* after 24 h is presented in figure 1. In concentration of 60 and 80 µl/ml, the mortality of *A. gossypii* treatment after 24 h was 63.0% and 87.4% respectively and it was significantly highest among different aphid species ($p \geq 0.05$). While the mortality were less than 2% and 35% in the *T. castaneum* treatments, respectively. The mortality of *A. fabae* treatments were 42.1% and 64.9% in concentration 60 and 80 µl/ml, respectively. The mortality percentage was significant higher in *A. fabae* and *M. persicae* than in *T. castaneum* treatments ($p \geq 0.05$).

Data from the present study on mortality of two concentration of acetonic leaf extract of *O. persica* on three aphid species and *T. castaneum* after two and three days are illustrated in table 1. After 72 hours, extract of *O. persica* caused 65.5 and 89.5% mortalities of *A. gossypii* in concentration of 60 and 80 µl/ml, respectively. In contrast, the mortalities occurred in *T. castaneum* treatments were less than 35% during the mentioned period. There was a steady progression in mortality with exposure to *O. persica* extract.

These results suggest that there may be different compounds in extracts possessing different bioactivities on different pests. On the other hand, some insects are sensitive to some plant extracts. Thus these compounds could be responsible of some features observed in *T. castuneum* or aphis species. Similar observations on other plant extracts effect on several insects have been reported. For example Jbilou et al (2006) have reported that *Tribolium castaneum* response varied with plant species. They showed that the adult of *T. castaneum* were more susceptible to extract of *Peganum harmala* L. (Zygophyllaceae) and *Ajuga iva* L.(Lamiaceae) than *Raphanus raphanistrum* L. (Brassicaceae) [10]. Compared with our data, *Artemisia tridentata* was less effective against *Sitophilus. oryzae* (L) [18], and *Artemisia scoparia* (Asteraceae) was less effective against *Sitophilus oryzae* (L) and *T. castaneum* than *Callosobruchus maculates* (Fab.) [15].

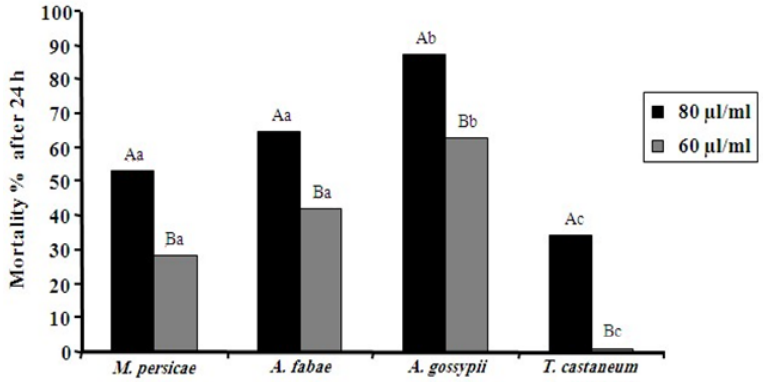

Fig. 1: Mortality of two concentration of acetonic leaf extract of *Otostegia persica* on three aphid species and *Tribolium castaneum* after 24 hours [Bars with different small letters indicate significant differences between the different biotests within the same concentrations. Bars with different capital letters indicate significant differences between the concentrations within the same biotests at $P \geq 0.05$ (one-way ANOVA)].

Table 1: Effect of two concentration of acetonic leaf extract of *O. persica* on three aphid species and *Tribolium castaneum* after 48 and 72 hours

Time after treated (hours)	Concentration (µl/ml)	Mortality% (Mean±SE)			
		M. persicae	*A. fabae*	*A. gossypii*	*T. castaneum*
48	60	28.6 ± 5.46^{Ba}	42.1 ± 4.01^{Ba}	62.9 ± 5.24^{Bb}	1.11 ± 0.73^{Bc}
	80	55.6 ± 4.16^{Aa}	68.4 ± 4.26^{Aa}	88.4 ± 3.17^{Ab}	34.4 ± 2.81^{Ac}
72	60	30.4 ± 5.49^{Ba}	44.2 ± 3.61^{Ba}	65.5 ± 3.90^{Bb}	1.11 ± 0.73^{Bc}
	80	57.9 ± 3.76^{Aa}	70.8 ± 4.50^{Aa}	89.5 ± 2.80^{Ab}	34.4 ± 2.81^{Ac}

Means in columns followed by different small letters indicate significant differences between the different biotests within the same concentrations. Means in columns followed by different capital letters indicate significant differences between the concentrations within the same biotests at $P \geq 0.05$ (one-way ANOVA).

The comparison between the percent total mortality of two concentrations on different treatments gives a good vision about the bioactivity of the *O. persica* acetonic leaf extract. The major concept of this study is to show the toxicity of the leaf extract on three aphid species and one stored product pest. The insecticidal activity varied with insect species, concentrations of the extract and time. The considerable differences in mortality of insects to acetonic extract, it maybe trend related to penetration and detoxification of *O. persica* acetonic leaf extract. In the case of the aphid species, even

though there seems to be greater activity against the more susceptible and smallest bulk *A. gossypii*.

Conclusion:

In conclusion, an attempt has been made to evaluate the role of plant extracts in pesticidal activity. The results reported here open the possibility of further investigations of efficacy on their pesticidal properties of natural product extracts. This naturally occurring plant extract could be useful for pest management of aphids. More studies are needed to bioassay the activity of each identified compound against aphid species and other pests.

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