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ORIGINAL ARTICLE

Study on Insecticide Effects of Some Medicinal Plant Extracts on the Population Rates of Eggs and Nymphs of the Common Pistachio Psyllid (*Agonoscena pistaciae*)

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KEYWORDS

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ABSTRACT: The common pistachio psyllid is one of the most important pests of pistachio trees throughout the pistachio-producing regions in Iran. In the present research, the extracts of some medicinal plants were used to study their inhibitory effect on oviposition and population rates of nymphs of common pistachio psyllid. To evaluate the effect of several medicinal herb extracts (Thyme, Eucalyptus, and Chamomile) on the psyllid, an experiment was conducted in a randomized complete block design with four replications. The extracts were sprayed at concentrations of 2.5, 5, and 7.5 per thousand. Sampling was performed in four stages; 3, 7, 14, and 21 days after spraying. The results showed that among different types of plant extracts, thyme extract was the most effective product on the reduction of population rate of eggs and nymphs of the common pistachio psyllid, although the extract of other medicinal plants, eucalyptus and chamomile, were also reduced the rates of egg and nymph. The dose of 7.5 per thousand of all extracts showed a better effect on the number of insect eggs and nymphs. In addition, the results indicated the better effect of these products on the number of insect eggs and nymphs appeared at 14 and 21 days after spraying.

INTRODUCTION

Pistachio, *Pistacia vera* L., is one of the most important horticultural products in Iran. The common pistachio psyllid, *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Psylloidea, Psyllidae), is a major pest of pistachio trees in all the areas of Iran where this plant is grown. The CPP occurs in many pistachio-growing regions around Iran's borders, including Armenia, Iraq, Turkmenistan, and Turkey, as well as Mediterranean regions such as Greece and Syria [1-3].

This pest sucks sap from the leaves, which damages the plant and reduces yield. This pest has several generations per year and is controlled by applying chemical insecticides several times per year [4, 5]. Intensive use of synthetic insecticides has become widespread at all farm levels [6].

Over the last 40 years, the common pistachio psyllid (CPP) has been the most economic pest of pistachio trees in Rafsanjan, the main pistachio production area in Iran. High population densities of CPP during the kernel development period from June to August cause severe

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reductions in pistachio yield and tree weakness [7].

The insect overwinters as an adult winter-form in pistachio orchards mainly beneath the plant residue. Adult psyllids appear in orchards from mid-March and their populations rapidly increase after bud-break in late March, but usually decrease in mid-May. The CPP actively reproduces and develops from early spring until mid-autumn, and is thus present in the pistachio orchard while the green parts of the plant such as leaves are present [8].

The use of biopesticides towards phytophagous insects has increased in recent years, particularly in cropping systems that rely on natural plant extracts as a major component of integrated pest management [9, 10]. The use of these natural compounds instead of conventional insecticides can reduce environmental pollution, preserve non-target organisms, and avert insecticide-induced pest resurgence that results from insecticide resistance.

The use of botanical pesticides in sustainable agriculture dates back at least two millennia in ancient China, Greece, Egypt, and India [11, 12]. The documented use of botanicals extends back more than 150 years in Europe and North America, pre-dating discoveries of the major classes of synthetic chemical insecticides (*e.g*. organochlorines, organophosphates, carbamates, and pyrethroids) in the mid-1930s to 1950s. However, the overuse of synthetic insecticides has led to numerous problems unpredicted such as acute and chronic toxicity of applicators, farm workers, and even consumers, destruction of fish, birds, and other wildlife; disruption of natural biological control and pollination; extensive groundwater contamination, potentially threatening to human and environmental health; and the evolution of resistance to pesticides in pest populations [13].

These changes in the environment appeared to heighten the impetus for the discovery and development of alternative pest management products including insecticides derived from plants. Indeed, the scientific literature of the past 25 years described hundreds of isolated plant secondary metabolites that showed feeding deterrent or toxic effects on insects in laboratory bioassays. Botanical insecticides have been the subject of several recent volumes [14].

Biochemical or microbial biopesticides are an important group of naturally occurring, often slow-acting crop protection products that are usually safer to humans and the environment than conventional pesticides and with minimal residual effects. Biochemical pesticides may include plant-derived pesticides (botanicals) that can interfere with the growth, feeding, or reproduction of pests or insect pheromones applied for mating disruption, monitoring, or attract-and-kill strategies [15].

Pistachio is an important agricultural product and has historical cultural significance [16]. The common pistachio psyllid, *A. pistaciae*, feeds on different parts of the plant and fruit, causing damage to the pistachio and hence, decreasing the yield [7]. This insect is controlled by chemical insecticides but consequently, the overuse of these pesticides has harmed the natural enemies and the environment. Also, the target pest has developed resistance to the insecticides and has increased in number. This has resulted in farmers being very interested in using natural compounds.

The increasing price of pistachios has resulted in the economic threshold of the pest to decrease and this has led most growers to use chemical pesticides to control the pistachio pest. Consequently, because of the high doses and the overuse of pesticides, the pest has become resistant to the pesticides, so the doses of the chemicals have increased and the number of pesticide applications has also increased each year. As a result of this increase in pesticide dose and application frequency, it has become necessary to use less-harmful pesticides, at low doses and, to apply them at appropriate times, such as when pest numbers reach a point that is above an acceptable level [17]. Botanical and microbial pesticides can control pests more effectively and are less harmful to the environment, people, and also for non-target organisms [18].

Plant extracts and phytochemicals have long been a subject of research to develop alternatives to conventional insecticides and in the present paper we focused on the effect of some plant extracts on the population rates of eggs and nymphs of the common pistachio psyllid.

MATERIALS AND METHODS

Experimental plan

This research was conducted in a randomized complete block design with four replications. The experimental farm was located in the village of Hossein Abad Haj-Ali-Naghi (longitude between 53 degrees and 15 minutes and 55 degrees and 20 minutes; and latitude between 34 degrees and 45 minutes and 36 degrees and 58 minutes at an altitude of 1170 meters above sea level). The long-term average rainfall was 286 mm and the maximum and minimum annual absolute temperatures were 42°C and -10°C, respectively.

To apply the experiment, four rows of a 40-hectare pistachio orchard (500 trees per hectare) were chosen that psylla density were nearly similar on them. The distance between two trees was three meters and the distance between rows was 7 meters. Each row contained 44 trees.

The effect of plant extracts on the number of insect eggs and nymphs

According to a literature review, eucalyptus, thyme, and chamomile plants were selected due to their insecticidal effect. Adequate amounts of these plants were freshly prepared and their phenolic compounds were extracted in the laboratory as standard methods [19-22].
Before spraying the plant extracts, random sampling was carried out from the pistachio leaves in each experimental unit (replication-treatment), and the number of eggs and nymphs of the psyllid was counted. The experimental treatments included: pure water (as a control) and the extracts of chamomile, eucalyptus, and thyme at concentrations of 2.5, 5, and 7.5 per thousand. Sampling of the experimental unit was performed in four stages (3, 7, 14, and 21 days after spraying of the extracts). Using the formula of Henderson & Tilton [23], the effect of each dose-extract combination on the number of insect eggs and nymphs was calculated in comparison with control and the data recorded before spraying time in each experimental unit.
Henderson & Tilton formula:

Reduction % = [1 - (I. % in Co. before treatment) × (I. % in T after treatment) / (I. % in Co. after treatment) × (I. % in T before treatment)] × 100

Where: I. % = infestation %, T = treated, Co. = control

Data was statistically analyzed using the SAS 9.2 program. The effect of the doses and the extracts on common pistachio psyllid was examined using analysis of variance (ANOVA), and; the Duncan's Multiple Range Test (DMRT) was employed to determine significant ($P < 0.01$ and 0.05) differences among mean values.

RESULTS

The effect of the plant extracts on the number of the insect eggs

The analysis of data showed that the effect of different doses-extracts on the number of insect eggs is statistically significant at the 1% probability level for all sampling times (Table 1). It means the dose-extract combinations are differentially effective on the rate of oviposition of common pistachio psyllid.

Table 1. ANOVA analysis for treatment effect on the number of common pistachio psyllid eggs.

Source of Variance	Degrees of freedom	F value for sampling time			
		3 days	7 days	14 days	21 days
Replication	3	9.23	24.08	4.81	11.33
Treatment	8	38.18**	94.28**	156.91**	656.23**
Error	24				
Total	35				
CV		4.32%	11.07%	4.30%	17%

**: Significant at the 1% probability level.

At 3 days after spraying, the analysis of mean values comparison showed different dose-extract combinations were classified into six groups. The minimum number of eggs (maximum oviposition reduction) was recorded for thyme at the dose of 7.5 with a 40.25% reduction of oviposition; and then for thyme at doses of 5 and 2.5

with 36.83 and 35.96% reduction of oviposition, respectively (Table 2). The maximum number of eggs (minimum oviposition reduction) was observed for eucalyptus at the dose of 7.5 and for chamomile at the dose of 5, with 27.10 and 27.25% reduction of oviposition, respectively. So, the thyme extract at the dose of 7.5 per thousand had the best control effect on the insect eggs.

Table 2. Mean values comparison for the effect of different extract-dose combinations on oviposition reduction at 3, 7, 14, and 21 days after spraying ($p < 0.01$).

Extract-dose	Sampling time			
	3 days	7 days	14 days	21 days
Chamomile 2.5	30.40 d*	7.71 c	75.24 ef	76.11 f
Chamomile 5	27.25 e	3.92 d	73.45 f	74.97 g
Chamomile 7.5	32.43 cd	12.51 b	78.71 cd	79.49 d
Eucalyptus 2.5	34.72 bc	14.38 b	79.66 c	78.75 e
Eucalyptus 5	31.96 cd	9.11 c	77.76 cd	78.49 e
Eucalyptus 7.5	27.10 e	5.44 d	76.89 de	78.21 e
Thyme 2.5	35.96 b	5.42 d	88.74 b	88.83 b
Thyme 5	36.83 b	12.28 b	88.68 b	86.67 c
Thyme 7.5	40.25 a	21.26 a	91.26 a	89.75 a

*: Similar letters in each column mean non-significant differences among values.

At 7 days after spraying, the comparison of mean values showed different dose-extract combinations were classified into four separate groups. The maximum oviposition reduction was recorded for thyme at the dose of 7.5, with a 21.26% reduction of oviposition (Table 2). The minimum oviposition reduction was observed for chamomile at the dose of 5, for thyme at the dose of 2.5, and for eucalyptus at the dose of 2.5, with 3.92, 5.42, and 5.44% reduction of oviposition, respectively. So, thyme extract at the dose of 7.5 had the best control effect on the insect oviposition.

At 14 days after spraying, the mean values were classified into seven groups. The maximum oviposition reduction was recorded for thyme at doses of 7.5 with 91.26% reduction of oviposition; and then for thyme at doses of 2.5 and 5 with 88.74 and 88.68% reduction of oviposition, respectively (Table 2). The minimum oviposition reduction was observed for chamomile at doses of 5 and 2.5, with 73.45 and 75.24% reduction of oviposition, respectively. So, thyme extract at the doses of 7.5, 5, and 2.5 had the best control effect on the insect oviposition.

At 21 days after spraying, the mean values were classified into seven separate groups. The maximum oviposition reduction was recorded for thyme at dose of 7.5 with a 89.75% reduction of oviposition; and then for thyme at doses of 2.5 and 5 with 88.83 and 86.67% reduction of oviposition, respectively (Table 2). The minimum oviposition reduction was observed for chamomile at doses of 5 and 2.5, with 74.95 and 76.11% reduction of oviposition, respectively. So, the dose of 7.5 for thyme extract had the best control effect on the insect oviposition. On the 21st day, the maximum and minimum oviposition reduction by different extract-dose combinations were similar to their arrangement at 14 days after spraying. As a result, the best time for maximum efficacy of the extracts on oviposition of the insect appeared at 14 and 21 days after spraying.

The effect of the plant extracts on the number of the insect nymphs

The analysis of data for the number of insect nymphs showed that the effect of dose-extract combinations is statistically significant at the 1% probability level for all sampling times (Table 3). It means the dose-extract combination is effective on the number of common pistachio psyllid nymphs.

Table 3. ANOVA analysis for treatment effect on the number of common pistachio psyllid nymphs.

Source of Variance	Degrees of freedom	F value for sampling time			
		3 days	7 days	14 days	21 days
Replication	3	0.56	0.22	0.71	0.78
Treatment	8	3461.66**	8422.29**	8152.34**	10324.26**
Error	24				
Total	35				
CV		3.5%	3.21%	2.04%	1.84%

**: non-significant and significant at the 1% probability level, respectively.

At 3 days after spraying, the analysis of mean values comparison showed different dose-extract combinations were classified into six separate groups. The minimum number of nymphs (maximum effect on the nymph population) was recorded for thyme at the dose of 7.5 with a 65.04% reduction of the nymph population; and then for thyme at doses of 2.5 and 5, with 64.04 and 63.68% reduction (Table 4). The maximum number of nymphs (minimum effect on the nymph population) was observed for eucalyptus at doses of 5 and 2.5, with 50.36 and 50.58% reduction of the nymph population. So, the thyme extract at the dose of 7.5 had the best control effect on the insect nymph population.

At 7 days after spraying, the comparison of mean values showed different dose-extract combinations were classified into seven separate groups. The maximum reduction in the nymph population was recorded for thyme at the dose of 7.5 with a 61.34% reduction; and then for thyme at the dose of 5 with a 59.03% reduction of the nymph population (Table 4). The minimum effect on the number of nymphs was observed for eucalyptus at doses of 2.5 and 5, with 41.60 and 41.71% reduction of the nymph population, respectively. So, the thyme extract at the dose of 7.5 had the best control effect on the reduction of the nymphs' number.

Table 4. Mean values comparison for the effect of different extract-dose combinations on nymph rate reduction at 3, 7, 14, and 21 days after spraying (p<0.01).

Extract-dose	Sampling time			
	3 days	7 days	14 days	21 days
Chamomile 2.5	60.10 d*	51.07 e	75.73 d	77.07 d
Chamomile 5	60.15 d	51.03 e	74.90 e	76.42 d
Chamomile 7.5	61.26 c	52.78 d	76.79 c	78.67 c
Eucalyptus 2.5	50.58 f	41.60 g	66.76 g	66.54 f
Eucalyptus 5	50.36 f	41.71 g	66.53 g	65.99 f
Eucalyptus 7.5	51.79 e	43.81 f	69.90 f	69.75 e
Thyme 2.5	64.04 b	58.07 c	82.42 b	82.20 b
Thyme 5	63.68 b	59.03 b	82.91 b	82.64 b
Thyme 7.5	65.04 a	61.34 a	83.76 a	84.13 a

*: Similar letters in each column mean non-significant differences among values.

At 14 days after spraying, the mean values were classified into seven separate groups. The maximum reduction in the nymph rate was recorded for thyme at the dose of 7.5 with a 83.76% reduction; and then for thyme at doses of 5 and 2.5 with 82.91 and 82.42% reduction of the nymphs' number (Table 4). The minimum reduction in the nymph population was observed for eucalyptus at doses of 5 and 2.5, with 66.53 and 66.76% reduction of the nymphs' number, respectively. On the fourteenth day, similar to 3 and 7 days after spraying, the doses of 7.5, 5, and 2.5 for thyme extract had the best efficacy on the reduction of the nymphs' rate.

At 21 days after spraying, the mean values were classified into six separate groups, and the arrangement of different extract-dose combinations was similar to their arrangement on 14 days after spraying. So, the doses of 7.5, 5, and 2.5 for thyme extract had the best control effect on the reduction of the common pistachio psyllid nymph population. As a result, the best time for maximum efficacy of the extracts on the population of the insect nymphs appeared at 14 and 21 days after spraying. Besides, thyme and then chamomile extracts were more effective than eucalyptus extract in the reduction of nymph populations.

DISCUSSION

In this research, an attempt was made to determine and compare the effects of some medicinal plant extracts such as thyme, chamomile, and eucalyptus on the oviposition and the nymph population of common pistachio psyllid, *A. pistaciae*. Generally speaking, among different types of plant extracts, thyme extract was the most effective product in the reduction of the population rate of eggs and nymphs of the common pistachio psyllid and the results indicated this product is significantly more effective than other tested plant extracts. Of course, other plant extracts eucalyptus and chamomile, also showed considerable effect on the oviposition and the number of nymphs, especially at 14 and 21 days after spraying. Generally, the dose of 7.5 per thousand of all three extracts had a better effect on the number of the insect eggs and nymphs, compared to the doses of 5 and 2.5 per thousand. In addition, sampling on the fourteenth and 21st day after applying the treatments showed the best effect of the extracts on the number of insect eggs and nymphs.

The discovery of environmentally safe products, including plant extracts, has created a new perspective on pest management. The insecticidal and antifungal effects of spices and aromatic plants have been known to man for a long time, and in ancient times, these compounds were used to preserve and increase the shelf life of agricultural products and food. Among the other applications of these materials, it could be mentioned the usage of the products for the control of behavior of pests, population monitoring, disrupting reproductive behavior, direct control of pests, and health uses in humans and animals.

The ovicidal, anti-nutritional, and repellent properties of neem extract mixed with food and spraying on plants have been positively evaluated on all sensitive stages of whitefly, except the egg stage [24]. The effect of lavender essential oil on the reduction of oviposition and the adult population of the first generation of the legume beetle, as well as the safety of plant essential oils for human health and their compatibility with the environment, were shown by Duke (1985) [25]. Nicotine has been used as a short-lived contact insecticide in the control of aphids, thrips, apple worms, and mealybugs, and nicotine extract is effective as a disinfectant to reduce nematode populations in the soil [26]. Contact toxicity of cardamom essential oil (*Elletaria cardamomum*) on adult stage of corn aphid (*Sitophilus zeamais*) and red flour bug (*Tribolium castaneum*) [27], essential oil of wormseed leaves (*Chenopodium ambrosioides*) on adult insects of common pulse weevil (*Callosobruchus maculatus*), Chinese bean beetle (*Callosobruchus chinensis*), bean beetle (*Acanthoscelides obtectus*), granary weevil (*Sitophilus granarius*) and maize weevil (*Sitophilus zeamais*) [28], essential oil of *Chamaecyparis obtuse* on adult insects of Chinese bean beetle and rice weevil [29] and the extract of *Cupressus sempervirens* and a type of eucalyptus (*Eucalyptus saligna*) on adult insects of red flour beetle and maize grain weevil [30] has been proved.

Investigating the effect of contact toxicity of the extracts of *Artemisis annua* L. and *Sambucus ebulus* L. on the flour beetle (*T. confusum*) indicates that these compounds are effective [31]. Research also shows that essential oils of Shirazi thyme and lavender are effective on the common pulse beetle [32]. Thirty-two oil extracts obtained from the plants that are commonly used in the food industry were used to control four strains of *Listeria monocytogenes* and one strain of *L. innacua* bacteria, and among them, cinnamon, clove, origanum, pimento, and thyme extracts showed antimicrobial properties [33]. Thyme and clove extracts have a great ability to reduce the growth of mycelia of blue mold fungi and reduce spore germination to zero in pure extracts [34-36].

Since the climatic conditions of Iran are suitable for the cultivation of many of these aromatic plants, therefore, in this study, an attempt was made to study the insecticidal effects of some natural plant extracts. As pistachio is a tree that is very important and valuable economically, the farmers use any type of method for pest controlling (e.g.

harmful pesticides) to produce more and raise economic gains. Using natural plant extracts, we can produce the qualitative and organic products; so, the application of this method is recommended to farmers and gardeners. Especially, the usage of thyme extract is suggested to control the common pistachio psyllid in integrated pest management programs. It is also suggested to study the side effects of these compounds on non-target organisms, the stability of the extracts in the environment, and other similar cases.

Conflict of interests

No conflict of interests.

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