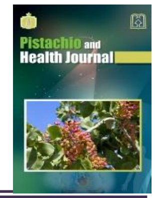

The effect of some plant extracts in comparison to Dinotefuran insecticide on the nymph of common pistachio psyllid (*Agonoscena pistaciae*) under field conditions

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Abstract

Background: Common pistachio psyllid (*Agonoscena pistaciae*) is the most important pest of pistachio orchards in Iran.
Material and Methods: In order to reduce chemical toxins in pistachio orchards, several experiments were conducted, including pre-tests to determine the concentration. In field experiments in the form of completely randomized block design, it has been attempted to investigate the effect of several plant extracts of Lavender, Stinking assa, and Tobacco with respectively 5.981± 1.48, 10. 397±5.58, and 18.127±2.83 LC_{50} and Dinotefuran (Starkle) 0.7 per thousand with 3 replicates on the percentage reduction of nymph population. Pre-test was conducted to determine the required concentration based on the logarithmic mortality percentage of 25 to 75%. After applying the treatments randomly, as many as 30 leaflets were selected from each treatment and transferred to the laboratory to count the nymphs.
Results: The results of analysis of variance and comparison of means indicated a significant effect of plant extracts on the percentage of pistachio nymph psyllid mortality at the level of one or five percent compared to that of the control. Using the plant extracts decreased the percentage of nymph population, and this decrease was different based on the type of plant species and concentration. The maximum time of nymph mortality was observed on day 14 and the lowest mortality was observed on day 3. The highest efficiency for reducing the population of nymphs was observed in Starkle pesticide, and the maximum percentage of nymph mortality was observed in Lavender, Stinking assa, and Tobacco, respectively.
Conclusion: The compounds tested in this study can be used to control this pest.

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1. Introduction

Pistachio (*Pistacia vera* L.) in Iran, as a strategic product, has a special position among agricultural products and accounts for a large part of non-oil exports. The export value of pistachios and their kernels in Iran has increased significantly compared to other agricultural products [1]. Pistachio trees, like other fruit trees, are not safe from pests; they cause a lot of damage to pistachio orchards every year. Among the various mites and insects that damage pistachios, the common pistachio psyllid *Agonoscena pistaciae* Burckardt & Lauterer (Hemiptera: Psyllidae) or dry sap is the most important pest and is regarded as one of the native pests of pistachio orchards; by feeding on plant sap, it causes severe damage and ultimately reduces crop yield. Feeding of psyllid from trees is accompanied by the secretion of a white substance called honeydew. This sticky substance absorbs dust and makes the branches and leaves of trees look bad. Loss of plant sap causes the general weakness of pistachio trees and the loss of leaves, buds, small seeds and an increase in the percentage of hollowness and closed-shell pistachios. One of the major damages of dry sap is the formation of incomplete kernel and sometimes completely hollow pistachio [2]. Today, this pest has become a serious problem for gardeners and imposes huge economic costs on them every year. In addition to the economic consequences of this pest, indiscriminate spraying with dangerous pesticides will undoubtedly lead to numerous environmental and health disasters [3].

Using chemical pesticides in psyllid pest control has long been common, but for environmental reasons, including the residual pesticides on the product, they bring about serious problems in production and especially the export of the product. Nowadays, using healthy and organic products is one of the general goals of producers. Therefore, it is necessary to produce healthy products without or using the least chemical pesticides to control pests and diseases [4]. In a study that investigated the effect of several non-chemical compounds on pistachio psyllid, satisfactory results were obtained to control this pest in pistachio orchards [5].

Using plant biological compounds for pest control seems to be essential and the type of substance and the usage concentration of biological compounds play an important role in pest control; more comprehensive studies are required to be conducted in this regard. Plant compounds have respiratory, contact, repellent and anti-nutritional toxicity, and given the effect of these compounds on the biological parameters of insects, their low risks to humans and mammals, their faster decomposition in nature, and their fewer environmental damages on the environment (compared to chemical pesticides), they have found a specific position in pest control [6]. Tobacco is one of the first plants to be known for its insecticidal properties, and Europeans used its extract to control pests around 1690 [7]. Nicotine and other tobacco alkaloids are used in the preparation of pesticides, natural fungicides, citric acid, vitamin B6 and niacin [8].

In a study, the insecticidal effect of acetonic extract of *Calotropis procera*, *Teucrium polium*, *Fumaria officinalis* and *Thymus vulgaris* leaves on pistachio psyllid nymphs of the fifth instar was investigated by leaf disc immersion method. It has been reported that the extracts of all four plants reduced the population of psyllid, and this population reduction was higher in *T. vulgaris* extract [9].

In another study, it was attempted to investigate using neem extract in controlling pistachio psyllid in laboratory conditions. Concentrations of 75 ppm of neem extract on nymphs and concentration of 100 ppm of neem extract on adult insects were reported to have the best control effects [10]. A study entitled "Determining the toxicity of Acetamiprid and alcoholic extract of Asafoetida on common pistachio psyllids *Agonoscena pistaciae* in laboratory conditions" was conducted in laboratory conditions, and it was reported that the efficiency of alcohol and Asafoetida was more effective than Acetamiprid [11]. In another study, it was attempted to investigate the effect of ethanolic extracts of oleander on pistachio psyllid nymphs, and it was concluded that oleander extract reduces pistachio psyllid nymphs compared to the control group [12].

In another study, it was attempted to investigate the effect of ethanolic extract of Chinaberry and neem on pistachio psyllid nymphs, it was reported that Chinaberry extract had the required ability to control ordinary pistachio psyllid in the nymph stage and can be a suitable alternative for neem extract [13].

Starkle, commonly known as Dinotefuran, is manufactured by Mitsui Chemical of Japan. It is a contact, gastrointestinal and systemics insecticide of the third generation of neonicotinoid insecticides. This compound causes insect death by inhibiting insect feeding for several hours after being used. After spraying, it is absorbed both through the leaves and through the roots and is transferred to all the organs of the plant. Starkle binds to acetylcholine receptors behind the synapse of the insect nervous system, and since this compound cannot be broken down by cholinesterase, the nerve cell is constantly under complete stimulation, leading to seizures, paralysis, anesthesia, and eventually death of the pest [14]. Starkle insecticide has a significant effect on sucking insects of Hemiptera family, including bugs, aphids, whiteflies and other pests of this family [15].

In the present study, it has been attempted to investigate the effect of some medicinal plant extracts (Lavender *(Lavandula* sp.), Stinking assa (*Ferula assa-foetida*) and Tobacco (*Nicotiana tabacum*)) with Starkle insecticide on pistachio psyllid pest control.

2. Materials and methods

Method of conducting bioassay experiments: using the tested compounds on pistachio psyllid nymphs

The tested plant extracts were prepared from Dana Gene Pazhouhan Karmania Company. The plant studied in this study include Lavender, Stinking assa, and Tobacco. Alcoholic extracts of Tobacco and Lavender were extracted from their leaves, and as for Stinking assa, its leachate was extracted. Then different concentrations of plant extracts were prepared using distilled water. Pistachio psyllid bioassay was conducted by contact method with the dry residue of plant compounds left on the leaf surface [16]. Preliminary tests were conducted to obtain a series of soluble concentrations in a suitable range and to determine the final concentrations. Based on the results of preliminary experiments, concentrations between 25 and 75% mortality were selected and intermediate concentrations were determined based on logarithmic intervals [17].

The bottom of petri dishes was covered with 0.7% agar and in the next step, the infected leaves were laid by counting the number of live nymphs. Concentrations of each extract were prepared using micropipette and beaker and subsequent concentrations were made using the concentration preparation formula. After spraying the concentrations on the nymphs, the lids of petri dishes were placed on them to prevent the escape of nymphs. For proper ventilation, the lid of the container was drilled with a diameter of 5 cm and then completely closed with a cloth net. Finally, the mortality effect of the plant extracts was evaluated 24 hours after treatment. Nymphs that could not walk when stimulated by the brush were considered as dead [18]. Then, the number of live nymphs in each treatment and replicate was separately counted and recorded. This experiment was repeated 3 times for each extract and each concentration of the compound.

Garden blocking and the method of applying treatments

Field experiment was conducted in a one-hectare pistachio orchard with Ohadi cultivar. In the selected orchard, at least from the beginning of spring to early August, no spraying was performed, and the its population of ordinary pistachio psyllid was, thus, high. The rows of the garden were blocked into 3 replicates (3 blocks) based on a randomized complete block design. In each replicate (block), determining the type of plant extracts, Starkle, and control were randomly conducted and a design map was prepared. In each block, for each treatment, 2 trees in each row were considered as replicate and 1 tree in each row was considered as guard. Foliar spraying was conducted in the early morning with 20-liter back sprayer.

Sampling

First, one day before spraying, 15 leaflets from each tree (30 leaflets per treatment in each replicate) were randomly selected as a sample and then the samples related to each tree were placed in separate containers and transferred to the laboratory. In the laboratory, nymphs of different instars of pistachio psyllid were counted on both upper and lower leaf surfaces by stereomicroscope.

3, 7, 14 and 21 days after foliar application, sampling of treated trees was conducted in the same way mentioned earlier. In this method, 30 leaflets from both trees (for each treatment in each replicate) were randomly selected and the number of live psyllid nymphs related to each treatment and replicate were counted and recorded separately. Psyllid nymphs were counted by stereomicroscope on both the upper and lower surfaces of the terminal leaflets. After calculating the average number of normal pistachio nymphs in each experimental unit, the percentage of effect of each of the pesticides used was calculated using Abbott formula and comparisons and statistical calculations were conducted as well [19].

$$\%\mathrm{R} = 1 - \frac{Ca - Ta}{\mathrm{Ca}} \times 100$$

%R: Effectiveness percentage of treatments (percentage of nymph mortality)

Ta: The number of nymphs after the treatment

Ca: The number of nymphs in the control samples after the treatments

Statistical analysis

This research was considered as a randomized complete block design with 3 replicates and 30 leaflets for each replicate. The obtained data were first entered into Excel software. Then, the percentage of population reduction of normal pistachio psyllid nymphs in each treatment was calculated using Abbott formula. Data were analyzed using SPSS software and the means were compared with Tukey test at one and five percent probability levels. Graphs were drawn using Excel software. To determine the values of LC_{10}, LC_{50} and LC_{90}, the probit method was used in the probit Analysis-Ms chart software (9006).

3. Results

Results of bioassays after 24 hours in the laboratory

The results of bioassays showed that the lavender treatment with LC_{50} = 5.981 ± 1.48 per thousand had the highest percentage of population reduction after spraying compared to Stinking assa and Tobacco treatments with LC_{50} = 10.397 ± 5.58 tobacco with LC_{50} = 18.127 ± 2.83 per thousand, respectively (Table 1).

Table 1- Bioassy testes on Pistachio psyllid nymphs 24 hours after consumption

Variable	LC_{50}	y-intercept	Line slope	Chi-square value	Degree of freedom	heterogeneity	Lower-Upper(LC_{50})
Lavender	5.981±1.48	4.18	0.214	1.053	4	0.52	0.00245-60.21
Stinking assa	10.397±5.58	0.824	1.03	3.34	4	3.34	0.013-99.939
Tobacco	18.127±2.83	16.53	5.04	1.877	4	0.56	9.416-39.393

The effect of the tested compounds on the percentage reduction of the population of pistachio psyllid nymphs 3 days after consumption

Based on the LC_{50} obtained in the laboratory, the effect of treatment of Tobacco, Lavender, and *Stinking assa* with LC_{50} dose and Starkle pesticide with recommended dose (0.7 per thousand) on the population of pistachio nymphs three days after consumption indicated a significant difference. The highest percentage of population reduction was 98.6 ± 7.9, 62.2 ± 7.4, 47 ± 8.8 and 28.3±1 in Starkle, Lavender, *Stinking assa* and Tobacco treatments, respectively. Lavender and *Stinking assa* treatments were in one statistical group and the highest percentage of population reduction was reported for Starkle treatment and the lowest percentage of population reduction was related to Tobacco treatment; *Stinking assa* and tobacco were in one statistical group (Fig. 1).

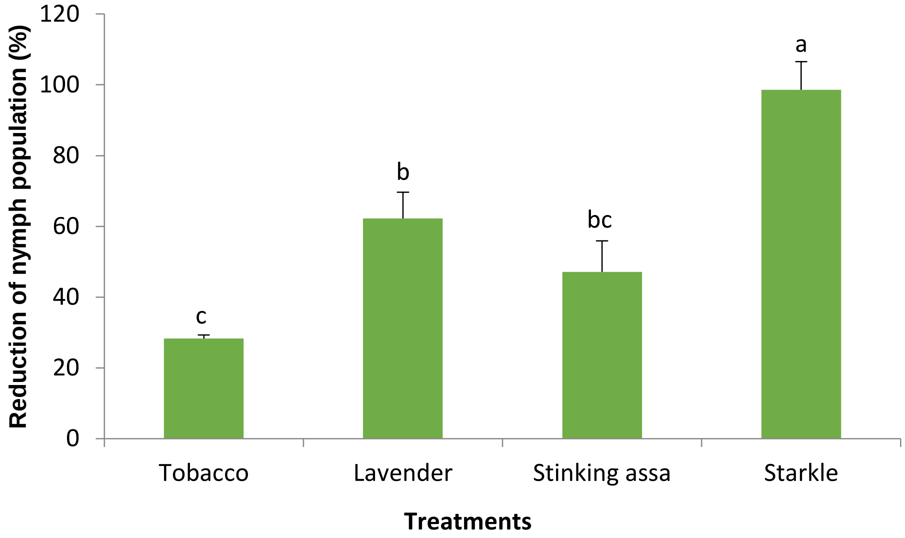

Fig. 1- Mean population reduction of *Agonoscena pistaciae* nymphs under the influence of different treatments 3 days after spraying

The effect of the tested compounds on the percentage reduction of the population of pistachio psyllid nymphs 7 days after consumption

The effect of Tobacco, Lavender, Stinking assa and Starkle treatments on pistachio psyllid nymph populations 7 days after consumption was significantly different from each other. The highest percentage of population reduction was 99.84 ± 0.15, 80.51 ± 4.62, 62.11 ± 6 and 50.34 ± 7.71% in Starkle, Lavender, Stinking assa and Tobacco treatments, respectively. Tobacco and Stinking assa treatments were in one statistical group (Fig. 2).

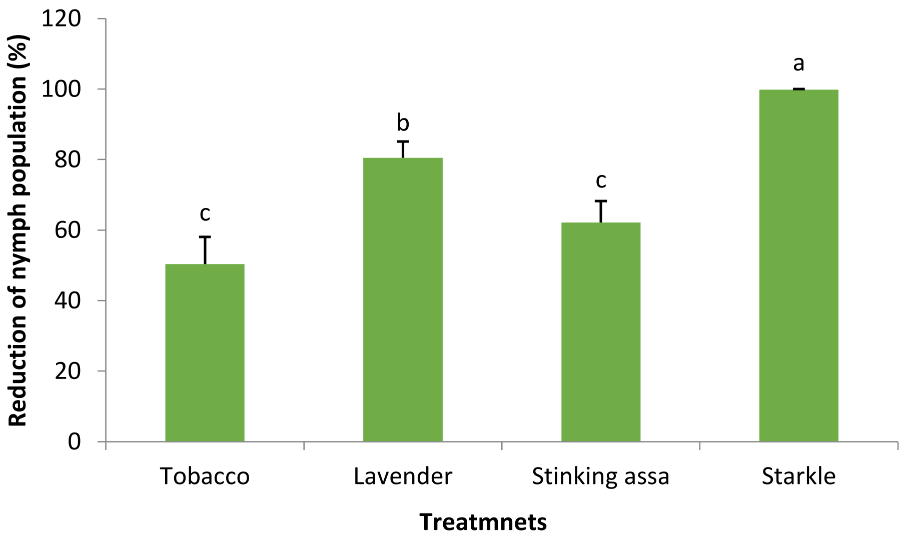

Fig. 2- Mean population reduction of *Agonoscena pistaciae* nymphs under the influence of different treatments 7 days after spraying

The effect of the tested compounds on the percentage reduction of the population of pistachio psyllid nymphs 14 days after consumption

The results of comparing the means showed that the type of toxin has a significant effect on the percentage of nymph population. The highest percentage of population decrease was 99.45 ± 0.32, 78.71 ± 6.38, 73 ± 6.3 and 59.2 ± 9.54 in Starkle, Stinking assa, Lavender and Tobacco treatments, respectively. Lavender and Stinking assa treatments were in one statistical group (Fig. 3).

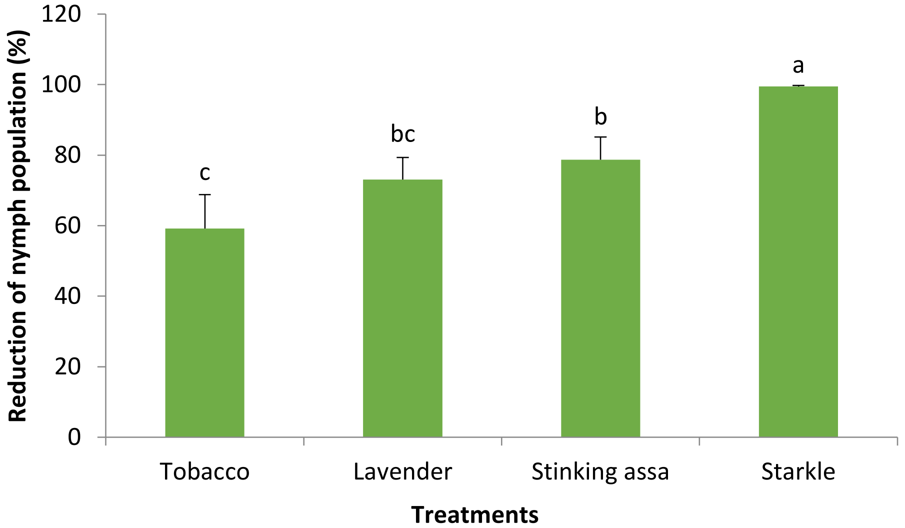

Fig. 3- Mean population reduction of *Agonoscena pistaciae* nymphs under the influence of different treatments 14 days after spraying

The effect of the tested compounds on the percentage reduction of the population of pistachio psyllid nymphs 21 days after consumption

The effect of Tobacco extract, Lavender, Stinking assa and Starkle treatment on pistachio psyllid nymph population 21 days after consumption was significantly different from each other. The highest mortality rates were 99.55 ± 0.44, 72.93 ± 4.52, 62.92 ± 7.16 and 60.12 ± 9.43% in Starkle, Lavender, Stinking assa and Tobacco treatments, respectively. Lavender and Stinking assa treatments were in one statistical group (Fig. 4).

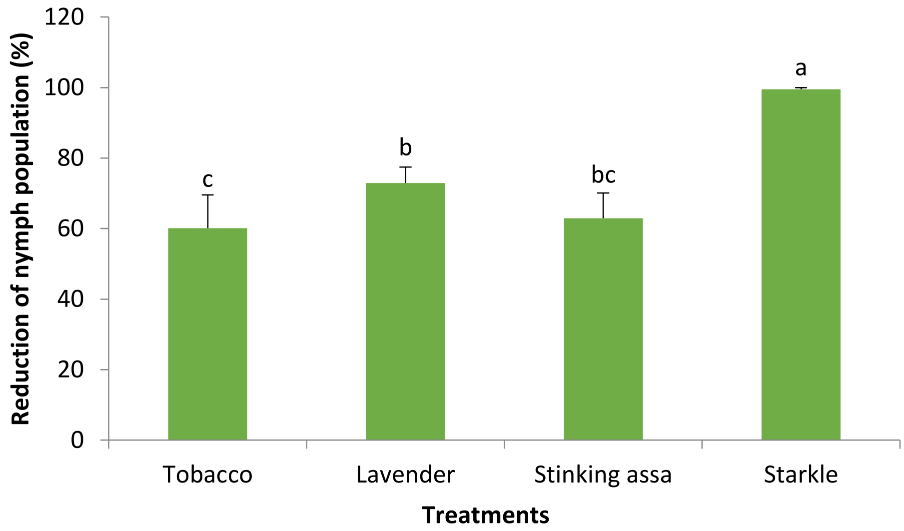

Fig. 4- Mean population reduction of *Agonoscena pistaciae* nymphs under the influence of different treatments 21 days after spraying

The effect of the tested compounds on the percentage reduction of the population of pistachio psyllid nymphs in the post- spraying period in orchard conditions

The effect of Tobacco, Lavender, Stinking assa and Starkle on pistachio psyllid nymph population was significantly different during the post-consumption period. The highest percentage of nymph population reduction in the post-consumption period was 99.36 ± 3.75, 72.17 ± 4.5, 62.70 ± 2.95 and 49.49± 4.5 in Starkle, Lavender, Stinking assa and Tobacco treatments, respectively. Lavender and Stinking assa treatments were in one statistical group (Fig. 5).

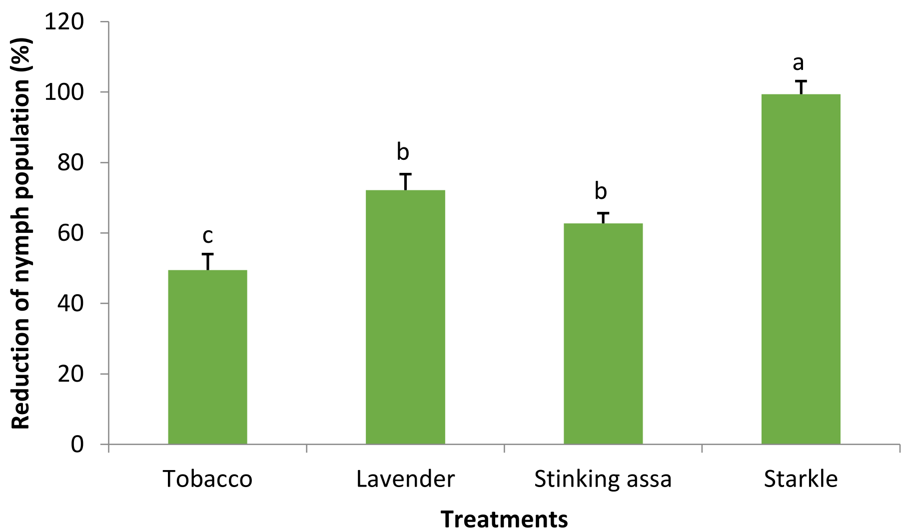

Fig. 5- Mean population reduction of *Agonoscena pistaciae* nymphs under the influence of different treatments in the post- spraying period

4. Discussion

Using chemical pesticides has always been associated with major problem for humans. Chemical compounds always threaten human health and the environment [20]. Therefore, today, there is an uregent need for safe, inexpensive, easy-to-use and environmentally friendly alternatives [21].

In the past few years, the use of plant extracts for pest control has received much attention. Plant extracts can have repellent and anti-nutritional effects and affect many pests. Toxins derived from plant extracts have little effect on mammals and are quickly degradable in the environment. Today, the desire to find new plants rich in biological insecticides is increasing all over the world; this is an effective step towards preserving the health of the environment.

This study was conducted to investigate the effect of three plant insecticides and compare them with Starkle (a chemical insecticide) on the reduction percentage of the population of common pistachio nymphs in orchard conditions. The results of the present study show that the effect of these insecticides on the reduction percentage of nymph population is different after 3, 7, 14 and 21 days.

Based on the results of analysis of variance tables, the use of extracts of Stinking assa, Tobacco, Lavender and Starkle had a significant effect on the population of pistachio psyllid nymphs and caused a significant difference in the population of nymphs compared to the control at the level of one percent. The concentration of extracts also caused a significant difference on the nymph population. Using plant extracts as well as Starkle reduced the population of pistachio psyllid nymphs; this reduction varied based on different concentrations. Moreover, the percentage of nymph mortality was significant and different in the study period. The highest percentage of mortality was observed by Starkle toxin and the lowest nymph mortality was observed in Tobacco plant extract. The highest mortality rate was on the fourteenth day and the lowest was on the third day. Given the significance of pistachio psyllids, especially the damage caused by the presence of nymphs, the control time and the compounds used are of high significance. The results of the present study indicate that using Starkle, Lavender and Stinking assa treatments two weeks after the emergence of nymphs has the best efficiency and reduces the population of nymphs. Similar results have been reported on the use of extracts of *C. procera*, *T. polium*, *F. officinalis* and *Th. vulgaris* on pistachio psyllids; it has been reported that thyme extract was more effective in controlling pistachio psyllid than the other three extracts [9].

Moreover, another study investigated the effects of two plant pesticides including insecticide soap (Palizin) and red pepper extract on pomegranate aphids and mites. They have reported that two concentrations of 2 and 2.5 per thousand of both compounds could reduce the population of pomegranate aphids and mites. Also, no significant difference was observed between the mentioned compounds and the concentrations used. Based on the obtained data, the effect of Palizin insecticide was higher [22].

In a study, the mortality effect of extracts of several species of medicinal plants (Thyme, Rosemary, Castor, Sophora) on common pistachio psyllids was investigated and the results were reported as follows. All extracts were confirmed to be effective in the control of pistachio psyllids and resulted in the reduced population of nymphs. Among the used extracts, Sophora extract showed more efficiency in reducing nymph population than other extracts [23]. Similar results have been reported by other researchers on other pests such as wheat aphids, two-spotted mite, Mealy bug and some storage pests; they have reported the effect of plant extracts on reducing the damage caused by the use of plant extracts compared to control groups. In one study, the pesticide effect of *C. procera*, *T. polium*, *F. officinalis* and *Th. vulgaris* was investigated by immersion method. The results estimated LC_{50} values to be 328.171, 409.726, 321.283 and 476.850 µl/ml, respectively [9].

The reasons for the percentage reduction in psyllid population by plant extracts may be specific insecticidal compounds in medicinal plants, especially monoterpene volatile compounds and alkaloids including nicotine in tobacco, which affect the action of acetylcholine at the nerve and muscle junction (synapse) and cause contraction, seizures and death [24]. The sulfur compounds in Stinking assa and the alcoholic volatile compounds in Lavender are a type of contact, fumigation insecticide; by affecting respiration and respiratory disturbances by blocking airways or disrupting insect growth regulators, they interfere cellular metabolism and growth hormone production act during metamorphosis (change in form).

Disrupted metabolism happens through affecting production (ATP), oxidative phosphorylation, separating or inhibiting adenosine triphosphate (ATP) production, which in turn reduces energy production and increases nymph mortality. Owing to the fact that pistachio psyllid nymphs do not have a hard shell (lack of hard shell), it is, thus, possible that the plant extract and also Starkle cause death by affecting the respiratory disorders of the nymphs. The reasons for the difference in the effectiveness of the extract of any plant at a certain concentration can be owing to specific secondary compounds in each plant or differences in the solvent used and the time of testing. Moreover, the effective compounds in plant essential oils are a function of species genetics, growth stage, location, the effect of stresses and some physiological factors. Respiratory toxicity of essential oil is positively and significantly correlated with essential oil concentration and duration of providing essential oils [25]. The results of the first experiment of the present study clearly show the significant difference in the dose of each extract. This difference expresses the effective dose (percentage of mortality) of each species and shows the fact that to achieve the desired result in reducing damage and pest control via medicinal plant extracts, the different levels of consumption should be determined based on the mortality of each species, type of pest, and pest life cycle of each plant. There are reports from some researchers indicating the effect of some plant extracts on other insects that have caused differences in their development and biological stages and have been effective on their mortality. The differences between these studies and the present one are related to the type of solvent, the type of extract, the type of pest and the time of testing. The results of this study indicate that some extracts can be selected as an option to produce a natural insecticide [26]. One of the important and significant results obtained in the present study was the maximum percentage of nymph mortality that was obtained in the second week (14 days after extract spraying); this indicates the fact that in the life cycle, each pest has sensitive stage against different insecticides including natural insecticides and plant extracts.

Similar results have been reported in a study investigating the effect of Sirinol, Tondexir and Palizin extracts on pistachio nymphs. In the results, they stated that all three insecticides reduced the population of psyllid nymphs compared to the control. The maximum nymph mortality during 2 and 7 days after foliar application was reported for Palizin treatment, and the maximum nymph mortality within 14 and 21 days after foliar application was reported in Tondexir. This indicates the difference in the effect of extracts of different plant species, concentration effect, time of maximum effect and interaction of time of use, pest life cycle, dose, place of application, type of solvent, amount of active substance, type of species (enzymatic, metabolic, … reactions). According to these issues, after the initial biometric tests and according to the percentage of mortality as stated in the results, a certain dose was investigated for each plant to achieve the desired result [27].

According to the results of the present study, Starkle showed the greatest effect on the mortality percentage of pistachio psyllid nymphs compared to the plant extracts used. In another study, the effect of several pesticides including Starkle (with a concentration of 0.5 per thousand) as a solution in soil, Oberon and Memory with concentrations of 0.5 and one per thousand as a spray on *Bemisia tabaci* and *Trialeurodes vaporariorum* were examined over a 28-day period. The results have indicated that among the treatments, Memory and Starkle insecticides were the most effective. This process continued for 28 days after spraying. Based on this experiment, in greenfly-infected greenhouses, Starkle, Oberon and Memory insecticides with a concentration of 0.5 per thousand are recommended for pest control [28].

The results of a study on mosquito larvae showed that Starkle was 10 times more toxic than Permethrin and 1000 times more toxic than Propoxur. Because neonicotinoids have different modes of action, these chemicals can also help better manage pest resistance to insecticides [14]. In another study, greenhouse whitefly was investigated by several neonicotinoid insecticides including Starkle SG 20%. The insects were adults. The first instar nymphs were assessed by bioassay experiments via immersion of infected leaves and the percentage of adult insect mortality was recorded 24 hours after having contact with infected leaves. The LC_{50} value obtained for Starkle in the experiments was determined to be 93.55 ppm based on the active substance. Sensitivity and percentage of first instar nymph mortality were assessed 72 hours after their first contact with the treated leaves. The LC_{50} value of Starkle insecticide in the experiments was determined to be 15.48 ppm based on the active substance. The results have indicated that this insecticide has more toxic properties on first instar nymphs of greenhouse whitefly compared to adult insects [29].

5. Conclusion

According to the results, the compounds tested in this study can be used to control this pest. However, it is necessary to reconsider the use of chemical pesticides and to find effective non-chemical compounds against this pest in order to reduce the use of pesticides. The plant compounds investigated in this experiment, including Stinking assa, Tobacco, Lavender, had a high insecticidal effect on pistachio psyllid nymphs. Therefore, based on the results of the present study and considering the environmental effects of pesticides, these plant extracts can be recommended and used to control pistachio psyllids. However, it is essential that all technical points such as concentration, ambient temperature, spraying time, and life cycle of the pest be taken into account. The benefits of these valuable plants can be used by conducting more extensive experiments on the effect of these plants on various aspects of the pest and examining the appropriate methods of using them in pistachio orchards.

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Conflict of interest

The authors acknowledge that they have no conflict of interest in publishing this study and no significant financial support has been provided for this study (to influence the findings).

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