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Toxicity of selected plant-derived pesticides to the citrus spider mites (Acari: Tetranychidae) and their predator, *Stethorus gilvifrons*, in the semi-field conditions

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ABSTRACT

To examine selective effectiveness of specific pesticides on the citrus spider mites and their predator, we evaluated the contact toxicity of different plant-derived pesticides including dishwashing liquid, Dayabon, Palizin, Palizin + Citrol oil, Tondexir + Bartar soap, cpirodiclofen, spiromesifen, pyridaben, chlorpyrifos-ethyl and abamectin and control on the adult of *Tetranychus urticae* and *Panonychus citri*, *Panonychus ulmi* and their predator, *Stethorus gilvifrons* in the semi-field conditions. The results revealed that at 24, 48 and 72 h after treatment, Tondexir treatment of 3 ml/L + Bartar 1ml/L had the highest mortality rate on the *T. urticae*, *P. citri*, and *P. ulmi*. This value was 98.13, 84.48, and 80.62%, respectively, for 72 h after treatment for these mites. In addition to the mentioned cases, this treatment caused 72.88% mortality in the larvae and 16.13% in the adult predator during 72 h after treatment, while at the same time, the rate of mortality of chlorpyrifos-ethyl and abamectin treatments on ladybirds was 100%. The results of this study showed that herbal treatments have a high efficiency for integrated management programmes of the major harmful citrus mites and have little impact on their natural enemies and can replace the high-risk chemical pesticides.

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Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most common and dangerous pests of agricultural crops (Jeppson et al. 1975). This species is a major pest which infests on more than 1,100 plant species (Piraneo et al. 2015).

The citrus red spider mite, *Panonychus citri* McGregor (Acari: Tetranychidae), is one of the most important pests of citrus in most parts of the world. Its original habitat is not exactly clear, but Southeast Asia is postulated (Bedford et al. 1998). It is reported as a first-class citrus pest from China, Japan, Taiwan, Italy, Spain, Brazil, and South Africa, and is considered to be the key pest of citrus in Northern Iran, necessitating the control of this pest. The citrus red mite has 15 to 19 generations per year in Mazandaran, Iran (Damavandian 2012).

The European red mite, *Panonychus ulmi* Koch (Acari: Tetranychidae), is one of the main pests in apple, pear, citrus, and quince orchards as well as some other fruit trees and ornamental plants worldwide (Botha and Learmonth 2005; Khodabandehloo and Asgari 2016).

Adult mites and immature stages of the listed species feed on plant nutrients and in large populations can damage the citrus sap of citrus trees. Damage of these mites reduces the quantity and quality of the product (Brandenburg and Kennedy 1987; Baniameri 2008).

The predator beetle- *Stethorus gilvifrons* Mulsant (Coleoptera: Coccinellidae), is one of the two recorded Iranian *Stethorus* species (Modares Awal 2001; Mossadegh and Kocheili 2003) that successfully control various spider mites (Obrycki and Kring 1998).

Organic insecticides or naturally occurring pesticides containing herbal compounds (such as garlic extract, pepper, citrus peel, eucalyptus, etc.), inorganic compounds (sulphur, kaolin, calcium, potassium, copper, iron, etc.) as well as soaps and edible oils, are today the most widely used pesticides in organic farming (Kabiri Raeis Abad and Amiri Besheli 2014). Unlike synthetic chemical toxins, they often act selectively and are harmless to humans and other non-target organisms and do not contaminate the environment. Their application to biological and plant pathogens is far less costly and complex. They can be easily decomposed in nature and do not have any toxic effects, such that after a short period of time, they can be consumed with oral food (Danay-Tous et al. 2014). Due to their mild toxic effects, beneficial insects may not be affected after coming in contact with the sprayed plants. Unlike synthetic chemical toxins, their effects on the pests do not cause resistance (Damavandian and Asghari Jafarabadi 2007). Therefore, the purpose of this study was to investigate the effect of the combination of three plant insecticides Palizin (Coconut oil), Tondexir (Garlic and Pepper Extract), and Dayabon (Castor oil) at various concentrations with synergists such as Citrate oil, Soybean oil, Citrus peel extract, and soaps (non-ionic and orthophosphoric surfactants). Another objective was to compare the fate of these compounds with other chemical agents at different times.

Materials and methods

Preparation of mite's colony and rearing

Citrus red spider mite, *Panonychus citri*, two-spotted spider mite, *Tetranychus urticae*, and European red mite, *Panonychus ulmi*, were reared to establish their colonies. For the rearing of citrus red mite, 2-year-old orange seedlings (Thomson-Novel variety) were used. Thompson Novel orange seedlings planted in plastic pots and kept at 25 ± 2°C, 65 ± 5% relative humidity, and 16D:8L h photoperiods were used as the host for the citrus red spider mite. The primary colony of citrus red spider mites was collected from citrus orchards infested with these pests in Sari, Iran and transferred to the laboratory. To obtain a similar population of pest, adult mites were placed on the leaves of citrus, and after 24 h, adult mites were removed from the leaves. Plant leaves infested with mite eggs were placed in the germinator. Twelve days later, the emerged adult mites were used for bioassay tests.

The primary colony of two-spotted spider mite was collected from the strawberry fields infested with the pest in the

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Bahnemir city of Babolsar, Mazandaran, Iran and transferred to the laboratory. To obtain an adult population, adult mites were placed on leaves of strawberry (Camaraso cultivar) (3 weeks after leaf germination) and after 24 h, adult mites were separated from the leaves. Plants infested with mite eggs were placed inside the germinator. Twelve days after the emergence of adult mites, they were used for bioassay tests. The effect of these plant compounds was also investigated on the mites' eggs, simultaneously.

The primary colony of European red mites was collected from apple orchards infested with these pests in the city of Sari, Iran and transferred to the laboratory. To obtain a similar population of pest, adult mites were placed on the leaves of the apple seedlings (red apple cultivar Red DeLish), and after 24 h, adult mites were removed from the leaves. Plants infested with mite eggs were placed in the rearing chambers. 8 days later, emerged adult mites were used for bioassay tests.

Rearing of the predator ladybird Stethorus gilvifrons

The primary colony of the predator ladybird was collected from citrus gardens and strawberry fields of Mazandaran province of Iran; once identified and confirmed, these population were reared. For the purpose of rearing, wooden cages of 200 × 200 × 200 cm (height, length, and width) were used. The cage was covered with a fabric net, but the roof was glazed to allow light to pass lightly. Strawberry bushes were infested with *T. urticae* mite for rearing of the ladybirds. To maintain the colonies of the ladybird and to avoid cannibalism between different stages, each week, 5–6 new bushes infested with *T. urticae* mite were placed in the cage. Strawberry bushes were planted in nylon pots of 18 × 20 cm in diameter with 50:50 peat moss and clay soil. The test conditions were at 25 ± 2°C, 65 ± 5% relative humidity, and 16D:8L h photoperiods.

Pesticides compounds

In this study, 21 different plant-derived and chemical treatments were used according to Table 1.

Bioassay tests

In order to investigate the effect of treatments on the adult and egg stages of the citrus red mite, cages were made in the same way as cages for ladybird. For this purpose, the plants were grown in pots and pots were placed in the cages and in each one, leaves of 10 two-year seedlings were infested with the citrus red mite. Mites were collected from the established colonies in the lab to be used in the bioassay. Then, 2 ml of the desired herbal compound was sprayed separately on each tree leaves with hand sprayer. After spraying, 20 leaves were randomly selected from each seedling and observed to be completely moistened on the leaves. The experiments on the adult and egg stages of the European red mite and the two-spotted spider mite were the same as above experiment, the difference was that the infested seedlings of apples and strawberry bushes were used. Also, experiments on larvae and adult insects of ladybirds were performed on strawberry bushes, separately.

All the experiments were replicated thrice. The number of live and dead mites was counted 24, 48, and 72 h after treatment and the mortality rate was evaluated. The leaves were not cut for counting from seedlings but were counted at each time by a pocket binocular at magnification of ×60. For each leaf count, 40 leaves of each seedlings was randomly selected and live and dead mites were counted on the back and on it. Mites that were unable to move their legs against hot needles were considered as dead insects. The test method for eggs was the same as adult tests, but the time to calculate the mortality rate of eggs was 15 days after treatment so that an egg which was not hatched was considered dead.

Statistical analysis

$$\text{Corrected}(\%) = \left(1 - \frac{\text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}} \times 100\right)$$

Where: n = No. of insect or mite density, T = Treated and Co = Control

The data were subjected to a two-way analysis of variance (ANOVA) and the means were compared by Tukey Multiple Range Test at 0.05 levels, using SPSS version 24 programmes (SPSS 2006). Before statistical analysis, mortality data were normalized to Arcsin√x for the stabilization of variance (Snedecor and Cochran 1989).

Results

The results of bioassay experiments on the effects of different compounds on the adult and egg stages of *P. citri, T. urticae*, and *P. ulmi* are listed in Tables 2–4. The results of the data analysis of the adult stage of citrus red spider mite revealed a significant difference between the different treatments at 24 h (df = 19;

Table 1. Different plant-derived and chemical treatments were used in the experiments.

Treatment	Chemical name	Ingredient or formulation	Applied concentration
1	Dishwashing liquid (Ave)	Sodium hydroxide	10 ml/L
2	Dayabon	Castor oil	5 ml/L
3	Dayabon	Castor oil	6 ml/L
4	Dayabon	Castor oil	7 ml/L
5	Dayabon	Castor oil	8 ml/L
6	Dayabon	Castor oil	9 ml/L
7	Dayabon	Castor oil	10 ml/L
8	Palizin	Coconut oil	1.5 ml/L
9	Palizin	Coconut oil	2 ml/L
10	Palizin	Coconut oil	2.5 ml/L
11	Palizin+Citrol oil	Coconut oil +Soybean oil and citrus peel extract	1.5 ml/L + 5 ml/L
12	Palizin+Citrol oil	Coconut oil +Soybean oil and citrus peel extract	2 ml/L + 5 ml/L
13	Palizin+Citrol oil	Coconut oil +Soybean oil and citrus peel extract	2.5 ml/L + 5 ml/L
14	Tondexir+Bartar wetting agent	Red pepper extract+ Non-ionic surfactants ortho-phosphoric acid	2 ml/L + 1 ml/L
15	Tondexir+Bartar wetting agent	Red pepper extract+ Non-ionic surfactants ortho-phosphoric acid	3 ml/L + 1 ml/L
16	Spirodiclofen	Envidor EC 24% (tetronic acid derivative)	0.5 ml/L
17	Spiromesifen	Oberon SC 24% (tetronic acid derivative)	0.5 ml/L
18	Pyridaben	Sanmite WP 20% (a pyridazinone derivative) (Organochlorine insecticide)	0.5 mg/L
19	Chlorpyrifos-ethyl	Dursban EC 40.8% (organophosphate insecticide)	2 ml/L
20	Abamectin	Vertimec EC 1.8% (derived from soil bacterium Streptomyces avermitilis)	0.5 ml/L
21	Control	water	-

F = 327.473; *P* < 0.001), 48 h (df = 19; F = 221.122; *P* < 0.001), and 72 h (df = 19; F = 349.133; *P* < 0.001) following the treatments. Based on these results, the treatment (Tondexir 3 ml/L + Bartar wetting agent 1 ml/L) had the highest observed mortality rate on 24, 48, and 72 hours, which was 82.16, 89.92, and 98.13%, respectively. Among different Dayabon treatments, 10 ml/L treatment had the highest mortality rate at different times, whereas at 72 hours after treatment, ~ 80.17% of the adult mites were dead. In the dishwashing liquid treatment, 10 ml/L, with lengthening the time from 24 to 72 h, the mortality rate increased from 25 to 43.32% (Table 2).

According to the results of analysis of variance, the data obtained from bioassay of the two-spotted spider mite indicated a significant difference between all treatments at tested times for 24 h (df = 19; F = 141.710; *P* < 0.001), 48 h (df = 19; F = 265.227; *P* < 0.001), and 72 h (df = 19; F = 181.292; *P* < 0.001) post-treatment. The results of the comparison suggested that the treatments, Tondexir 2 and 3 ml/L + Bartar wetting agent 1 ml/L and also Palizin 2.5 ml/L + Citrol oil 5 ml/L at 72 h after the treatment had the highest mortality rates of 84.40, 82.39, and 75.83%, respectively on the two-spotted spider mites. As with citrus red spider mites, among different treatments of Dayabon, 10 ml/L had the maximum mortality rate on the two-spotted spider mites, such that the mortality was 71.90% at 72 h post-treatment. For Spirodiclofen treatment, the mortality rates were 2.64, 14.41, and 60.16% at 24, 48, and 72 h, respectively (Table 3)

According to the analysis of variance of the data obtained from the European red mite, a significant difference was seen between all treatments at the tested times for 24 h (df = 19; F = 186.073; *P* < 0.001), 48 h (df = 19; F = 152.003; *P* < 0.001), and 72 h (df = 19; F = 219.205; *P* < 0.001). Based on the results, the treatment (Tondexir 3 ml/L + Bartar wetting agent 1 ml/L) had the highest mortality rates at 24, 48 and 72 h which were 57.91, 68.87, and 80.62%, respectively. Other treatments including Pyridaben, Abamectin, Spirodiclofen, and Spirodiclofen demonstrated 52.00, 45.45, and 64.68% mortality at 24, 48, and 72 h, respectively (Table 4)

The results of the comparison of the average mortality rate in Table 5 suggest that there is a significant difference between the treatments on the citrus red spider mite eggs 15 days after treatment (df = 19; F = 393.004; *P* < 0.001)). The highest mortality rate (100%) was observed in treatments Tondexir 2 and 3 ml/L + Bartar wetting agent 1 ml/L, Palizin 2.5 ml/L + Citrol oil 5 ml/L, Pyridaben 0.5 mg/L and Spiromesifen 0.5 ml/L, with no significant difference

Table 2. Mean (±SE) mortality of the citrus red mite, Panonychus citri treated with different chemical and botanical insecticides at different time intervals.

Treatment	Mean (±SE) mortality at different time intervals		
	24 h	48 h	72 h
Dishwashing liquid (Ave) 10 ml/L	1.70% ^{e} ± 25.0	2.57% ^{g} ± 36.42	2.62% ^{h} ± 43.32
Dayabon 5 ml/L	1.49% ^{d} ± 33.19	1.65% ^{f} ± 49.23	1.52% ^{g} ± 54.58
Dayabon 6 ml/L	1.66% ^{c} ± 44.35	2.57% ^{f} ± 52.50	1.47% ^{f} ± 63.20
Dayabon 7 ml/L	1.64% ^{c} ± 44.42	1.66% ^{ef} ± 55.72	2.53% ^{f} ± 64.05
Dayabon 8 ml/L	1.90% ^{bc} ± 50.15	1.99% ^{de} ± 64.68	1.78% ^{ef} ± 67.53
Dayabon 9 ml/L	0.62% ^{b} ± 57.18	1.92% ^{d} ± 66.33	1.41% ^{de} ± 74.44
Dayabon 10 ml/L	1.59% ^{b} ± 56.04	0.76% ^{cd} ± 71.49	1.30% ^{cd} ± 80.17
Palizin 1.5 ml/L	1.30% ^{fg} ± 10.41	2.13% ^{h} ± 13.92	0.55% ^{ij} ± 17.89
Palizin 2 ml/L	1.68% ^{f} ± 14.12	1.85% ^{h} ± 18.64	2.60% ^{i} ± 25.94
Palizin 2.5 ml/L	1.85% ^{de} ± 31.03	2.36% ^{g} ± 31.30	1.47% ^{h} ± 37.49
Palizin 1.5 ml/L + Citrol oil 5 ml/L	0.81% ^{de} ± 28.10	1.02$^{\%}$ ^{g} ± 32.91	2.12% ^{h} ± 41.64
Palizin 2 ml/L + Citrol oil 5 ml/L	0.50% ^{c} ± 43.41	1.70% ^{f} ± 51.77	0.32% ^{fg} ± 60.32
Palizin 2.5 ml/L + Citrol oil 5 ml/L	1.92% ^{c} ± 48.54	1.86% ^{bc} ± 77.84	0.96% ^{bc} ± 87.51
Tondexir 2 ml/L + Bar-Tar wetting agent 1 ml/L	1.37% ^{a} ± 80.92	1.43% ^{ab} ± 86.41	1.55% ^{ab} ± 91.42
Tondexir 3 ml/L + Bar-Tar wetting agent 1 ml/L	0.50% ^{a} ± 82.16	1.27% ^{a} ± 89.92	1.07% ^{a} ± 98.13
Spirodiclofen 0.5 ml/L	0.41% ^{h} ± 2.46	0.96% ^{h} ± 12.93	1.48% ^{de} ± 75.21
Spiromesifen 0.5 ml/L	0.73% ^{gh} ± 3.90	0.71% ^{i} ± 12.56	1.84% ^{h} ± 35.86
Pyridaben 0.5 mg/L	1.49% ^{c} ± 48.45	2.0% ^{g} ± 35.25	0.65% ^{jk} ± 41.49
Chlorpyrifos-ethyl 2 ml/L	0.14% ^{f} ± 13.10	1.87% ^{g} ± 32.82	1.15% ^{k} ± 38.34
Abamectin 0.5 ml/L	1.20% ^{f} ± 16.51	1.78% ^{g} ± 17.32	0.73% ^{j} ± 38.32

*Means were compared by Tukey's multiple range test at 0.05 level. The similar letters indicate no significant difference.

Table 3. Mean (±SE) mortality of the two-spotted spider mite, Tetranychus urticae treated with different chemical and botanical insecticides at different time intervals.

Treatment	Mean (±SE) mortality at different time intervals		
	24 h	48 h	72 h
Dishwashing liquid (Ave) 10 ml/L	1.61% ^{cdef} ± 42.95	0.96% ^{efg} ± 51.38	2.12% ^{efg} ± 54.91
Dayabon 5 ml/L	2.74% ^{ghi} ± 31.84	1.13% ^{g} ± 45.32	1.78% ^{gh} ± 49.96
Dayabon 6 ml/L	0.14% ^{fgh} ± 36.83	0.47% ^{fg} ± 47.09	0.58% ^{fgh} ± 50.85
Dayabon 7 ml/L	3.83% ^{ghi} ± 32.03	2.57% ^{ef} ± 53.19	1.77% ^{cd} ± 64.71
Dayabon 8 ml/L	2.59% ^{def} ± 41.76	0.83% ^{de} ± 55.49	2.15% ^{bcd} ± 67.08
Dayabon 9 ml/L	1.26% ^{cde} ± 48.19	0.65% ^{cd} ± 62.06	0.86% ^{cde} ± 63.47
Dayabon 10 ml/L	1.92% ^{cd} ± 50.98	0.70% ^{bc} ± 67.13	0.64% ^{bc} ± 71.90
Palizin 1.5 ml/L	0.71% ^{m} ± 5.64	1.03% ^{k} ± 12.25	0.69% ^{k} ± 14.35
Palizin 2 ml/L	1.64% ^{lm} ± 11.24	1.67% ^{jk} ± 18.69	1.54% ^{j} ± 26.12
Palizin 2.5 ml/L	1.55% ^{jk} ± 21.34	0.97% ^{ij} ± 25.90	0.31% ^{ij} ± 33.98
Palizin 1.5 ml/L + Citrol oil 5 ml/L	1.44% ^{hij} ± 29.32	1.84$^{\%}$ ^{h} ± 35.59	1.11% ^{hi} ± 41.78
Palizin 2 ml/L + Citrol oil 5 ml/L	1.11% ^{efg} ± 40.21	0.52% ^{efg} ± 47.97	1.20% ^{defg} ± 59.51
Palizin 2.5 ml/L + Citrol oil 5 ml/L	2.27% ^{a} ± 51.95	3.22% ^{b} ± 69.98	1.21% ^{ab} ± 75.83
Tondexir 2 ml/L + Bar-Tar wetting agent 1 ml/L	2.65% ^{b} ± 62.82	0.76% ^{b} ± 72.73	2.51% ^{a} ± 82.39
Tondexir 3 ml/L + Bar-Tar wetting agent 1 ml/L	1.17% ^{a} ± 74.01	0.77% ^{a} ± 80.71	0.97% ^{a} ± 84.40
Spirodiclofen 0.5 ml/L	0.29% ^{m} ± 2.64	1.44% ^{k} ± 14.41	4.11% ^{def} ± 60.16
Spiromesifen 0.5 ml/L	0.61% ^{m} ± 3.39	0.74% ^{l} ± 3.79	4.10% ^{j} ± 29.40
Pyridaben 0.5 mg/L	1.91% ^{b} ± 63.78	1.20% ^{fg} ± 47.10	0.67% ^{j} ± 51.10
Chlorpyrifos-ethyl 2 ml/L	1.26% ^{ijk} ± 23.09	1.25$^{\%}$ ^{i} ± 27.86	1.0% ^{k} ± 33.70
Abamectin 0.5 ml/L	1.52% ^{k} ± 18.79	2.31% ^{hi} ± 32.27	1.25% ^{k} ± 44.08

*Means were compared by Tukey's multiple range test at 0.05 level. The similar letters indicate no significant difference.

Table 4. Mean (±SE) mortality of the European red mite, Panonychus ulmi treated with different chemical and botanical insecticides at different time intervals.

	Mean (±SE) mortality at different time intervals		
Treatment	24 h	48 h	72 h
Dishwashing liquid (Ave) 10 ml/L	2.76% ^{d} ± 39.33	1.12% ^{de} ± 45.31	1.59% ^{de} ± 48.44
Dayabon 5 ml/L	1.26% ^{gh} ± 18.10	3.24% ^{fg} ± 35.24	0.44% ^{f} ± 42.44
Dayabon 6 ml/L	1.72% ^{fgh} ± 22.39	0.93% ^{fg} ± 26.98	1.12% ^{f} ± 32.65
Dayabon 7 ml/L	0.69% ^{ghi} ± 18.54	1.99% ^{fg} ± 23.71	1.77% ^{fg} ± 25.38
Dayabon 8 ml/L	1.30% ^{d} ± 39.33	1.16% ^{e} ± 41.18	0.59% ^{d} ± 50.51
Dayabon 9 ml/L	1.11% ^{cd} ± 44.22	0.52% ^{cd} ± 51.93	1.65% ^{bc} ± 60.78
Dayabon 10 ml/L	1.73% ^{bc} ± 48.63	2.45% ^{bc} ± 57.10	1.41% ^{b} ± 67.08
Palizin 1.5 ml/L	0.43% ^{j} ± 6.36	1.36% ^{ij} ± 7.82	0.93% ^{j} ± 10.83
Palizin 2 ml/L	0.64% ^{ij} ± 10.45	0.55% ^{hi} ± 14.27	2.52% ^{gh} ± 20.69
Palizin 2.5 ml/L	0.74% ^{hi} ± 16.02	2.43% ^{gh} ± 20.63	3.57% ^{fg} ± 27.10
Palizin 1.5 ml/L + Citrol oil 5 ml/L	0.78% ^{efg} ± 24.82	1.16$^{\%}$ ^{f} ± 30.79	0.59% ^{e} ± 41.52
Palizin 2 ml/L + Citrol oil 5 ml/L	1.80% ^{e} ± 29.97	3.10% ^{e} ± 42.24	0.22% ^{cd} ± 53.79
Palizin 2.5 ml/L + Citrol oil 5 ml/L	2.10% ^{d} ± 38.65	1.48% ^{bc} ± 56.66	1.33% ^{b} ± 63.98
Tondexir 2 ml/L + Bar-Tar wetting agent 1 ml/L	1.28% ^{a} ± 56.27	0.90% ^{ab} ± 60.86	0.84% ^{a} ± 77.57
Tondexir 3 ml/L + Bar-Tar wetting agent 1 ml/L	1.68% ^{a} ± 57.91	0.98% ^{a} ± 68.87	1.32% ^{a} ± 80.62
Spirodiclofen 0.5 ml/L	0.57% ^{j} ± 4.07	0.69% ^{j} ± 5.16	2.78% ^{b} ± 64.68
Spiromesifen 0.5 ml/L	0.33% ^{j} ± 4.14	0.53% ^{j} ± 5.76	0.78% ^{f} ± 32.57
Pyridaben 0.5 mg/L	0.68% ^{cd} ± 41.52	1.68% ^{e} ± 42.53	1.20% ^{hi} ± 44.0
Chlorpyrifos-ethyl 2 ml/L	1.34% ^{fgh} ± 18.36	0.71$^{\%}$ ^{f} ± 31.28	2.01% ^{hi} ± 35.07
Abamectin 0.5 ml/L	1.0% ^{ef} ± 25.41	2.10% ^{de} ± 45.45	0.55% ^{ghi} ± 49.21

*Means were compared by Tukey's multiple range test at 0.05 level. The similar letters indicate no significant difference.

Table 5. Mean (±SE) mortality percentage of the citrus red mite Panonychus citri, the twospotted spider mite, Tetranychus urticae, and European red mite Panonychus ulmi eggs treated with different chemical and botanical insecticides at 15 days post-treatment.

	Mean (±SE) mortality at different time intervals		
Treatment	*P. citri*	*T. urticae*	*P. ulmi*
Dishwashing liquid (Ave) 10 ml/L	1.69% ^{fg} ± 40.98	1.47% ^{e} ± 53.78	0.61% ^{def} ± 55.07
Dayabon 5 ml/L	1.92% ^{hi} ± 31.88	1.26% ^{g} ± 32.49	1.85% ^{ij} ± 33.25
Dayabon 6 ml/L	1.82% ^{fgh} ± 38.58	0.93% ^{fg} ± 38.85	1.17% ^{efg} ± 49.24
Dayabon 7 ml/L	2.05% ^{f} ± 44.10	1.07% ^{e} ± 52.20	0.14% ^{de} ± 57.07
Dayabon 8 ml/L	0.80% ^{de} ± 62.34	1.84% ^{cd} ± 67.51	1.44% ^{c} ± 73.03
Dayabon 9 ml/L	0.76% ^{cde} ± 64.23	1.43% ^{bcd} ± 71.62	1.86% ^{b} ± 80.83
Dayabon 10 ml/L	0.62% ^{b} ± 81.62	1.93% ^{b} ± 89.68	2.27% ^{a} ± 92.88
Palizin 1.5 ml/L	0.93% ^{j} ± 15.82	1.02% ^{fg} ± 36.86	2.56% ^{j} ± 36.91
Palizin 2 ml/L	2.62% ^{i} ± 24.88	0.34% ^{ef} ± 46.69	0.84% ^{de} ± 57.46
Palizin 2.5 ml/L	1.31% ^{gh} ± 35.47	1.60% ^{d} ± 65.40	1.63% ^{d} ± 66.11
Palizin 1.5 ml/L + Citrol oil 5 ml/L	1.10% ^{fg} ± 41.82	5.91% ^{bc} ± 77	3.61% ^{b} ± 86.48
Palizin 2 ml/L + Citrol oil 5 ml/L	1.36% ^{c} ± 71.80	0.0% ^{a} ± 100	0.0% ^{a} ± 100
Palizin 2.5 ml/L + Citrol oil 5 ml/L	0.0% ^{a} ± 100	0.0% ^{a} ± 100	0.0% ^{a} ± 100
Tondexir 2 ml/L + Bar-Tar wetting agent 1 ml/L	0.0% ^{a} ± 100	0.0% ^{a} ± 100	0.0% ^{a} ± 100
Tondexir 3 ml/L + Bar-Tar wetting agent 1 ml/L	0.0% ^{a} ± 100	0.0% ^{a} ± 100	0.0% ^{a} ± 100
Spirodiclofen 0.5 ml/L	3.84% ^{a} ± 72.07	2.0% ^{b} ± 80.24	1.52% ^{a} ± 93.29
Spiromesifen 0.5 ml/L	0.0% ^{a} ± 100	0.0% ^{a} ± 100	0.0% ^{a} ± 100
Pyridaben 0.5 mg/L	0.0% ^{a} ± 100	0.0% ^{a} ± 100	0.0% ^{a} ± 100
Chlorpyrifos-ethyl 2 ml/L	2.64% ^{e} ± 27.92	4.42$^{\%}$ ^{fg} ± 37.75	2.0% ^{hi} ± 47.66
Abamectin 0.5 ml/L	1.22% ^{cd} ± 69.21	2.40% ^{bcd} ± 72.40	1.16% ^{c} ± 75.70

*Means were compared by Tukey's multiple range test at 0.05 level. The similar letters indicate no significant difference.

observed between them. The lowest mortality rate was in Palizin 1.5 and 2 ml/L treatments obtained as 15.82 and 24.88%, respectively. Spirodiclofen chemical insecticide treatment caused the death of 92.7% of eggs, categorized in same group. Also, the results of the comparison of the mean mortality occurring in bioassay tests of different treatments on the two-spotted spider mites revealed that there was a significant difference between treatments at 15 days after the treatment (df = 19; F = 200.420; $P < 0.001$). The highest mortality rate (100%) was observed in treatments Tondexir 2 and 3 ml/L + Bartar wetting agent 1 ml/L, Palizin 2 and 2.5 ml/L + Citrol oil 5 ml/L, as well as Spiromesifen and Pyridaben with no significant difference observed between them (Table 5).

In the combination treatments palizin 1.5, 2, and 2.5 ml/L, palizin 1.5, 2, and 2.5 ml/L + citrol oil 5 ml/L, the results indicated that when palizin was combined with citrol oil, its effect was potentiated. The concentrations of palizin poison, 1.5, 2, and 2.5 times per thousand, resulted in mortality rates of 36.86, 46.69, and 65.4%, respectively. However, the same concentrations when combined with citrol oil caused 77.71%, 100%, and 100% mortality (Table 5).

According to the information obtained from Table 5, the mortality rate between treatments on the European red mite eggs was significant on 15 days after treatment (df = 19; F = 339.372; $P < 0.001$). In this regard, the highest mortality rate (100%) was found in treatments Tondexir 3 mL/L + Bartar wetting agent 1 ml/L and Palizin 2.5 ml/L + Citrol oil 5 ml/L as well as Spirodiclufen and Pyridaben, all categorized in the first group.

According to the results of analysis of variance of data from bioassay experiments on different treatments on the predator ladybird larvae, *S. gilvifrons* at different times, there was a significant difference between treatments at 24, 48, and 72 h after the treatment, for 24 h (df = 19; F = 77.363; $P < 0.001$), 48 h (df = 19; F = 69.016; $P < 0.001$), and 72 h (df = 19; F = 108.729; $P < 0.001$). According to the results presented in Table 6, the lowest percentage of mortality in the ladybird larvae occurred 24 h after the treatment by Palizin 2 and 1.5 ml/L, which were equal to 4.30 and 5.08%, respectively (Table 6).

Based on the results of analysis of variance of data from bioassay of different treatments on *S. gilvifrons* adult insects at different times, a significant difference was observed between the treatments at 24, 48, and 72 h after the treatment. It was observed for 24 h (df = 19;

Table 6. Mean (±SE) mortality of the predator ladybird larvae, Stethorus gilvifrons treated with different chemical and botanical insecticides at different time intervals.

	Mean (±SE) mortality at different time intervals		
Treatment	24 h	48 h	72 h
Dishwashing liquid (Ave) 10 ml/L	4.51% ^{bcdef} ± 23.54	3.97% ^{cdef} ± 33.98	1.13% ^{de} ± 42.07
Dayabon 5 ml/L	0.56% ^{cdefgh} ± 15.49	4.40% ^{defghi} ± 22.83	2.02% ^{efg} ± 27.57
Dayabon 6 ml/L	2.20% ^{bcdef} ± 24.32	5.60% ^{cdefg} ± 28.04	2.77% ^{ef} ± 29.03
Dayabon 7 ml/L	5.92% ^{bcde} ± 25.73	0.63% ^{cdefg} ± 28	2.68% ^{efg} ± 29.92
Dayabon 8 ml/L	1.99% ^{bcd} ± 29.04	2.89% ^{cdef} ± 34.31	1.01% ^{efg} ± 37.31
Dayabon 9 ml/L	2.91% ^{bcd} ± 31.10	2.85% ^{cd} ± 38.98	2.73% ^{de} ± 42.11
Dayabon 10 ml/L	1.58% ^{b} ± 35.54	1.80% ^{c} ± 39.87	3.46% ^{de} ± 43.96
Palizin 1.5 ml/L	0.93% ^{gh} ± 5.08	1.83% ^{i} ± 8.19	1.05% ^{fg} ± 9.01
Palizin 2 ml/L	0.59% ^{h} ± 4.30	1.91% ^{hi} ± 11.35	1.90% ^{efg} ± 15.60
Palizin 2.5 ml/L	0.43% ^{fgh} ± 8.41	1.32% ^{ghi} ± 14.69	3.82% ^{efg} ± 16.51
Palizin 1.5 ml/L + Citrol oil 5 ml/L	1.13% ^{defgh} ± 14.56	1.44$^{\%\ fghi}$ ± 18.49	0.66% ^{ef} ± 19.01
Palizin 2 ml/L + Citrol oil 5 ml/L	5.35% ^{bcdef} ± 24.54	2.40% ^{cdefg} ± 30.46	3.71% ^{de} ± 36.82
Palizin 2.5 ml/L + Citrol oil 5 ml/L	4.75% ^{bc} ± 32.49	3.90% ^{c} ± 40.40	1.98% ^{de} ± 45.20
Tondexir 2 ml/L + Bar-Tar wetting agent 1 ml/L	3.88% ^{bcdefg} ± 21.80	5.04% ^{cde} ± 36.73	0.73% ^{de} ± 45.63
Tondexir 3 ml/L + Bar-Tar wetting agent 1 ml/L	5.69% ^{bcd} ± 31.62	2.62% ^{cde} ± 37.97	2.22% ^{de} ± 38.67
Spirodiclofen 0.5 ml/L	1.01% ^{gh} ± 5.33	2.85% ^{cdefgh} ± 24.69	2.86% ^{d} ± 47.78
Spiromesifen 0.5 ml/L	2.52% ^{efgh} ± 9.75	3.14% ^{efghi} ± 22.33	4.37% ^{c} ± 64.27
Pyridaben 0.5 mg/L	4.47% ^{bc} ± 34.08	4.64% ^{i} ± 63.55	4.49% ^{b} ± 85.41
Chlorpyrifos-ethyl 2 ml/L	0.0% ^{a} ± 100	0.0$^{\%\ a}$ ± 100	0.0% ^{a} ± 100
Abamectin 0.5 ml/L	3.58% ^{a} ± 93.27	0.0% ^{a} ± 100	0.0% ^{a} ± 100

*Means were compared by Tukey's multiple range test at 0.05 level. The similar letters indicate no significant difference.

F = 201.092; $P < 0.001$), 48 h (df = 19; F = 120.322; $P < 0.001$), and 72 hours (df = 19; F = 197.912; $P < 0.001$). According to the results presented in Table 7, the lowest percentage of the mortality rate of adult insects was observed at 24 h after the treatment, by Palizin 1.5 ml/L, which were equal to 4.36%. However, at time 72 h after the treatment, in addition Palizin 2 ml/L treatment also yielded 6.77% mortality, which was differentiated in the grouping of the other treatments in the group (Table 7).

Discussion

Knowledge of the use of selective insecticides for pests and their natural enemies is very important in integrated management planning, particularly when compounds are used as pest control tools (Sterk et al. 2003). Pesticides must be effective on pests, but relatively safe for natural enemies. Also, knowledge about the combination of natural enemies affecting key pests and the impacts of pesticides on these creatures is also very important (Campbell et al. 1991). Previous studies using the two treatments on the two-spotted spider mites revealed that the highest percentage of mortality (64%) was found in Dayabon 10 ml/L and Palizin 2.5 ml/L + Citrol oil 5 ml/L (Toorani and Abbasipour 2016). Also, in other experiments conducted using the mentioned treatments on the citrus red spider mites, the highest mortality rate was observed in the treatment of Tondexir 3 ml/L + Bartar wetting agent 1 ml/L (91.67%), followed by Tondexir 2 ml/L + Bartar wetting agent 1 ml/L (90.48%) (Toorani et al. 2016). Compared to the research performed by the researchers, in this experiment, treatments Dayabon 10 ml/L and Palizin 2.5 ml/L + Citrol oil 5 ml/L yielded 56.04 and 48.44% mortality after 24 h of treatment, respectively, on the two-spotted spider mites. Therefore, it can be concluded that the mortality rate of similar treatments in the present study was less than that of Toorani and Abbasipour's study (2016). The reason for this discrepancy is the difference in the test conditions in the two comparative studies. For example, the mites tested in their research were collected directly from citrus orchards and strawberry farms, to which the treatments were applied. However, in the present study, the mites were reared in a laboratory for generations and then tested. This suggests that the pest's surrounding environment especially temperature (Katey et al. 2018) as well as the type of nutrition and host (Nauen et al. 2011; Schmehl et al. 2014) can affect its resistance to pesticides.

Table 7. Mean (±SE) mortality of the predator ladybird adults, Stethorus gilvifrons, treated with different chemical and botanical insecticides at different time intervals.

	Mean (±SE) mortality at different time intervals		
Treatment	24 h	48 h	72 h
Dishwashing liquid (Ave) 10 ml/L	0.46% ^{efg} ± 9.84	1.38% ^{efgh} ± 25.33	1.09% ^{defg} ± 33.38
Dayabon 5 ml/L	1.89% ^{efg} ± 10.90	1.86% ^{hijk} ± 15.96	1.08% ^{fg} ± 16.56
Dayabon 6 ml/L	1.88% ^{def} ± 15.92	2.33% ^{fghij} ± 22.68	1.39% ^{defg} ± 32.99
Dayabon 7 ml/L	2.85% ^{cde} ± 18.39	2.36% ^{ghij} ± 20.18	1.73% ^{efg} ± 27.93
Dayabon 8 ml/L	2.39% ^{cd} ± 22.90	1.83% ^{def} ± 32.95	2.66% ^{de} ± 38.08
Dayabon 9 ml/L	2.18% ^{cd} ± 22.14	3.49% ^{defg} ± 31.58	3.0% ^{d} ± 32.30
Dayabon 10 ml/L	1.73% ^{cd} ± 21.96	3.18% ^{cd} ± 37.68	2.88% ^{de} ± 38.64
Palizin 1.5 ml/L	0.42% ^{g} ± 4.36	1.02% ^{k} ± 6.78	0.45% ^{g} ± 8.37
Palizin 2 ml/L	0.90% ^{g} ± 5.14	0.85% ^{k} ± 6.12	0.59% ^{h} ± 6.77
Palizin 2.5 ml/L	0.26% ^{g} ± 4.75	0.80% ^{jk}±12.57	0.37% ^{fg} ± 16.12
Palizin 1.5 ml/L + Citrol oil 5 ml/L	0.67% ^{fg} ± 7.62	1.22$^{\%\ ijk}$ ± 13.31	0.74% ^{fg} ± 16.39
Palizin 2 ml/L + Citrol oil 5 ml/L	1.83% ^{cde} ± 18.26	1.40% ^{fghij} ± 23.11	1.76% ^{de} ± 29.29
Palizin 2.5 ml/L + Citrol oil 5 ml/L	1.81% ^{c} ± 25.60	2.09% ^{efghi} ± 26.46	1.87% ^{de} ± 28.14
Tondexir 2 ml/L + Bar-Tar wetting agent 1 ml/L	2.12% ^{def} ± 15.50	2.31% ^{efghij} ± 23.58	2.59% ^{de} ± 31.40
Tondexir 3 ml/L + Bar-Tar wetting agent 1 ml/L	2.57% ^{c} ± 26.98	1.95% ^{de} ± 34.92	1.94% ^{cd} ± 46.13
Spirodiclofen 0.5 ml/L	0.60% ^{g} ± 5.71	0.62% ^{hijk} ± 15.94	2.76% ^{de} ± 32.87
Spiromesifen 0.5 ml/L	0.50% ^{g} ± 5.36	1.32% ^{ijk} ± 13.58	4.17% ^{b} ± 68.09
Pyridaben 0.5 mg/L	3.05% ^{b} ± 39.21	2.51% ^{i} ± 47.83	3.73% ^{c} ± 64.21
Chlorpyrifos-ethyl 2 ml/L	0.0% ^{a} ± 100	0.0$^{\%\ a}$ ± 100	0.0% ^{a} ± 100
Abamectin 0.5 ml/L	2.56% ^{b} ± 68.91	5.16% ^{b} ± 82.41	0.0% ^{a} ± 100

*Means were compared by Tukey's multiple range test at 0.05 level. The similar letters indicate no significant difference.

To corroborate this theory, the effects of Tondexir (pepper extract) in the present study on the mites showed that this toxin had less of an impact on the two-spotted spider mite than on the citrus red spider mite. It could be due to the fact that pepper is a plant host for *T. urticae* and so it has little resistance to pepper-based ingredients. Expectedly according to this theory, in the treatment of Citrol oil, the percentage of mortality of the red citrus spider mite was less than that of the other mites, but indeed it was not so, as the Citrol oil is a mixture of extracts of citrus and soybean oil. In particular, this product plays a synergistic role and intensifies the effect of palizin, a major cause of lethal toxicity of mites. Possibly, if the Citrol oil was used alone as a treatment against mites, according to the abovementioned theory, it would have a greater effect on the two-spotted spider mite than on the citrus red spider mite.

In this study, in contrast to other investigations, in experiments performed on citrus red spider mite, the mortality rate in the treatment of Tondexir 3 ml/L + Bartar wetting agent 1 ml/L was 74.01%, while in the Tondexir 2 ml/L + Bartar wetting agent 1 ml/L it was 62.82%. The results of the comparison of the mortality rate of larvae of the citrus leafminer, *Phyllocnistis citrella* Stainton showed that 24 h after treatments with Tondexir, Sirinol, Palizin, 2000 ppm along with 5000 ppm of mineral oil, and 750 ppm of Spinosad caused 31, 75, 35, 75, and 76% mortality, respectively (Amiri-Beheshti 2009). In the present study, Tondexir 2 ml/L + Bartar wetting agent 1 ml/L and Palizin 2 ml/L + Citrol oil 5 ml/L caused 62.82 and 40.21% mortality on the two-spotted spider mites, respectively. The values were 80.92 and 43.41% on the citrus red spider mite and 56.27% and 29.97% on the European red mites, respectively, indicating that the mites used in the present research exhibited far less resistance than citrus leafminer larvae in Amiri-Beheshti (2009). The reason for this is the mode of action of the toxins since the Palazin and Tondexir agents are contact insecticides. On the other hand, in the experiment of larvae of the citrus leafminer, the larvae were left in the plant tissue and were relatively protected by leaf tissue to reduce the contact of tested toxins with the larval body, and thus a lower mortality rate was observed. In the same study, Spinosad insecticide caused 76% mortality of the larvae of citrus leafminer, as this toxin also affects the digestive tract causing paralysis of the insect. This insecticide acts by activating nicotinic acetylcholine receptors in the insect's nervous system.

The study of the effect of herbal compounds of coconut oil (Palazin), garlic oil (Sirinol) extract, and red pepper extract (Tondexir) with a concentration of 2,000 ppm in pomegranate orchards of Iran indicated that the use of the above compounds reduced the population of the biological stages of the pomegranate false spider mite, *Tenuipalpus punicae*, Pritchard, and Baker, by 85, 80, and 85%, respectively (Farazmand 2012). Based on studies conducted in vitro, the LC_{50} values of the chilli pepper, *Capsicum annum*, and garlic extracts for the two-spotted spider mite, *T. urticae* were 4.448 and 473.11 ppm, respectively. The results of this study revealed that the pepper extract had a greater effect on the two-spotted spider mite (Kazem and El-Shereifi 2010). Seifi et al. (2015) studied the contact toxicity effect of the new insecticide, Dayabon, on the immature and adult stages of the two-spotted spider mites at concentrations of 5000 and 8000 ppm, with the mortality counted 24 h after the treatment. The percentage of mortality at the concentration of 5000 ppm on the immature and adult stages was 50 and 62.5%, respectively; at 8000 ppm, the values were 66.81 and 66.80%, respectively, which had a significant difference with the control. The ovicidal effect of this pesticide at a concentration of 8000 ppm was studied on one, two, and three-day-old eggs for 10 days. The results revealed a significant difference with the control, where 49.7% of the eggs did not hatch. The experiment also showed that there was no significant difference between egg ages and egg mortality percentages (Seifi et al. 2015). The results of these studies suggested that, as in the present study, Palizin and Tondexir (garlic extract and pepper extract) have a high efficiency in reducing the mite populations and lead to a high percentage of deaths, as the impurities of the pest's body with these compounds cause problems in gaseous metabolism exchange, body skin damage, and eventually mortality. In the present study, concentration of 8 ml/L of Dayabon on the citrus red spider mite, two-spotted spider mite, and European red mites caused mortality rates of 62.33, 67.51, and 67.33%, respectively on the mite eggs, which were similar to the results of the Seifi et al. (2015) study.

New plant pesticides such as Dayabon, Palazin, and Tondexir had a very minor effect on the predator ladybird, *S. gilvifrons* in the present study. In previous studies, garlic extract (Sirinol) with a concentration of 2500 ppm had less of an impact in comparison with the Muspilan and Consalt insecticides on natural enemies of pistachio psyllid, *Agonoscena pistaciae* Burckhardt & Lauterer such as coccinellid predator, *Oenopia conglobate* (L.) and parasitic wasp, *Psyllaephaus pistaciae* Ferrière (Kabiri et al. 2012). In the present study, as stated above, the Palizin 1.5 and 2 ml/L, containing the coconut oil, was less effective than other chemical insecticides. With the lowest losses due to this treatment at 72 h after treatment, the mortality rate was 4.37 and 4.77%, respectively. On the other hand, the highest mortality rate with Chlorpyrifos-ethyl at 24 to 72 h, and Abamectin at 24 to 72 h after the treatment were 100%, and 82.41–100%, respectively.

Previous researchers suggested that the effect of herbal pesticides such as Palizin on the citrus black aphid, *Toxoptera aurantii* Boyer, caused mortality of 79.75, 95.59, and 96.39% of the pests at 24, 48, and 72 h after the treatment, respectively (Gholamzadeh-Chitgar and Pourmoradi 2017). Similar to the current study, the effect of plant pesticides grew over time from 24 h to 72 h, causing more losses in pests. The results of this study, in accordance with the results of Gholamzadeh-Chitgar and Pourmoradi (2017), revealed that the effect of plant-based toxins with a physical and contact effect including Dayabon, Palizin, and Tondexir, as well as dishwashing liquid, rises over time from 24 h to 72 h, with higher mortality rates observed in the tested pests (Tables 2–4). Nevertheless, in almost all cases, the lethal effect of these treatments was significantly reduced after 72 h, most likely due to the loss of the effect of these chemicals over time. It seems that the molecular structure of these toxins decomposes after 72 h in the test environment and its effect is reduced or deactivated. The results across all tables suggest that in Palizin, Dayabon, and Tondexir treatments, 72 h post-treatment was the deadliest time for the studied mites, and the highest concentration of used toxins was the deadliest concentration.

Previous studies, as in the present study, suggested that the botanical pesticides used in this research have a minor effect on the natural enemy. For example, the results of the research revealed that the highest and lowest mortality rates of the Mealybug Ladybird, *Cryptoloemus montrouzieri* Mulsant, were observed in chlorpyrifos-ethyl 2 ml/L (76.66%) and Dayabon 5 ml/L as well as Palizin 2 ml/L (3.33%) (Heydari et al. 2016a). Also, for the Vedalia ladybird, *Rodalia cardinalis* Mulsant, the highest mortality rate (83.33%) was found in the chlorpyrifos-ethyl 2 ml/L treatment, while the rest of the botanical treatments caused mortality rates below 10% (Heydari et al. 2016b). In addition to the mentioned cases, in the research conducted under field conditions with the same treatments, the highest and lowest mortality rates were observed for *C. montrouzieri* in the treatment of Chlorpyrifos-ethyl insecticide at the concentration of 2 ml/L (76.66%) and Dayabon 5 ml/L Palizin 2 ml/L (3.33%). Nevertheless, in other treatments, except for chlorpyrifos-ethyl treatment, no significant difference was observed. The highest and lowest mortality rates for the Mealybug Ladybird, *C. montrouzieri* larvae, were estimated in the chlorpyrifos-ethyl 2 ml/L (83.33%) and Dayabon 5 ml/L (3.33%) treatments, respectively (Toorani et al. 2017).

In conclusion, botanical insecticides used in this study killed different species of mites but were less toxic on the predatory beetle. Mortality of the mites could have resulted from the penetration of chemicals into the bodies of mites through contact or through other modes of action. They were soft on the ladybug predators, due to species-specific effect.

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No potential conflict of interest was reported by the author(s).

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