

Article

Effect of water-washing on control of citrus cottony scale, *Pulvinaria aurantii* Cockerell (Hemiptera: Coccidae) in field conditions

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Abstract

The citrus cottony scale, *Pulvinaria aurantii* Cockerell damages citrus trees in Mazandaran province every year. The study was conducted in two separate experiments in Miandorood city. The experimental design was completely randomized in both experiments and four treatments (control, two, three and four times of water-washing) and four treatments (control, Chlorpyrifos at a concentration of 2L/1000L, water-washing and mineral oil at a concentration of 1%) in 5 replications were considered in the first and second experiments, respectively. In first experiment, the lowest egg sac was obtained in the two times of water-washing with an average of 3.80 ± 0.37 eggs per 20 leaves and the lowest number of nymphs was observed in the four times of water-washing with an average of 247.80 ± 7.32 . In second experiment, the highest number of egg sacs in different dates was obtained for control with average of 30.60 and 21.20 egg sacs per five leaves and the highest number of egg sacs was obtained for water-washing with an average of 25.60. The highest number of live nymphs in all dates was obtained for control treatment. Also, the lowest number of nymphs in different dates were counted for Chlorpyrifos treatment with an average of 450.4, 351.0 and 156.0, respectively. The lowest number of nymphs was obtained for mineral oil treatment with an average of 9.4.

Keywords citrus cottony scale; water-washing; mineral oil; ovisac; nymph.

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1 Introduction

The citrus cottony scale (CCS), *Pulvinaria aurantii* Cockerell (Hemiptera: Coccidae) is a polyphagous insect that damages a wide range of fruit and ornamental (non-fruit) plants in addition to citrus trees (Helyer et al., 2003). Currently, this insect is the most important citrus pest in Mazandaran province (northern Iran), causing great damage to citrus orchards in this province every year (Rajabpour et al., 2008; Damavandian, 2014). The

CCS was first reported on citrus trees in Bandar-e Anzali and Rasht cities, then it spread to the east of Mazandaran province (Halaji Sani, 1999; Behdad, 2002).

In addition to sucking and reducing the sap of the plant, *P. aurantii* causes leaf and fruit fall and even the host plant drying in outbreak conditions (Halaji Sani, 1999). Also, this pest causes the growth of sooty mold (*Capnodium citri* Penz.) by secreting a large amount of honeydew, which reduces the marketability of the fruits by forming a black layer on citrus fruit, in addition to reducing the level of photosynthesis (Helyer et al., 2003; Damavandian, 2006). The CCS has two generations per year. The first generation (called the summer generation) appears from June to September, and the second generation (called the autumn generation) appears from September to June in next year (Halaji Sani, 1999).

On the one hand, the increase in the intensity of infestation and damage caused by this pest in a large part of the citrus orchards of Mazandaran province (more than 100,000 ha) has caused the widespread use of organophosphate and hormonal pesticides to control it (Damavandian, 2010). On the other hand, the repeated use of these pesticides has resulted in an outbreak of the CCS and increased the pest resistance and the destruction of the natural enemies of this pest, including predatory mites, the ladybug *Cryptolaemus montrouzieri* Mulsant, and the parasitoids of the Encyrtidae and Aphelinidae families (Damavandian, 2003; Damavandian, 2005; Maleki and Damavandian, 2015). It is also worth mentioning that repeated spraying in the north of Iran and environmental pollution as a result of that (Damavandian, 2007) is worrying for the health of the residents of this region. Therefore, the use of low-risk and environmentally friendly methods to control the citrus pests, especially the CCS is necessary.

During the past decades, the use of chemical insecticides, such as Chlorpyrifos (Dursban® EC 40.8%), Diazinon (EC 60%) and Buprofezin (Applaud® SC 40%) has been the main strategy for controlling *P. aurantii* in Mazandaran province (Damavandian, 2007). It should be noted that repeated and high-volume spraying by organophosphate compounds or newer insecticides will also cause *P. aurantii* outbreaks (Bedford et al., 1998). In addition to the outbreak of the pests, the emergence and increase of pest resistance to pesticides as a result of their repeated use is a very important problem at the global level (Roush and Tabashnik, 1990). The harmful effects of synthetic pesticides on humans, the environment, and non-target organisms, such as natural enemies and resurgence of pests, have made it necessary to identify alternative control strategies in integrated pest management programs (Maleki and Damavandian, 2015; Zhang, 2018, 2025). Mineral oils are an essential component of integrated pest management programs for agricultural crops worldwide because they do not have the harmful effects of synthetic pesticides (Helmy et al., 2012; Damavandian and Kiaeian Moosavi, 2014) and is considered as a valuable alternative to chemical pesticides for controlling important citrus pests in the orchards (Kiss et al., 2005; Kim et al., 2010). Although, Damavandian (2014) recommended only mineral oils to control the CCS in special conditions, but the use of chemical pesticides is still common in the region, which results in the high cost of pest control and environmental pollution. According to Davidson et al. (1991), mineral oils have an insecticidal effect on different developmental stages of the mealybugs, and scale insects, including eggs and different nymphal instars, and control them. On the one hand, the field studies show that the CCS can be controlled only by mineral oils and non-applying synthetic pesticides (Damavandian, 2010), and on the other hand, many natural enemies have been reported from citrus orchards that are active (Davies and Jackson, 2009; Jacas and Urbaneja, 2010) and their conservation has a special importance for controlling citrus pests, including *P. aurantii*.

There have been studies on the effect of water-washing on the control of various pests. Saeidi and Noorbakhsh (2010) investigated the effect of tree washing on the control of the almond spider mite, *Schizotetranychus smirnovi* Wainst and stated that washing trees with soap and water at a ratio of 1L/1000 L, caused more than 90% mortality in the population of this mite. Arbabi et al. (2010) by evaluating the effect of

water-washing in controlling the population of date palm spider mite, *Oligonychus afrasiaticus* McGregor in different regions of Iran reported that the highest efficacy was calculated for water-washing in a interval of seven days in Kerman region and the most stable control was recorded for 25 days in this region. Li et al. (2015) used hot water treatment to control storage pests of persimmon fruit. The results showed that the use of hot water treatments can reduce damage caused by the pests, as well as reduce cold damage. Norris et al. (2002) found that natural rainfall was effective in controlling thrips population. Drees (1997) stated that water-washing for 12 days was effective in controlling rose spider mite population. Drees (2004) reported that many pests, especially aphids, thrips, and various caterpillars, can be controlled using high-pressure water spray. Therefore, this study was aimed to evaluate the effect of water-washing and mineral oil on the control of CCS during the emergence of ovisacs and nymphs.

2 Materials and Methods

This research was conducted in two separate experiments.

2.1 Geographical characteristics

The citrus orchard was located in Miandorud county (52° 45'E, 36° 43'N), Mazandaran province, northern Iran and at 7 meter height above sea level. This garden had a area of 6500 m² and citrus trees were 10-year-old Thomson navel grafted on *Citrus aurantium* root stock with an inter-row distance of approximately 6 m and the distance between trees within a row about 4 m, and there were a total of 200 trees on 20 rows in this garden. This study was conducted at two different experiments in years of 2019 and 2020, respectively. The dominant pest in the studied orchard was CCS.

2.2 First experiment

This experiment was conducted at a completely randomized basic design with four treatments in five replications. The experimental treatments were as follows:

- 1) Control (without water-washing)
- 2) two times of water-washing
- 3) three times of water-washing
- 4) four times of water-washing

First, 20 trees were sampled before applying the treatments, so that 20 leaves (five leaves from each geographical direction) were randomly selected and sampled from each tree. The samples were placed in plastic bags and after transported to the laboratory. The number of live nymphs was counted and recorded under a stereomicroscope. About 15 days after the appearance of the first ovisac and before hatching the eggs, the trees were washed for the first time on May 31, 2019 by using a 500 L tractor sprayer at a pressure of 25 Pa with a volume of water consumption of 20 liters for each tree (a total of 400 liters of water). Then, for the four, three and two times of water-washing treatments, trees were washed every week, once every 10 days, and once every 15 days, respectively. After applying the treatments, sampling was done on July 11, 2019, and the leaves sampled from the trees were examined under a stereomicroscope and the number of ovisacs and live and dead nymphs were counted and recorded. To determine whether the nymphs are alive, first was pressurizing the body of the nymphs with a needle, and if the body was juicy, it indicated that the nymph was alive, otherwise, their dry and brown body was the criterion for the death of the nymphs.

2.3 Second experiment

This experiment was also conducted at a completely randomized basic design with four treatments in five replications. The treatments studied in this experiment included:

- 1) Control (without water-washing and oil spraying)
- 2) Applying Chlorpyrifos at a recommended concentration (2 L/1000 L) based on the manufacturer's

recommendation on June 9, 2020

3) Only water-washing (two times of water-washing on May 30 and June 9, 2020, respectively)

4) Applying mineral oil at a rate of 1% on June 9, 2020

In this experiment, 20 trees infested by CCS were selected and 5 trees were considered for each treatment. Before applying the treatments, 5 leaves were randomly sampled from each geographical direction (a total of 20 leaves from each tree). The samples were placed in a plastic bag and transferred to the laboratory for counting. The number of live first nymphal instars of CCS was counted and recorded under a stereomicroscope in the laboratory and the data were entered in the relevant tables. The treatments were applied by a 500 L tractor sprayer at a pressure of 25 Pa with a volume of water consumption of 20 liters for each tree (a total of 400 liters of water). Sampling before applying the treatments was done on May 30, 2020. After applying treatments, the sampling was replicated for four times on June 16, July 17, August 10 and 31, 2020. The live first nymphal instars on each leaf were counted under a stereomicroscope and their number was recorded separately by treatment, tree and geographical direction in the relevant tables. The criterion for live or dead nymphs was the same as in the first experiment. First, the body of the nymphs was pressurizing with a needle, and a juicy body showed that the nymph was alive; otherwise, their dry and brown body was the criterion for the death of the nymphs.

2.4 Statistical analysis

The mortality percentages were converted by $\text{Arc sin } \sqrt{x}$ formula. The data were analyzed based on a completely randomized design using one-way analysis of variance (ANOVA) with the help of SAS software (version 9.4 m3, SAS Institute, 2017). The mean was also compared by using Duncan's multiple range test.

3 Results

3.1 First experiment

The results of analysis of variance before applying the treatments showed that the difference between the number of alive nymphs on the selected trees was not significant ($p > 0.05$). The means obtained for control, two, three and four times of water-washing were 34.00, 41.60, 32.80 and 44.00 nymphs per 20 leaves, respectively.

3.1.1 Evaluation of the effect of water-washing on the reduction of ovisacs of CCS

Based on the results obtained from the variance analysis, the effect of the studied treatments on the number of alive egg sacs of citrus, *P. aurantii* after the applying was statistically significant ($p < 0.01$). The results showed that after applying the treatments, there were statistically different numbers of ovisacs of this pest on the treated trees and therefore, the water-washing had a significant difference in terms of effectiveness compared to the control. Also, based on the mean comparison results (Fig. 1), the highest number of ovisacs was obtained in the control (without water-washing), which had a significant difference with other treatments. On the other hand, the lowest number of alive ovisacs was obtained for the treatment of two times of water-washing, which was not significantly different from the treatments of three and four times of water-washing.

3.1.2 Evaluation of the effect of water-washing on the reduction of CCS nymphs

The results of variance analysis on the number of alive *P. aurantii* nymphs showed that the effect of water-washing treatments on the number of alive CCS nymphs on the treated trees after applying the treatment was statistically significant ($p < 0.01$). Based on these results, after applying the treatments, there were different numbers of alive nymphs on the trees. The mean comparison results of the number of alive nymphs are shown in Fig. 2. According to the obtained results, the highest number of alive CCS nymphs counted on the leaves was related to the control. The lowest number was obtained in the trees treated by four times of water-washing, which was not significantly different from the treatment of two times of water-washing and they were placed

in a statistical group.

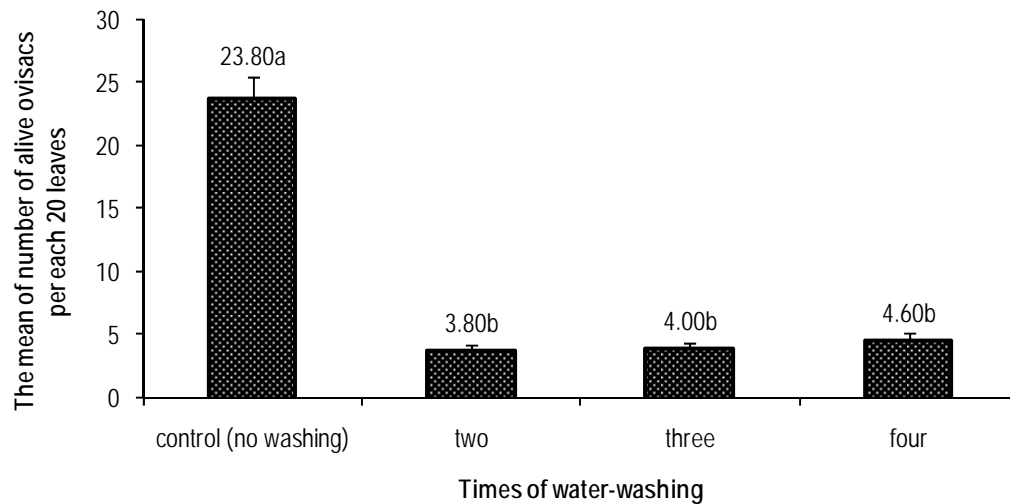


Fig. 1 Mean comparison of number of alive ovisacs of *P. aurantii* after the treatment.

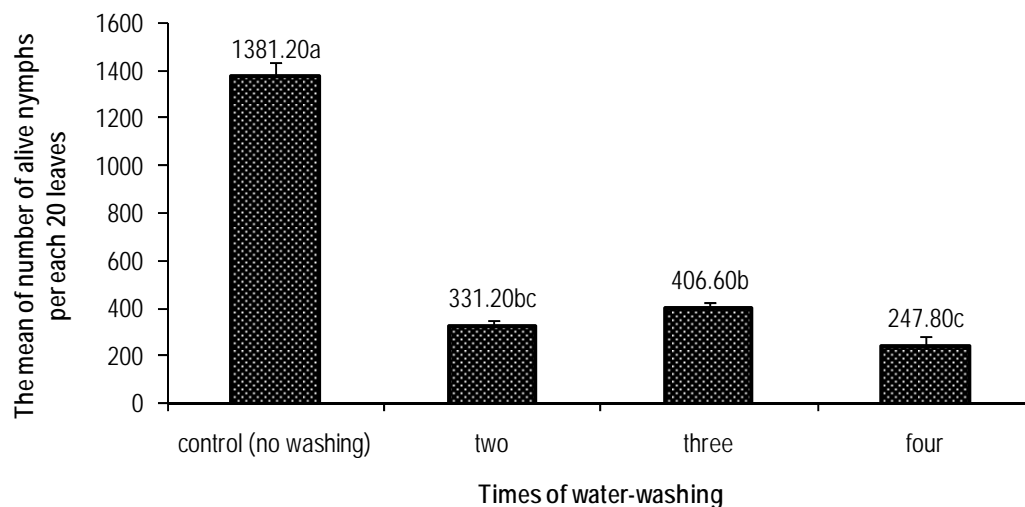


Fig. 2 Mean comparison of number of alive nymphs of *P. aurantii* after the treatment.

3.1.3 Evaluating the effect of geographical directions on the population density of ovisacs and nymphs after treatment on control trees

The results of analysis of variance on the number of alive ovisacs of *P. aurantii* showed that the number of alive ovisacs in different geographical directions was not significantly different ($p > 0.05$). According to the mean comparison results in Fig. 3, despite there was no significant difference between different geographical directions, but the highest and lowest number of ovisacs was obtained in the west and east direction, respectively. Based on the results obtained from the variance analysis of the number of alive nymphs of *P. aurantii* in different geographical directions on the control trees, there was a significant difference between the

directions ($p < 0.01$). According to the mean comparison results presented in Figure 4, the highest number of alive nymphs was obtained in the west direction, which had not a significant difference with the number of alive nymphs counted in the east and south directions. Also, the lowest number of alive nymphs was obtained for the north, which had a significant difference with other directions.

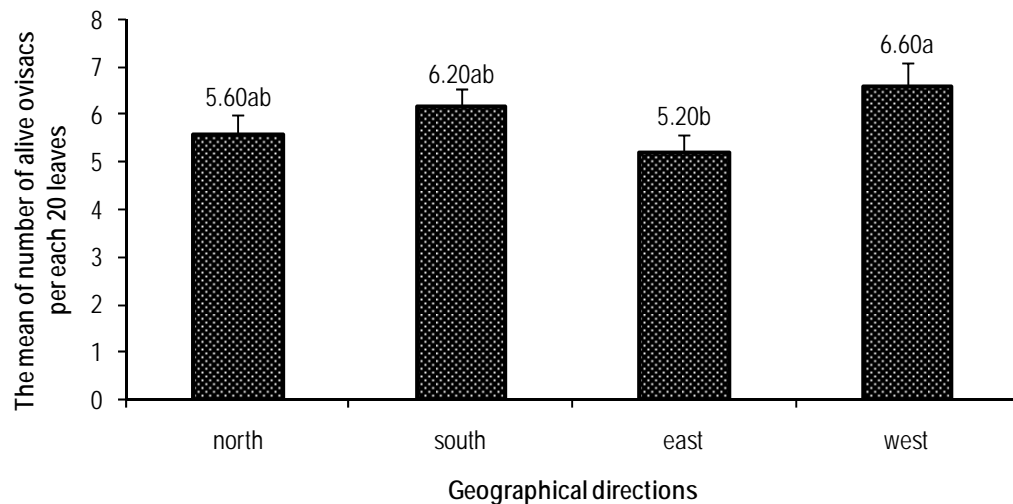


Fig. 3 Mean comparison of number of alive ovisacs of *P. aurantii* in different geographical directions on control trees.

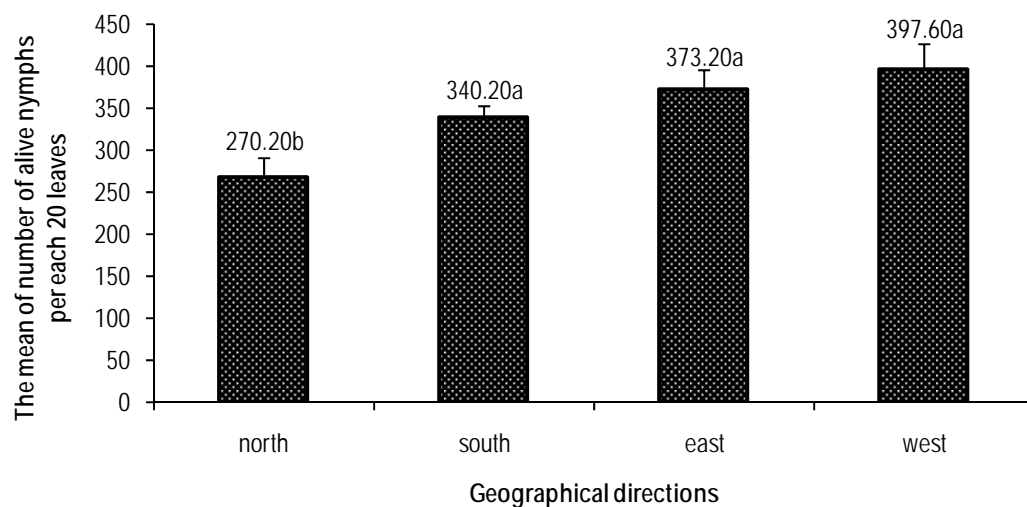


Fig. 4 Mean comparison of number of alive nymphs of *P. aurantii* in different geographical directions on control trees

3.2 Second experiment

3.2.1 Evaluation of the effect of treatments on the reduction of ovisacs of CCS

Before applying the treatments, there was no significant difference between the numbers of alive ovisacs on the studied trees. The results after applying the treatments showed that the effect of the treatments on the number of alive ovisacs in the studied dates was significant ($p < 0.01$). In other words, the treatments had significant differences with each other in terms of controlling and reducing the number of alive CCS ovisacs.

The mean comparison results on the number of alive CCS ovisacs in the studied dates are given in Table 1. As it is clear from the results, before applying the treatment, there was no significant difference in the number of alive ovisacs on the studied trees in the treatments (Table 1). After applying the treatments, the highest number of alive ovisacs on June 16, 2020 was obtained for the control treatment with an average of 30.60 ovisacs per five leaves, which there was a significant difference between the control and other treatments. On this date, the difference between Chlorpyrifos, water-washing and mineral oil was no significant, although the lowest number of alive ovisacs was obtained for mineral oil (Table 1). The highest number of alive ovisacs counted on July 17, 2020 was related to the control treatment with an average of 21.20 ovisacs per five leaves. On the other hand, the lowest number of alive ovisacs was obtained for the mineral oil with an average of 9.20 ovisacs per five leaves, which was not significantly different from the Chlorpyrifos treatment (Table 1).

Table 1 Mean comparison of number of alive ovisacs of *P. aurantii* on the studied dates affected by different control methods.

Control methods	Mean number of egg sacs per five leaves (M±SE) [‡]		
	May 30, 2020 (Before treatment)	June 16, 2020	July 17, 2020
Control (no spary)	10.80±0.87a	30.60±1.06a	21.20±1.02a
Chlorpyrifos	15.40±0.69a	16.60±0.92b	10.20±0.66c
Water-washing	10.80±0.55a	17.20±0.88b	15.00±0.73b
Mineral oil	10.80±0.71a	16.00±0.80b	9.20±0.28c

[‡]Different letters in each column indicate a significant difference at the probability level of 1%

Table 2 Mean comparison of number of alive first nymphal instars of *P. aurantii* on the studied dates affected by different control methods

Control methods	Mean number of alive first instar nymphs per five leaves (M±SE) [‡]			
	June 16, 2020	July 17, 2020	August 10, 2020	August 31, 2020
Control (no spary)	1674.00±19.04a	2151.20±25.87a	347.20±4.75a	343.80±5.32a
Chlorpyrifos	450.40±7.95b	351.00±5.20d	156.00±4.95d	59.20±2.85b
Water-washing	1601.20±15.55a	1165.80±15.59b	190.20±2.56c	23.00±0.84c
Mineral oil	560.60±3.99b	922.80±6.28c	359.40±1.03b	9.40±0.51c

[‡]Different letters in each column indicate a significant difference at the probability level of 1%

3.2.2 Evaluation of the effect of treatments on the reduction of CCS nymphs

The results of the analysis of variance on the number of alive CCS nymphal instars showed that the effect of the treatments on the number of nymphs in all the studied dates after applying treatments was significant ($p<0.01$). In other words, different pest control methods had significant differences with each other in terms of reducing number of nymphs.

The mean comparison results of the number of first CCS nymphal instars in the studied dates are presented in Table 2. As it is clear, the highest number of alive nymphs counted on June 16, 2020 was related to the control treatment with an average of 1674.00 nymphs per five leaves, which there was no significant difference between control and water-washing. The lowest number of alive nymphs on this date was obtained for the

Chlorpyrifos with an average of 450.40 nymphs per five leaves, which was not significantly different from the mineral oil (Table 2).

The highest number of alive nymphs on July 17, August 10 and 31, 2020 was also obtained for the control with the averages of 2151.20, 347.20 and 343.80 nymphs per five leaves, respectively, which was significantly different from other treatments. Also, the results showed that the lowest number of alive nymphs counted on July 17 and August 10, 2020 was related to the Chlorpyrifos with the averages of 351.00 and 156.00 nymphs per five leaves, respectively, which had a significant difference with other treatments. The lowest number of alive nymphs counted on August 31, 2020 was related to mineral oil with an average of 9.40 nymphs per five leaves, which had not a significant difference compared to water-washing (Table 2).

3.2.3 Evaluating the effect of geographical directions on the population density of ovisacs and nymphs after treatment on control trees

The results of variance analysis on the number of ovisacs showed that the effect of geographical directions on the number of ovisacs on the tree on June 16, 2020 was not statistically significant ($p > 0.05$), the number of ovisacs in the different directions on July 17 and August 31, 2020 was significantly different ($p < 0.05$). It is necessary to mention that due to the fact that no ovisacs were observed on the trees on August 10, 2020, therefore, the analysis result was not presented on this date.

The results of comparing the means on the number of ovisacs on the tree are given in Table 3. As can be seen, on June 16, 2020, the number of ovisacs in different geographical directions did not differ significantly, although the highest and lowest number of ovisacs were observed in the north and west directions with averages of 9.20 and 6.00 ovisacs per tree, respectively. The results of the mean comparison on July 17, 2020 showed that the highest number of CCS ovisacs was obtained in the south direction of the trees with an average of 6.60 ovisacs per tree, which was not significantly different from the average ovisacs counted in the north and west directions. On the other hand, the lowest number of ovisacs counted was related to the east direction with an average of 3.60 ovisacs per tree, which had a significant difference with other directions. Also, the highest number of ovisacs on August 31, 2020 was obtained for the north direction with an average of 8.60 ovisacs per tree, while there was a significant difference between north and other directions. On the other hand, the lowest number of ovisacs was counted in the west direction with an average of 4.20 ovisacs per tree, which was not significantly different from the averages obtained for the south and east directions (Table 3). The results of variance analysis on the number of nymphs showed that the effect of geographical directions on the number of first nymphal instars on the tree on June 16, 2020 was statistically significant ($p < 0.05$). On the other hand, the number of nymphs counted in the geographical directions on July 17, August 10 and 31, 2020 was significantly different from each other ($p < 0.01$). The results of mean comparisons of the number of first nymphal instars on the tree in different direction are given in Table 4. As can be seen, on June 16, 2020, the highest number of nymphs was obtained in the north direction with an average of 538.00 nymphs per tree, while the lowest number of nymphs in the south direction was 378.60 nymphs per tree, which there was a significant difference between them. The results of the mean comparison on July 17, 2020 showed that the highest number of CCS nymphs was found in the south direction of the trees with an average of 694.40 nymphs per tree, which was not significantly different from the average obtained in the west direction. On the other hand, the lowest number of nymphs counted was related to the east direction with an average of 336.60 nymphs per tree, which had a significant difference compared to other direction. According to the obtained results, the highest number of nymphs on August 10, 2020 was obtained in the east direction with an average of 104.60 nymphs per tree, which was not significantly different from the north direction. Also, the highest number of nymphs on August 31, 2020 was obtained for the east direction with an average of 108.00 nymphs per tree. On the other hand, the lowest number of nymphs on August 10 and 31, 2020 was related to the west

direction with the averages of 65.00 and 54.00 nymphs per tree, respectively, while there was no significant difference between west and south direction on August 10, 2020 (Table 4).

Table 3 Mean comparison of the number of ovisacs of *P. aurantii* in cardinal directions of the tree on studied dates.

Cardinal directions	Mean number of egg sacs per tree (M±SE) [‡]		
	June 16, 2020	July 17, 2020	August 31, 2020
North	9.20±0.35a	5.40±0.16a	8.60±0.27a
South	8.60±0.31a	6.60±0.22a	5.60±0.19b
East	6.80±0.25a	3.60±0.08b	5.20±0.16b
West	6.00±0.26a	5.60±0.24a	4.20±0.10b

[‡]Different letters in each column indicate a significant difference at the probability level of 1%

Table 4 Mean comparison of the number of first nymphal instars of *P. aurantii* in cardinal directions of the tree on studied dates.

Cardinal directions	Mean number of first instar nymphs per tree (M±SE) [‡]			
	June 16, 2020	July 17, 2020	August 10, 2020	August 31, 2020
North	538.00±9.54a	492.40±7.62b	93.20±2.82a	88.40±2.45b
South	378.00±5.87b	694.40±8.43a	77.00±2.24b	91.40±2.70b
East	386.60±6.06b	366.60±6.01c	104.60±2.90a	108.00±2.72a
West	389.40±5.81b	597.80±8.69ab	65.00±2.20b	54.00±2.15c

[‡]Different letters in each column indicate a significant difference at the probability level of 1%

4 Discussion

The suitable weather conditions in the north of Iran, which are favorable for different types of pests, force farmers to apply chemical pesticides to preserve their crops every year based on the timetable. Concerns resulting from excessive use of pesticides, such as disrupting the biological balance, the destruction of natural enemies, and the outbreak of pests, lead to the use of sustainable strategies in which crop production costs and environmental risks (water and soil pollution) are simultaneously reduced and the income of farmers is improved and also, it follows the health of the environment and society. One of the alternative strategies to control CCS is water-washing.

The results of the first experiment showed that two times of water-washing reduced the density of ovisacs compared to the control, and four times of water-washing yielded the lowest number of nymphs compared to the control, although there was no significant difference between two and four times of water-washing. Arbabi et al. (2010) evaluated the effect of water-washing on controlling the date palm spider mite, *O. afrasiaticus* and reported that the highest effect of water-washing was obtained at seven days interval and the most stable and longest control by water-washing was recorded up to 25 days. The results of study conducted by Shakrmi et al. (2013) on the effect of water-washing on the fig spider mite, *Eotetranychus hirsti* Pritchard & Baker (Acari: Tetranychidae) in Pol-e-dokhtar city, Lorestan province, Iran showed that one time of water-washing caused a significant reduction (47.32% mortality on 3 days after treatment) of this pest compared to the control. These researchers also stated that the mortality of mites treated by two times of water-washing on 29 days after treatment was 62.21%. In the first experiment of the present study, two times of water-washing resulted in a

significant decrease in the number of nymphs (from 1381.20 to 331.20 nymphs per 20 leaves) compared to the control, which is consistent with the results of the above study. Norris et al. (2002) in evaluating the effect of water-washing caused by natural rainfall against the of thrip populations showed that by increasing the duration of rainfall from 30 to 120 min, the pest populations was significantly controlled. Based on the results reported by Brunner and Burts (1981), three times of high-pressure water-washing on pear trees in Washington DC caused the destruction of *Cacopsylla pyricola* Förster (Hemiptera: Psyllidae) population on branches, leaves and fruits. Jaferi (2017) found that one time of water-washing on plane trees every two weeks from early July to early September is enough to control the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). This researcher also stated that water-washing had a negative effect on the number of eggs and adults of *T. urticae*. Stoyenoff (2001) by studying on the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) in USA reported that high-pressure water-washing on rose flowers for three weeks significantly reduced the aphid population, so that the population decreased by 50 to 100% due to the initial washing. Jamieson et al. (2010) also stated that water-washing with a pressure between 50-200 Pa reduced the population of mealybugs on apple and avocado trees by 84%. The results of the first experiment of the present study also indicated the effectiveness of water-washing and reducing the population of CCS on citrus trees.

Regarding the effect of water-washing on the activity of natural enemies, Damghani & Arbabi (2003) and Arbabi et al. (2010) reported that water-washing not only limits the activity of ladybug *Stethorus* spp. which is the predator of the date palm spider mite, *O. afrasiaticus*, but accelerates the activity of this predator by favoring the environmental conditions. Considering that predatory ladybugs, *C. montrouzieri* and *Chilocorus bipustulatus* Linnaeus are important predators in citrus orchards of Mazandaran (Halaji Sani, 1999; Saeidi et al., 2015), therefore, water-washing can be effective in increasing the activity of these ladybugs.

The results obtained in the first experiment showed that by knowing the biological stages of CCS and at least two times of water-washing with 15-day interval after about 15 days from the appearance of the first ovisacs and before hatching them, can destroy up to 84% of ovisacs and 76% of nymphs and reduce the CCS population. Since, the economic injury level (EIL) of CCS reported about 135 ovisacs per tree (Amozegar et al., 2016), the number of live ovisacs affected by the water-washing after applying treatment was always lower than the EIL (Authors' observations), therefore, the gardener did not apply chemical control until the end of the season and satisfied with the health of the trees.

The results of the second experiment showed that the application of mineral oil to control CCS has achieved the best results, because in the studied dates, the lowest number of ovisacs was obtained in the mineral oil treatment. Control by applying the Chlorpyrifos ranked second and produced the lowest number of ovisacs after mineral oil. Water-washing on trees in order to control CCS got the third rank in terms of the impact. Meanwhile, gardeners were satisfied about the effect of water-washing in controlling CCS on the trees and its population was lower than the EIL. Also, the results of the second experiment showed that the highest number of live nymphs at all dates was obtained for the control treatment. The lowest number of nymphs on June 16, July 17 and August 10, 2020 was obtained for Chlorpyrifos treatment. At these dates, mineral oil ranked next in terms of effect on decreasing the number of nymphs. The lowest number of nymphs on August 31, 2020 was obtained in mineral oil treatment. Halaji Sani et al. (2019) indicated that the mineral oil increased the insecticidal effect of Chlorpyrifos, Acetamiprid and Pyriproxyfen on first nymphal instar of *P. aurantii*, when combined with these insecticides. It has been proven that mineral oils have the same efficiency of chemical insecticides in citrus pest control (Liang et al., 2010; Leong et al., 2012; Damavandian and Kiaeian Moosavi, 2014; Baliota and Athanassiou, 2023).

In the first experiment, despite there was no significant difference between the number of ovisacs in different geographical directions, the highest and lowest were obtained in the west and east directions,

respectively, while the effect of the geographical directions on the establishment of live nymphs was significant and the highest and lowest numbers of that was related to the west and north directions. In the second experiment, a significant difference was observed between the number of ovisacs in geographical directions on July 17 and August 31, 2020, so that the highest and lowest number of ovisacs on July 17, 2020 was counted in the south and east directions and those on August 31, 2020 was in the north and west directions, respectively. Also, the highest number of nymphs on June 16 and July 17, 2020 was recorded in the north and south directions, respectively, while the lowest number on both dates was counted in the east direction. The highest and lowest numbers of nymphs On August 10 and 31, 2020 were related to the east and west directions of the trees, respectively.

Also, according to the obtained results, the geographical direction has a significant effect on the distribution of CCS, but this significance depends on factors such as the distance between the trees and the position of the trees between the rows in the orchards. Therefore, when monitoring trees, experts should examine all geographical directions to make a more accurate assessment of the presence of the pest and estimate its density.

The severe infestation and high damage of CCS during the past years has caused that the registered and recommended pesticides are applied annually to control the pest in a large part of the citrus orchards of Mazandaran province, northern Iran. Increasing pesticides resistance is a very critical and important problem at the global level (Roush and Tabashnik, 1990; Damavandian, 2006). Studies by Montazeri and Alavi (2002) indicated that CCS can be controlled by mineral oil alone, which is consistent with the results of the present study.

The predatory mite, *Allothrombium pulvinum* Ewing (Acari: Trombidiidae) is native to Mazandaran and is effective in controlling CCS (Saboori et al., 2003) and since the harmful effect of mineral oils on the natural enemies of pests is much less compared to chemical pesticides (Davidson et al., 1991), Therefore, their use can be very effective in maintaining and supporting the mentioned *A. pulvinum*. As it is known, mineral oil is less harmful than synthetic pesticides for natural enemies such as predatory mites, but if it is possible to replace water-washing as a control method, undoubtedly, more suitable and favorable conditions will be provided for activity of biological control agents present in citrus orchards, which will result in the stability of the population of other species, including pests.

Arbabi et al. (2010) stated that a gradual increase in the effect of water-washing on the controlling date palm spider mite during 25 days of sampling was observed in all the investigated areas with some difference. The average efficiency of water-washing (72%) compared to some insecticides such as amitraz (68.85%) caused more mortality (Arbabi et al., 2010; Arbabi et al., 2017). The effect of three times of high-pressure water-washing on controlling *C. pyricola* in Washington showed that water-washing pear trees destroys *Psylla* nymph population from foliage and fruit and prevents honeydew produced by the pest (Brunner and Burts, 1981). Therefore, Brunner and Burts (1981) concluded that three times of water-washing at a high pressure by a sprayer has an reducing effect on the population of pear psylla, and this method can be a suitable alternative to chemical pesticides and is compatible with integrated management programs. Washing with water at a pressure between 50-200 Pa reduced up to 84% of mealybug populations on apple and avocado and was recognized as a management approach in New Zealand (Jamieson et al., 2000). It should be mentioned that the results of the second experiment also showed the positive effect of water-washing on controlling CCS, so the gardener did not agree to spray chemical pesticides and was completely satisfied with the health of the produced fruits.

The results of the present study showed that the number of CCS ovisacs remaining after water-washing trees was always lower than the EIL, so the gardener did not apply chemical control until the end of the season

and was satisfied with the health of the trees. Also, according to the results of two experiments, the geographical direction has a significant effect on the distribution of CCS, but this significance depends on other factors such as the distance between the trees and the position of the trees between the rows in the orchard. Therefore, when monitoring trees, experts should examine all geographical directions to make a more accurate assessment of the presence of the pest and estimate its density.

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