Article

Determination of application time and concentration of mineral oil against *Pulvinaria aurantii* (Hemiptera: Coccidae) in northern Iran

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Abstract
To determine the most appropriate concentration and application time of mineral oil against *Pulvinaria aurantii* Cockerell, the reaction rate of egg hatching and the emergence of mobile cushion citrus instars to different concentrations of mineral oil in the laboratory conditions with a temperature of 4 ± 25 °C, relative humidity 5 ± 75 percent and 12 hours of light every 24 hours over four days after applying the treatments was studied. In this study, four different concentrations of mineral oil including 0.2, 0.4, 0.6 and 0.8% (namely 200, 400, 600 and 800 ml /100 liter water) and control (water without mineral oil) were selected as treatments and tested on the samples of citrus cushion eggs in various stages of hatching (10, 30, 50, 70 and 90%). The results showed that the most appropriate time to control is when 50 percent of citrus cushion eggs are hatching and the best mineral oil concentration is 0.77%.

Keywords *Pulvinaria aurantii*; first instar; mineral oil.

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1 Introduction

In recent years, *Pulvinaria aurantii* Cockerell has been considered as the most important pest in citrus orchards in Mazandaran province (northern Iran) (Damavandian, 2010). This pest entered Iran from 1937 to 1939 and was first observed in Rasht and Bandar-e Anzali cities on citrus trees in 1976 and is currently distributed from Bandar-e Anzali to Gorgan (Behdad, 1991). *Pulvinaria aurantii* by feeding latex, on the one hand, causes tree weakness and, on the other hand, causes the growth of the fumagine fungus (*Cladosporium* sp.) because of the secretion of large amounts of honeydew, Which in turn causes a severe falling-out or loss of the quality of the fruit (Damavandian, 2006). This pest has two generations each year, the first generation appears from the middle of June to the middle of September and the second generation appears from mid-September to June of the following year (Behdad, 2003). During the past years, due to the severity of pollution and the high level of damage caused by pests have annually used phosphorus and hormonal pesticides to control the pests in most of citrus orchards in Mazandaran province, which is more than 100,000 ha (Amozegar, 2013; Kheirodin et al., 2012).

Over the past decades, the using chemical insecticides of Dursban, Diazinon and Boprofosin have been the main control strategy of *P. aurantii* in Mazandaran (Damavandian, 2007). It should be noted that repeated application and high levels of sprays by using organic phosphorus or new insecticides will cause an outbreak of *P. aurantii* (Bedford et al., 1998). In addition to the pest outbreak, the occurrence and increase of pest resistance to pesticides caused by their repeated application is a very important global problem (Roush and Tabashnik, 1990). The harmful effects of synthetic pesticides on humans, the environment and nontarget organisms, such as natural enemies and the renewed outbreak of pests, identification of alternative control strategies in integrated pest management plans has become a need (Maleki and Damavandian, 2015). Due to the lack of the destructive effects of mineral oils compared to synthetic pesticides, they are an integral part of integrated pest management programs for agricultural products around the world (Beattie, 2005; Damavandian and Kiaeian Moosavi, 2014; Helmy et al., 2012) and are considered as a worthy alternative of Chemical pesticides to control important pests in citrus orchards (Kim et al., 2010; Kiss et al., 2005). According to Davidson et al. (1991), mineral oils affect the different developmental stages of coccids, including eggs and various nymphal stages, and can control them. On the one hand, study of Damavandian (2010) indicates that *P. aurantii* can be controlled without the use of synthetic pesticides and only by using mineral oil, and on the other hand, many natural enemies have been reported from citrus orchards (Damavandian, 2003; Davies and Jackson 2009; Jacas and Urbaneja, 2010), whose supporting them for the control of citrus pests, including *P. aurantii*, is of particular importance.

Since the harmful effect of mineral oil on the natural enemies of pests is much less than that of pesticides (Bedford et al., 1998; Helmy et al., 2012; Suma et al., 2009), so they use to conserve and protect natural enemies of this pest such as *Cryptolaemus montrouzieri* Mulsant and predatory mites, including *Allothrombium pulvinum* Ewing, which is a native to Mazandaran citrus orchards, is essential (Saboori et al., 2003). One of the important issues related to the effectiveness of mineral oils is their application at the appropriate time (Beattie, 2005; Damavandian, 2012; Damavandian and Kiaeian Moosavi, 2014) and the selection of suitable concentrations of mineral oil (Beattie, 2005; Damavandian and Kiaeian Moosavi, 2014). The highlighted difficulties reveal the importance of research to reduce the use of pesticides and to prevent the irregular application of pesticides. Therefore, in this research, it is trying to determine the most suitable mineral oil concentration and the exact time of the control against *P. aurantii*.

2 Material and Methods

To determine the number of eggs in each ovisac producing by the second generation of *P. aurantii*, following the appearance of ovisacs in June, 20 ovisacs were randomly collected from citrus orchards and the eggs were checked and counted under the stereomicroscope. Considering the average number of egg per each ovisac and counting the number of nymphs emerged, the egg hatching percentage was determined for each treatment. Meanwhile, ovisacs with the same conditions (from a geographic region and homogeneous) were selected and, after a preliminary experiment, the final concentrations were calculated. After that control of eggs and nymphs was performed at each stage of 10, 30, 50, 70 and 90% hatching. For each stage of control, the treatments consisted of 200, 400, 600 and 800 ml of mineral oil (Tehran oil company, Tehran, Iran) in 100 liters of water and water without mineral oil (control). The mineral oil used had 80% effective ingredient and 20% emulsifier with 92% sulfonation degree (Volck®). Each replication included an ovisac and nymph on citrus leaves. In this study, a potter tower with a spray pressure of 1 bar/inch^2 was used, and the volume of water and mineral oil sprayed on the leaves containing ovisacs and nymphs of *P. aurantii* was 500 µl/10 cm^2.

These experiments were repeated for 5 times for each treatment. The data were collected about 2 hours before the application of the treatments to sure that eggs and nymphs are alive, after the samples were treated. Due to the fact that mineral oils penetrate the body of the pests and control them in contact method, so, the mortality and emerging the nymphs were recorded per 24 hours and in 4 consecutive days. Changes in egg color, wrinkling and non-hatching were the criteria for the death of eggs and the dehydration of the body and the immobility were the criteria for the death of nymphs. Natural mortality was first calculated from the control and then, the percentage of mortality was corrected using Abbott's formula as follow:

$$\text{Corrected mortality} = (T\text{-}C/100\text{-}C) \times 100 \qquad (1)$$

where T and C are the numbers of dead insects in treatment and control, respectively (Abbott, 1925). The corrected mortality data of egg and nymph were used to estimate different lethal concentrations (LC_{50} and LC_{90}) using Probit analysis method and by computer program P / PROBAN LSTATS (Van Ark, 1983).

3 Results

On average, 432 eggs were counted in each ovisac in June. A maximum and minimum number of counted eggs were 654 and 268, respectively. The LC_{50} and LC_{90} calculated for mineral oil in different stages of egg hatching of *P. aurantii* are presented in Table 1. The most homogeneous data were obtained at 30 and 50% hatching stages, so that estimation of fiducial limits was possible at these stages (Table 2) (Van Ark, 1983). At 30 and 50% hatching stages, the concentration of mineral oil used which caused 50 and 90% mortality of eggs and nymphs of *P. aurantii* is presented in Table 2. It should be noted that due scattering and heterogeneity of the data at stages of 10 and 70% egg hatching, estimation of fiducial limits was not possible.

Table 1 LC_{50} and LC_{90} estimated of mineral oil for different stages of *P. aurantii* egg hatching.

Egg hatching (%)	N*	Slop±SE	LC_{50} (%)	LC_{90} (%)
10	200	0.3678±0.01	1.38	4.68
30	350	1.0595±0.13	0.94	2.3
50	530	3.0372±0.25	0.35	0.77
70	950	0.6884±0.03	0.046	1.39

* Numbers of insects tested

Table 2 The fiducial limit of mineral oil (%, namely liter/100 liter water) for the predicted mortality (%) of *P. aurantii* instars in stages of 30 and 50% egg hatching.

The predicted mortality (%)	Fiducial limit (%)			
	30% egg hatching		50% egg hatching	
	upper	lower	upper	lower
50	1.21	0.8	0.55	0.06
90	3.31	1.82	3.09	0.57
95	3.91	2.1	4.07	0.65
99	6.3	3.23	5.92	0.78

The results of egg hatching and settling nymphs in the various stages of hatching egg of *P. aurantii* (Fig. 1) show that there was a significant different among mean number of nymphs emerged in different treatments (F=996603, df=24, p=0.0001). According to Fig. 1, the population of nymphs deployed in the 10 and 30% egg hatching stages up to 96 hours after the initial counting was constantly increasing. When the eggs hatched up to 50% and more, the nymphs settled in the leaf area had a decreasing trend. The mean comparison of mortality percent (Fig. 2) show that there was a significant different among mortality percent calculated in different treatments (F=74835.7, df=24, p=0.0001). According to the results, the highest percentage of mortality caused by treatments applied was observed at 50 and 70% of egg hatching stages and in 0.8% concentration of mineral oil (Fig. 2).

In the obtained results of the *P. aurantii* response in the 90% egg hatching stage, when on average 388 eggs (373-403) were hatched from each ovisac and nymphs deployed at the leaf surface, Despite the use of existing transformations, calculation of LC_{50} and LC_{90} was not possible due to the heterogeneity of the data, and the highest mortality rate was obtained 42% (Fig. 2).

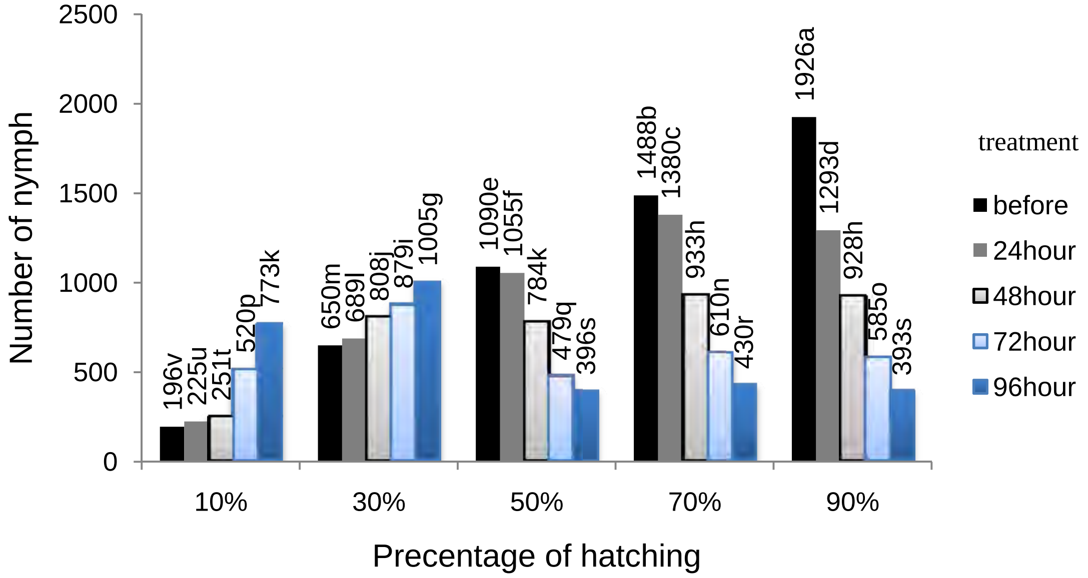

Fig. 1 The number of instars deployed on the surface of five leaves over five days in the stages of 10, 30, 50, 70 and 90% egg hatching (Different letters above the columns indicate significant difference among treatments at p=0.05, Tukey test, one-way ANOVA).

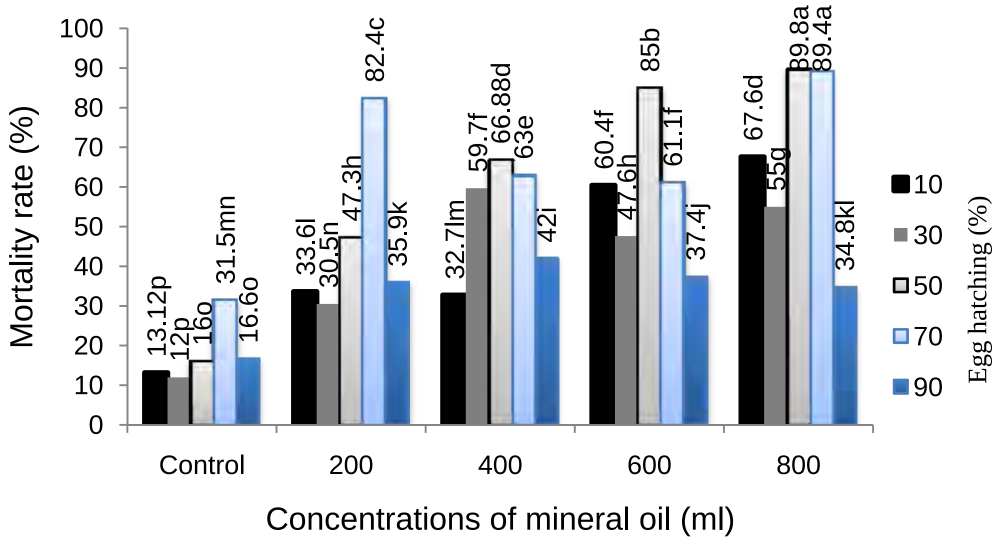

Fig. 2 Comparison of mortality rate (%) of instars caused by treatments applied in the different stages of *P. aurantii* egg hatching (Different letters above the columns indicate significant difference among treatments at p=0.05, Tukey test, one-way ANOVA).

4 Discussion

It is concluded from laboratory results that 90% of *P. aurantii* nymphs' population can be inhibited by the lowest concentration of mineral oil, which is 0.77% when 50% of the *P. aurantii* eggs were hatched (Table 1).

If control takes place earlier or later than 50 to 70% of hatching, on the one hand, the concentration of mineral oil and the limit of its consumption are increased (Table 1, 2), and on the other hand, the mortality rate will also decrease (Fig. 2). As shown in Table 2, the concentration of mineral oil caused mortality in the range of 50 to 99 percent in the 30% hatching egg is significantly higher than that in 50% hatching egg.

The reason for increasing the concentration at earlier times than 50% of hatching eggs may be related to the presence of eggs in the ovisacs and their greater resistance to control. Meanwhile, a few days after the control in the stages of 10% and 30% of hatching eggs, as shown in Fig. 1, the population of *P. aurantii* is constantly increasing, indicating the new nymphs emerged even in laboratory conditions, and on the other hand it has been proven that mineral oils need to be contacted to pests to have a lethal effect (Beattie, 2005; Daviason et al., 1991), hence the nymphs that have appeared after oil spraying will continue to live. Therefore, it seems that the control at the beginning of egg hatching (10 and 30%) would not be very efficient, and we need to use high concentrations of mineral oil (Table 1).

As shown in Fig. 1, when hatching eggs in the ovisacs increases from 50 to 70% or more, the mortality of the nymphs increases in the control. This is likely to be due to interspecies competition, since prickly nymphs occupy appropriate places on the leaves that contain better sap and thus have more growth rate, while others have not succeeded in finding the best location and nutrition in this competition, and occupy areas that contain less nutritious food and sap, and as a result, their growth slows down (Radjabi, 2008).

According to Halaji Sani (1999), after hatching, nymphs tend to be positioned along the main and secondary veins, but in high densities, they occupy other leaf areas. Therefore, leading nymphs have a higher aging rate due to more suitable and prolonged feeding and hence becomes more resistant. Therefore, LC_{90} of mineral oil increased is 39.1% in the 70% egg hatching stage (Table 1). In the 90% egg hatching stage due to reasons such as age of nymphs and their resistance, emergence of new nymphs after the control, and increased heterogeneity, there was no possibility of calculating LC_{50} and LC_{90} by the concentrations applied, And as Table 3 shows, the mortality rate decreased to 40.08%, and to increase mortality, the concentration of mineral oil should be increased, so it seems that the most appropriate time to control against *P. aurantii* is between 50% and 70% egg hatching stages (Fig. 2). The 50% egg hatching stage is preferable to 70% egg hatching stage for several reasons, The most important reason is that if the expert controlling against based on 70% egg hatching stage, he will not have an opportunity to error, and if the percentage of egg hatching exceeds 70%, the mortality rate of the pest will be greatly reduced, and another, by increasing the egg mass from 50 to 70%, the concentration required to control 90% of the population will increase from 0.77% to 1.39% of the mineral oil in water, so at 70% of egg hatching or higher, the probability of mortality of the pest and the desired efficiency of the control will reduce.

Damavandian (2006), the concentration of mineral oil that caused the mortality of 50% and 90% of the second and third instar of *P. aurantii* calculated 0.593 and 1.013 *L*/ 100 *L* of water, which can be used these concentrations of mineral oil in the later stages of the control, when have increased the age of the nymphs and the fruits are larger and more resistant.

For many years, Azinphos-methyl insecticide was used to control *P. aurantii* in citrus orchards, which led to the removal of natural enemies and outbreaks of other pests (Damavandian, 2006). The lethal effect of mineral oils on natural enemies is less than that of pests due to their high mobility (Beattie, 2005; Davidson et al., 1991; Helmy et al., 2012; Maleki and Damavandian, 2015; Suma et al., 2009), Therefore, by removing common insecticides and controlling *P. aurantii* by mineral oil, it is possible that supported *Allothrombium pulvinum*, which are natural enemies of *P. aurantii* (Saboori et al., 2003), as well as Phytoseiid predatory mites and the increase of their population after the pest control (Damavandian, 2010) and decreased the natural balance level of pests (Maleki and Damavandian, 2015). Predatory mites are also able to protect themselves from the risk of mineral oils and do not suffer any damage (Bedford et al., 1998). Despite the highly diverse species of predatory mites in Mazandaran citrus orchards, the use of broad-spectrum pesticides caused the mortality of them and other natural enemies, increased the natural balance level of pests and finally led to The outbreak of various types of pests in the citrus orchards of the province (Damavandian, 2007).

Bedford et al. (1998) reported that repeated and high spraying by phosphorus and hormonal insecticides eliminated parasitoid wasps, *Coccophagus pulvinaria* Compere and *C. basilis* Compere and *Metaphycus helvolus* Compere, and eventually led to the outbreak of *Pulvinaria aethiopica* Deletto. On the other hand, the lethal effect of mineral oils and its residual on parasitoid wasps is negligible (Rae et al., 2000). On the one hand, the highest population density of predatory mites is from late spring to early autumn (Damavandian, 2010) and on the other hand, *P. aurantii* nymphs appear from early June and about 100% of the first-age-old nymphs appear in late May (Damavandian, 2014), therefore, it is recommended that experts monitor the citrus orchards from the beginning of June and control the pest when egg hatching was observed between 50 and 70 percent, which, of course, it depends on the weather conditions of each year.

If pest control is carried out at the appropriate time, its population will be reduced to a degree that will probably not be needed to control the summer generation in September or October (Authors' observations). Beattie (2005) reported that, in addition to determining the time, the target pest density should be acceptable in the leaf area in oil spraying. According to the Maleki and Damavandian (2015), in citrus orchards whose pests are controlled by natural enemies and mineral oil, the economic injury level of *P. aurantii* per tree are at most 135 ovisacs in June, while in traditional citrus orchards, which pest control depends on using different synthetic pesticides, the economic injury level was reduced to 110 ovisacs per tree (Amozegar et al., 2017). Therefore, experts should consider the economic injury level. It should be noted that the distribution of *P. aurantii* in citrus orchards in the north of Iran is highly cumulative and about 8% of the trees are infected with this pest in June when the first control takes place at this time (Damavandian, 2014).

Therefore, the controlling *P. aurantii* can have two mistakes: first, it is done at the wrong time, when the egg hatching is outside the range of 50 to 70%. Second, it is done on a wider scale, regardless of contaminated sites, while, if the control is necessary, it should be done locally. This control method, in addition to protecting the natural enemies, will also reduce the use of pesticides. Therefore, the proper pest management along with the use of mineral oils at the appropriate time, on the one hand, will promote the biological safety of citrus orchards, on the other hand, it will not cause the unusual outbreak of other citrus pests (Damavandian, 2016).

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