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Effect of white oil on the behavior of citrus mealybug parasitoid *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae)

Beyaz yağın turunçgil unlubiti parazitoiti *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae)'nin davranışı üzerine etkileri

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ABSTRACT

In this study, the efficacy of the white oil widely used in citrus orchard plantations on host searching and parasitization behavior of citrus mealybug parasitoid *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) were determined in laboratory conditions at 28±1 °C, 65±10% RH and 16:8 h light:dark photoperiod. Airflow Y tube olfactometer was employed to determine behavior of host searching activity. In choice testing, the behavioral response of 2-3 day-old mated female *L. dactylopii* adults to fresh air, white oil, only mealybug and mealybug applied with white oil was evaluated. Parasitoids preferred fresh air compared to white oil, but host odor was found more attractant than white oil. For determining the parasitization behavior of *L. dactylopii*, third instar (12-day old) and young female mealybug stages were used. In these experiments, recommended rate of white oil were treated to the hosts, and parasitoids were released at 0, 6, 12, 18, 24-hour interval after application. As a result, the host number parasitized by *L. dactylopii* was increased directly proportional with exposure time in both host ages.

INTRODUCTION

Citrus is one of the most important agricultural products in the world. The major of citrus producing countries were China, Brazil, India, Mexico, USA, Spain, Egypt, Turkey, Nigeria and Iran (Anonymous 2016a). The Aegean and Mediterranean coasts of Turkey were the first plantation fields of citrus. World citrus production was about 146 million tons in 2016 and Turkey ranked eighth with 4 million 293 thousand tons (Anonymous 2016b).

Biotic factors, such as diseases, pests, and weeds are considered one of the major problems in citrus because they cause serious fruit yield loss. In Turkish citrus orchards; 34 diseases, 89 pests, 16 nematodes, and 155 weed species were detected that could adversely affect citrus production. Among these species, 17 pests, 8 diseases, 1 nematode, and 10 weeds are economically important that have to be considered as key pests (Uygun and Satar 2008).

The major pests of citrus are citrus mealybug, red and yellow scale, the Mediterranean fruit fly. Other economic pests include citrus red mite, citrus rust mite, citrus bud mite, carob moth, honeydew moth, citrus leafminer, aphids, citrus whitefly, and cottony cushion scale. The citrus mealybug *Planococcus citri* Risso (Hemiptera: Pseudococcidae) is polyphagous (Ben-Dov 1994) and harmful pest particularly in citrus, greenhouses, and nurseries (Ben-Dov 1994, Blumberg et al. 1995, Karacaoğlu and Satar 2017a, Karacaoğlu and Satar 2017b). Its nymphs and adults may cause damage by feeding the plant sap in foliage, flower, fruit, and stems. Furthermore, the citrus mealybug causes leaf chlorosis, yield reduction and quality losses especially on the fruit of the citrus trees (Karacaoğlu 2016, Karacaoğlu and Satar 2017b). The Citrus mealybug excretes honeydew, which stimulates the growth of sooty mold fungi. In addition to primer damage, *P. citri,* also reported as an important virus vector, has economic importance even at the low population level (Cabaleiro and Segura 1997).

Chemical control of citrus mealybug using pesticides is difficult because this pest protects itself by wax that covers the body. Moreover, the feeding location such as under leaves or fruit calyx provides a shelter for them against pesticides. Therefore, biological control is an alternative control method against citrus mealybug in practice (Çalışır et al. 2005, Yayla and Satar 2012).

Biological control of citrus mealybug in Turkey has been successfully carried out with the parasitoid *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) and the predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) imported from the USA in 1969 (Anonymous 2012). Both of them could not overwinter in natural flora, so they must be produced in insectarium and then released to nature (Yiğit et al. 1994, Yiğit and Canhilal 1998). *L. dactylopii*, an effective parasitoid, can easily find mealybug and settle on the host even at the low population density. This behavior makes the parasitoid a perfect biological control agent (Cloyd 1997).

Predator and parasitoid populations are seriously affected by the usage of intensive insecticides in order to control pests. White oils, accepted as compatible with integrated pest management, are commonly used for years to control scale insects, mealybugs, whiteflies, and mites. White oils block the breathing pores of insects (adult, nymphs, eggs) causing suffocation and death when they are applied. Pesticide resistance in insects and mites has not occurred due to the different effect mechanism. In addition, they are safe to human and environment compared to other control methods (Davidson et al. 1991). On the other hand, while white oils are used against these harmful pests, they can directly or indirectly affect natural enemies present in the environment. Considering the presence of parasitoids and predators intensively in citrus gardens, the effects of white oils on natural enemies have become more important. The aim of this study is to determine how the white oil effects the behavior of citrus mealybug parasitoid *L. dactylopii* in laboratory conditions.

MATERIALS AND METHODS

Rearing of Planococcus citri Risso

Planococcus citri populations obtained from Department of Plant Protection of Ankara University and then kept in the climate room at 28±1 °C and 65±10% RH with a 16:8 h L:D photoperiod. The potatoes used as a diet of citrus mealybug were placed at 4 °C for two weeks and then sprouted at 15-18 °C in the incubator. Mealybug eggs were gently transferred to the potatoes using the fine paintbrush then they were placed into plastic containers (13×16×6 cm). Ovisacs obtained from mealybugs, completed their development on approximately 20-23 days were transmitted on potato sprouts to keep insect cultures (Figure 1).

Figure 1. a. Rearing of *Planococcus citri*, a.potato sprouting cabinet, b. Infection with ovisacs, c. Rearing containers

Rearing of Leptomastix dactylopii Howard

Leptomastix dactylopii populations obtained from Department of Plant Protection of Ankara University and then kept in the climate room at 28±1 ºC and 65±10% RH with a 16:8 h L:D photoperiod. *L. dactylopii* was reared on both third instar nymphs and young females of *P. citri*. The mealybug infested potatoes were settled in plastic boxes with ventilation holes and then 30♀, 30♂ parasitoids were released into the boxes. Parasitoids emerging from parasitized mealybugs were steadily reared under laboratory conditions (Figure 2).

Figure 2. *Leptomastix dactylopii* production containers

Preparation of white oil

The white oil (Koruma Summer Oil), licensed by the Ministry of Agriculture and Forestry, was diluted with recommended amount of water and applied at label-recommended rate (1200 ml/100 l).

Behavior treatments

Adult parasitoids, emerged from 18 day-old mealybugs, were selected in treatments. Newly emerged 2-3 day-old (female+male) parasitoids were placed in ventilated plastic containers. They were allowed to feed and mate for 24 hours.

Choice test on airflow Y tube olfactometer

The choice test was performed using an airflow Y tube olfactometer (Akol et al. 2003) (Figure 3). The source of test odors was placed in a glass jar (250 ml capacity). Two pressure pumps (Cole-Parmer Air cadet vacuum/pressure station) pumped air into and out of the system.

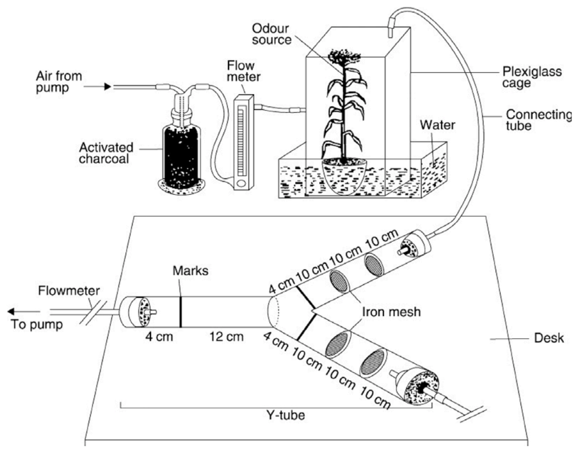

Figure 3. Y-tube olfactometer

Air from the pressure pump was passed through a carbon filter for purification, then through a flowmeter (Cole-Parmer Instrument Co., Vernon Hills, Illinois, USA) and finally split into two currents with each flow passing into an odor source flask. A second flowmeter was connected between the stem of the olfactometer and a second pump, which exhausted air out of the system. Airflow into the olfactometer was set at 100 ml/min and at the exit at 500 ml/min.

Behavioral response of mated 2-3 day-old female parasitoids to fresh air, white oil, only mealybug and mealybug with white oil was evaluated. The filter papers were sprayed with white oil and allowed to dry before using in the tests. Female parasitoids were introduced individually into the stem of the olfactometer and allowed 5 minutes to choose one of the arms of the olfactometer. Parasitoids that passed the finish line (4 cm past the section) were recorded as made a choice. Distilled water was used as a control. The experiments were conducted using three replicates including ten individuals.

Effect of white oil application on the rate of parasitization of Leptomastix dactylopii in time

2-3 day-old parasitoids were fed with honey for 24 hours. White oil at the recommended rate was applied to third instar and young mealybugs with a sprayer. One host, which was exposed with white oil, was put onto the petri dish and then one female parasitoid was released at 0, 6, 12, 18, 24-hour intervals. Parasitism behavior (host searching and parasitism) was observed for 30 minutes (Figure 4). The experiments were carried out at three replicates, each with ten hosts. Distilled water was used as a control.

Effect of white oil on mortality of Leptomastix dactylopii

Two different applications were used to determine the mortality of *Leptomastix dactylopii*. At first application, the parasitoids at 2-3 day-old were fed with honey for 24 hours and used for the experiment. Nine cm diameter filter papers in 9 cm diameter petri dishes with ventilation hole were used to determine the direct effect of the white oil. Honey-water mixture (1:1) was smeared to the lid of petri dishes to feed the parasitoid. White oil was applied to each filter paper at the recommended application rate (Figure 5) then five male and five female parasitoids were released into different petri dishes. To record the dead parasitoids, they were daily monitored for three days. The experiment was repeated at 0, 1, 3 and 5-hour intervals after application.

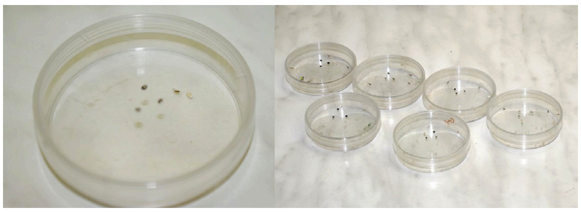

Figure 4. Experiments to determine the parasitization in time

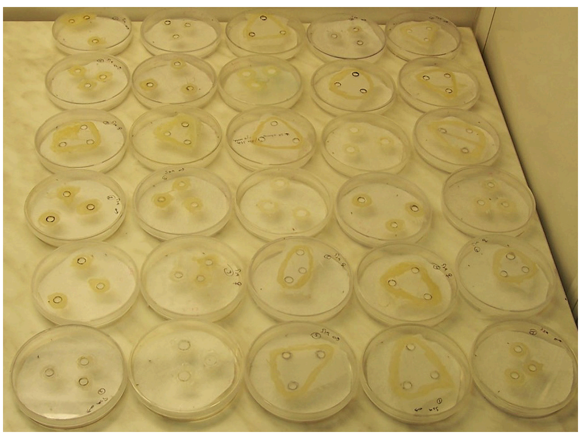

Figure 5. Studies on parasitoid mortality

At second application, the direct effect of spraying white oil on adults was also determined. Five female and five male parasitoids were released into the petri dishes and kept in the refrigerator for two minutes to stop the parasitoid activity. Then, white oil was directly sprayed to the parasitoids.

The number of parasitoids, died after both applications, was recorded. Both of the experiments were conducted using five replicates. Distilled water was sprayed to the filter paper as a control.

Statistical analysis

The difference between the two independent variations in the Y tube olfactometer selection test was determined by the Z-test. Abbott (%) formula was used to calculate adult mortality rates (Abbott 1925).

The results of the mortality rates were evaluated using the classification of the IOBC for the effects of chemicals on the natural enemies for laboratory conditions [In this scale: 1: Harmless or less harmful (<30), 2: Moderately harmful (31-79%), 3: Harmful (80-99), 4: Very harmful (>99). The Abbott formula was given below.

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

(n = number of insects)

RESULTS

Behavior treatments

Choice test with airflow Y tube olfactometer

The results obtained from the choice test were given in Figure 6 showing the tendency of parasitoids randomly occurred when fresh air (F.A.) was delivered from both tubes of the olfactometer (P>0.05).

In case of application of fresh air in one tube and host with white oil (A.H.) in the other tube, the host's preference percentage was 53.30% and this ratio was not statistically significant (P>0.05). More than half of parasitoids tended to the tube with fresh air instead of the tube with white oil (W.O.) (P<0.05). Host exposed with white oil (A.H.) was not preferred by the ratio of 66.70% compared to non-treated host (N.H) (P<0.05).

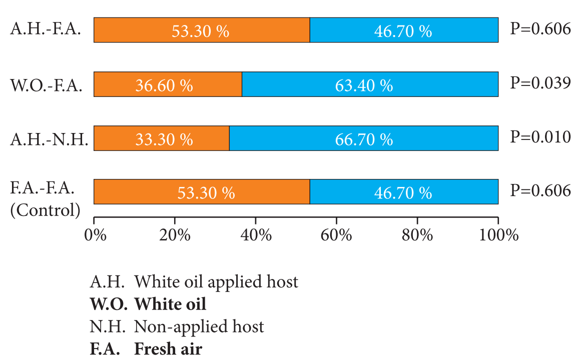

Figure 6. Choice test on airflow Y-tube olfactometer

Effect of white oil application on parasitization of Leptomastix dactylopii in time

The parasitization rates of *L. dactylopii* released at different time intervals to applied mealybugs were shown in Table 1. The results showed that the rate of parasitization in white-oil-treated mealybugs (third instar and young female) was increased in a time depended on manner. In both host ages, the rate of parasitization reached 90% after 24 h.

Table 1. Parasitization rate of *Leptomastix dactylopii* emerged from the third nymph and young female mealybugs applied with white oil according to the duration

Application time	Parasitization rate (%)	
	Third nymph n=30	**Young female n=30**
0. hour	%46.6	%33
6. hour	%46.6	%40
12. hour	%53.3	%50
18. hour	%76,6	%70
24. hour	%90	%90
Control	%96.6	%93.3

Effects of white oil on the mortality of Leptomastix dactylopii adults

According to the second application method, after spray application, all of the male and female parasitoids died. Results of the first method were shown in Table 2. Adult mortality was calculated according to the Abbott formula to determine the direct effect. The mortality ratio at female parasitoids was 91.66% immediately after treatment, 58.30% at one and three hours after application, and 29.16% at five hours after application. According to IOBC classification, the application of immediately after treatment (0-hour) was recorded as harmful, the first and the third hours' applications were determined as moderately harmful and the application of the fifth hour was classified as harmless or less harmful.

Mortality rates of male parasitoids were 95.65%, 56.50%, 47.80%, and 8.70% at immediately after treatment, 1 hour after application, 3 hours after application, and 5 hours after application, respectively. The classifications, based on IOBC criteria of the male parasitoids, were very similar to the results obtained from female individuals.

Table 2. Effect of white oil on mortality of female and male *Leptomastix dactylopii* calculated by Abbott % formula (the first method's results

Application time	Parasitization rate (%)	
	Female mortality (%) n=25	**Male mortality (%) n=25**
0. hour	91.66	95.65
1. hour	58.30	56.50
3. hour	58.30	47.80
5. hour	29.16	8.70
Control	4	8

DISCUSSION

Behavior studies have shown that although the white oil had repellent effect for parasitoids, the host showed an attractant effect for them. On the other hand, even if white oil was applied to the host, parasitoids were attracted by host odor. This can be considered as an advantage for parasitoid because *L. dactylopii* can survive even at oil-treated hosts. Baaren and Nenon (1996) investigated parasitoid's preference among the non-parasitized host, parasitized host, non-infected plant with mealybug and infected plant with mealybug, and found that *L. dactylopii* was tended to infected plant and non-parasited mealybug. In our study, the similar results were found in terms of the applied mealybug and non-applied host.

The behavior of *L. dactylopii* parasitizing *P. citri* nymphs of different ages with white oil varied at various time after treatment. When parasitoids were released at certain intervals on the white oil applied area, the number of parasitized individuals increased as the time elapsed. It was observed that when parasitoid released immediately after oil application, they started to search host after a long time cleaning behavior, but did not reach the host because of the oil droplets on the hosts. Parasitoid can find the host in a shorter time application due to the oil evaporation; the parasitism rate, therefore, steadily increased in time. It has been observed that white oil effect on adult parasitoids declined after a certain period because white oils are so volatile, and easily disappear from the application area. It was found that female parasitoids were more sensitive than male parasitoids to white oils. The reason for this is thought that because the female individuals are larger than the male ones and they are in contact with the oil in greater amounts.

One of the most appropriate spraying time is to release parasitoid short time after oil application, or to avoid white oil application at times when parasitoid activity is intense in order to increase the effectiveness of parasitoid. Siscaro et al. (2006) studied the effects of white oils on *L. dactylopii* adults after 24, 48 and 72 hours in laboratory conditions and found that more than half of the adults treated died. In another study, Suma et al. (2009) determined that the mortality of adult parasitoids was 53.2% after 72 hours of application of the recommended rate of white oil under laboratory conditions, by dry film method.

According to IOBC classification, the effect of white oils on adults of *L. dactylopii* was found "less harmful". As a result of these experiments, it was determined that parasitoid adults were directly or indirectly affected by the chemical application. For the direct applications, a death of adult parasitoids reveals that the releases must be done sometime after spraying.

In conclusion, white oil applications have adverse effects on parasitoids. These negative effects can be minimized by the regulation of the release time and planned white oil application during periods when parasitoids are active period. Additionally, white oils do not affect host finding behavior of parasitoid and block host odor. Due to the repellent effect of white oil, the parasitoids stay away from the environment for a while that prevents parasitoid adults from being affected by the oil. It may be possible to reduce the adverse effects of white oils with measures to be taken.

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ÖZET

Yapılan bu çalışmada; laboratuvar koşullarında (28±1 °C sıcaklık, 16:8 saat aydınlık:karanlık ve %65±10 orantılı nem) turunçgil bahçelerinde yoğun olarak kullanılan beyaz yağın Turunçgil unlubiti parazitoiti *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae)'nin konukçu bulma ve parazitleme davranışına etkileri araştırılmıştır. Konukçu bulma davranışı çalışmalarında hava akışlı Y tüp olfaktometre kullanılmıştır. Onsekiz gün yaşındaki unlubitlerden çıkış yapan 2-3 günlük ve çiftleşmiş dişi parazitoitlerin temiz hava, beyaz yağ, beyaz yağ uygulanmış konukçu ve beyaz yağ uygulanmamış konukçu arasındaki tercihi belirlenmiştir. Parazitoitlerin beyaz yağa oranla daha çok temiz havayı tercih ettiği ancak konukçu kokusunun beyaz yağ kokusuna göre daha baskın olduğu saptanmıştır. Parazitleme davranışının belirlenmesinde 12 ve 18 gün yaşındaki unlubitler kullanılmıştır. Uygulama dozundaki beyaz yağ konukçulara uygulanmış ve uygulamadan 0, 6, 12, 18, 24-saat sonra parazitoitler beyaz yağ uygulanmış bu konukçulara verilerek parazitleme davranışları incelenmiştir. Çalışma sonucunda *L. dactylopii*'nin parazitlediği konukçu sayısının her iki konukçu yaşında da uygulama zamanı ile doğru orantılı olarak arttığı belirlenmiştir.

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