

Efficacy of biorational insecticides against Dubas bug, *Ommatissus lybicus* (Hem.: Tropicuchidae) in a date palm orchard and evaluation of kaolin and mineral oil in the laboratory

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Abstract

In this study, mineral oil and kaolin as alternatives to conventional pesticide (diazinon) were evaluated to manage Dubas bug, *Ommatissus lybicus* de Bergevin, in the field and laboratory. In the field experiment, effect of four treatments was evaluated against population density (first and second nymphal stages) and honeydew production (as main damage) of Dubas bug. A single application of each product was applied at the recommended rates. Results showed that kaolin, mineral oil and diazinon were statistically similar in decreasing the population density and damage of Dubas bug. This result makes kaolin and mineral oil as potential alternatives to conventional insecticides in date palm orchards. In the laboratory, mineral oil was effective on egg mortality. In settling choice test, the ability of Dubas bug to discriminate between kaolin-treated and kaolin-untreated leaflets increased significantly over the time and females laid more eggs on untreated leaflets than kaolin-treated leaflets. Results of no-choice test revealed that the leafhoppers could lay eggs on treated leaflets as many as on untreated leaflets.

Keywords: kaolin, mineral oil, date palm, Dubas bug, biorational pesticides

چکیده

تأثیر حشره‌کش‌های کم‌خطر روی زنجبرک خرما، *Ommatissus lybicus* (Hem.: Tropicuchidae). در باغ خرما و ارزیابی کائولین و روغن معدنی در شرایط آزمایشگاهی

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در این تحقیق، به‌منظور مدیریت زنجبرک خرما، *Ommatissus lybicus* de Bergevin، آفت‌کش‌های کم‌خطر روغن معدنی و کائولین به‌عنوان جایگزین آفت‌کش متداول (دiazinon) در باغ خرما و شرایط آزمایشگاهی بررسی شدند. در شرایط باغ، اثر چهار تیمار علیه تراکم جمعیت (سن ۱ و ۲ مرحله پورگی) و تولید عسلک (به‌عنوان خسارت اصلی) زنجبرک خرما بررسی شد. کاربرد تیمارها در یک نوبت و در مقادیر توصیه‌شده انجام شد. نتایج نشان داد اثر تیمارهای diazinon، کائولین و روغن معدنی در کاهش تراکم جمعیت و خسارت آفت مشابه بود. این نتایج، کائولین و روغن معدنی را به‌عنوان جایگزین‌های بالقوه حشره‌کش‌های متداول در باغ‌های خرما معرفی می‌کند. در بررسی‌های آزمایشگاهی، روغن معدنی در مرگ و میر تخم زنجبرک خرما مؤثر بود. در آزمایش‌های انتخابی، که برای استقرار و تخم‌ریزی زنجبرک خرما بین برگچه‌های شاهد و تیمار شده با کائولین انجام شد، توانایی زنجبرک در تشخیص میزبان با گذر زمان بیش‌تر شد و حشرات ماده تعداد تخم بیش‌تری در برگچه‌های شاهد گذاشتند. در آزمایش‌های غیر انتخابی، زنجبرک خرما در هر دو تیمار شاهد و کائولین به یک اندازه تخم گذاشت.

واژگان کلیدی: کائولین، روغن معدنی، درخت خرما، زنجبرک خرما، آفت‌کش‌های کم‌خطر

Introduction

The Dubas bug, *Ommatissus lybicus* de Bergevin, is a key pest of date palm, *Phoenix dactylifera* L., in Iran (Gharib, 1966), several countries in the Middle East and North Africa (Hussain, 1963; Gharib 1966; Bitaw & Ben-Saad, 1990). The nymphs and adults suck the sap and secrete heavy honeydew over the leaf surface and fruits. The fruits of infested trees are less sweet and small in size (Hussain, 1963).

The most common method used by date palm growers to control the pest is foliar application of pesticides such as diazinon, chlorpyrifos methyl and sometimes imidacloprid (Anonymous, 2008). Due to

the ecological and human health risks of insecticide treatments, there is a great tendency toward using of biorational pesticides.

Mineral oils can be an alternative treatment to control Dubas bug. They seem to be promising insecticides for IPM programs, because they are not expensive, are highly toxic to insects, have very low toxicity to vertebrates, have few deleterious environmental effects, and no insect resistance has been reported (Davidson *et al.*, 1991; Fernandez *et al.*, 2006). Kaolin has also been introduced as a novel way to suppress arthropod pests of food crops (Glenn *et al.*, 1999; Unruh *et al.*, 2000; Showler, 2002; Glenn &

Puterka, 2005; Karagounis *et al.*, 2006; Hall *et al.*, 2007). When plants are sprayed with kaolin, a physical barrier is formed on plant surfaces that can act as a deterrent to insect settling, oviposition and feeding (Glenn & Puterka, 2005). As a result of its mode of action, the side effects of kaolin on non-target insects and spiders are generally considered to be low (Showler & Setamou, 2004; Glenn & Puterka, 2005). Despite these suppressing effects on a range of pest species, some studies suggest that kaolin can increase insect infestation rates (Showler & Armstrong, 2007; Marko *et al.*, 2008).

The goals of this study were (1) to demonstrate the efficacy of conventional and biorational pesticides against Dubas bug nymphs and honeydew production in the field, (2) to determine the deterrent effect of kaolin particle film on Dubas bug oviposition and adult settling on date palm leaflets in the laboratory, and (3) to determine the effects of kaolin and mineral oil on viability and hatching rate of Dubas bug eggs.

Materials and methods

Field trial

This trial was conducted in date palm groves of Farashband (Southern part of Fars Province, Iran) during April to May of 2011. Ten-year old date palm trees (variety 'Zahdi' - 6 m high) were used in this study. The trees were at least eight meters apart. The experimental design was a randomized complete block with four treatments, three blocks and three palms in each plot.

Treatments used in this study were: kaolin (Sepidan[®] WP, a.i. 95%, Kimia Sabzavar Co., Tehran, Iran) at 50 g/L; diazinon (Diazinon[®] EC, a.i. 60%, Golsam Gorgan Chemicals Co., Tehran, Iran) at 2 ml/L; and mineral oil (Volck-92[®], a.i. 80.2%, Iran Chemical Production Co., Tehran, Iran) at 10 ml/L. The untreated trees served as a control. A single application of each product was applied at the company recommended rates on 24 April 2011. Insecticide sprays were applied using a motorized hydrolic sprayer equipped with a lance. A continuous

agitator kept the material in suspension. It had a cone nozzle and a pressure of 300 psi. Spraying was conducted on the first and second nymphal stages of the population.

To evaluate the efficacy of treatments against nymphal stages and honeydew production of Dubas bug, two sampling methods were used. For the sampling of leafhopper nymphs, four leaflets were excised carefully from the middle part of the second frond row at four main geographical directions of each palm (a total of 16 leaflets per palm). The leaflets were placed in plastic bags and were taken to the laboratory for recording the number of nymphs per leaflet. The records conducted one day before and three times after treatments at 8 to 9-day intervals.

For sampling honeydew production, a scale of 0 to 4 was used as follows: 0 = not detected, 1 = low (only on leaves), 2 = scattered droplets on fruit stalk and bunches, 3 = more than 50% of bunches were covered, and 4 = bunches and fruits were completely covered (Puterka *et al.*, 2005; Awamleh *et al.*, 2009). Sampling of honeydew was recorded only on two final sampling dates due to the lack of honeydew in the beginning of this trial.

Number and honeydew scores of leafhopper nymphs were analyzed with two-way analysis of variance (ANOVA) using the SPSS version 10 (SPSS, 1999) and significant differences among means were compared using the Fisher's LSD test. The data were normally distributed and did not require transformation before statistical analysis. The effectiveness of different treatments was estimated as the percentage reduction in population according to the Henderson-Tilton formula (Henderson & Tilton, 1955): Efficacy % = $1 - (T_a/T_b \times C_b/C_a) \times 100$, where T_b and C_b are pre-treatment densities and T_a and C_a are post-treatment densities of nymphs in the treated (T) and control (C) plots, respectively.

Laboratory tests

The experiments were carried out in the laboratory (27 °C, 70% RH, and a 14L: 10D

photoperiod), Agricultural Jihad Organization of Darab (Fars Province, Iran). Plants were obtained from a commercial orchard as offshoots which separated from mature date palms by experts and were planted in plastic pots (38 × 38 × 27 cm). 'Khasui' variety of date palm was used in the experiments. The potted plants were irrigated and fertilized for one year to establish completely and be suitable for laboratory experiments. Insects were collected from date palm orchards and were reared in the laboratory on the potted plants. To obtain adult leafhoppers with the same age, final instars were confined in clip cages until adult emergence. After emergence, females were paired individually with single males emerged on the same day. Males were differentiated from females by the black ovipositor of females. The effect of kaolin on adult oviposition rate, and on settling of adults on leaflets over time (in both choice and no-choice tests) were studied. The rate of egg viability and hatching were determined in kaolin and mineral oil in the laboratory.

Choice test

Leaflets and small slivers of the wood underneath them were individually cut from midrib and then a piece of wet cotton wool was wrapped around the slivers. This kept humidity close to saturation so that the leaflets remained fresh for at least two weeks. To do the test, one experimental arena, a round plastic cage with a diameter of 70 cm and height of 21 cm was used. In each replication (cage), eight leaflets were used, half of which were dipped for 20 seconds in a 5% slurry kaolin powder and the other half were dipped in tap water and allowed to dry before inserting into the cage. Treated and untreated leaflets were arranged with altering placement on the periphery of the cage and 30 adult leafhoppers (15 males and 15 females) were released at the centre of the cage. The cage was enclosed with fine mesh screen. Observations were made daily and the number of adult leafhoppers, settled on each leaflet of treatments, and the number of eggs

deposited by females on each leaflet were recorded for 10 days. A total of eight replicates were completed.

Independent samples *t*-test was used to compare the adult settling and oviposition between control and kaolin-treated leaflets for each day separately. For each treatment, repeated measures ANOVA was used to assess changes over time in daily number of adult settling and of eggs laid by female leafhoppers. Fisher's LSD test was used to compare treatment means (SPSS, 1999). Data were subjected to arcsine square-root transformation and other data were log transformed (data + 1).

No-choice test

In this test, leaflets were prepared and were treated as described above in the choice test. Four leaflets were dipped into either a 5% slurry kaolin powder or tap water and allowed to dry before inserting into a cage (23 × 12 × 6 cm). Ten adult leafhoppers (five males and five females) were released into the cage. Observations were made daily for seven days and the number of eggs deposited by females in each treatment were recorded. The plot design was a completely randomized design with eight replications.

Independent samples *t*-test was used to compare the number of eggs in control and kaolin-treated leaflets for each day separately. Repeated measures ANOVA was also used to compare the number of eggs laid in different days for each treatment separately. Fisher's LSD test was used to compare treatment means (SPSS, 1999).

Effect of kaolin and mineral oil on egg viability and hatching rate

One-year old potted plants with at least six appropriate palm leaves were used for this experiment. The experimental design was a completely randomized design with three treatments and three replications (each plot consisted of three potted plants). On each potted plant 20 leaflets were marked and individually caged in a plastic cylinder cage (18.5 × 2.5 cm). The

cages were covered with enough fine mesh screens for ventilation and were sealed by foam on leaflets. More than 15 mated females were released in each cage and were allowed to oviposit for two days. The number of eggs on each leaflet was counted, and then the whole potted plants were sprayed with kaolin (50 g/l), mineral oil (10 ml/l) or tap water as a control until run off by using a hand-held sprayer. After plants were dried, the marked leaflets containing leafhopper eggs were caged again and monitored to determine the number of hatched, unhatched-unviable or unhatched-viable eggs. The monitoring lasted 70 days and unhatched eggs which were more than 70 days old were considered as unhatched-viable (when egg appeared fresh and eye spots within the egg were present) or unviable (when an egg was shriveled or turbid).

Data in the experiment were transformed before statistical analysis. The data were subjected to one-way ANOVA and means were compared using Fisher's LSD test (SPSS, 1999).

Results

Field trial

The efficacy of pesticides against Dubas bug nymphs at the four observations performed in 2011 is shown in table 1. There were no significant differences among treatments ($F_{3,6} = 1.25$, $P = 0.37$) or blocks ($F_{2,6} = 1.16$, $P = 0.38$) before application of treatments. All products were significantly effective to control the pest at the first and second post-treatment observation. This is reflected in the mean number of leafhoppers or efficacy. However, mean number of leafhoppers at the

last post-treatment observation was not different among treatments (table 1).

Effect of insecticides on the honeydew production rate (main damage) of Dubas bug is shown in table 2. On the 17th and 25th day after treatment, efficacy of kaolin, mineral oil and diazinon treatments were significantly different from control.

Laboratory tests

Choice test

On all days, *t*-test analysis showed that more adults settled on the control leaflets than on the kaolin-treated leaflets. Percentage of settled adults varied significantly in time within control ($F_{9,63} = 5.64$, $P < 0.001$) and kaolin-treated leaflets ($F_{9,63} = 5.64$, $P < 0.001$), as it increased gradually through days in control ($R = 0.57$, $n = 80$, $P < 0.001$) and decreased in kaolin ($R = -0.57$, $n = 80$, $P < 0.001$) treatment (fig. 1). This indicated that adult ability to discriminate between treated and untreated hosts would increase over time.

In the case of number of eggs laid per day by females, *t*-test showed significant differences between control and kaolin treatments on all days tested except days 1 and 3 (fig. 2). Repeated measures ANOVA revealed no significant changes over time in daily number of eggs in both the control and kaolin treatment ($F_{9,63} = 1.113$, $P = 0.367$; and $F_{9,63} = 1.994$, $P = 0.055$, respectively); however, the lowest number of eggs in control and kaolin treatments were found on the first and last day after treatment, respectively (fig. 2).

Table 1. Mean (\pm SE) number of *Ommatissus lybicus* nymphs per leaflet (density) and efficacy of treatments in the field trials.

Treatments	8 days after treatment		17 days after treatment		25 days after treatment	
	Density	Efficacy	Density	Efficacy	Density	Efficacy
Kaolin	0.95 (\pm 0.57) a	82.7	0.56 (\pm 0.23) a	80.0	0.15 (\pm 0.03)	81.5
Mineral oil	2.22 (\pm 0.96) a	66.4	0.48 (\pm 0.39) a	85.7	0.03 (\pm 0.03)	96.6
Diazinon	0.66 (\pm 0.20) a	95.7	0.57 (\pm 0.09) a	92.9	0.28 (\pm 0.19)	87.8
Control	6.53 (\pm 0.33) b	-	3.33 (\pm 0.29) b	-	0.97 (\pm 0.57)	-
	$F_{3,6} = 27.27$ $P = 0.001$	$F_{2,4} = 2.66$ $P = 0.184$	$F_{3,6} = 29.17$ $P = 0.001$	$F_{2,4} = 0.14$ $P = 0.87$	$F_{3,6} = 2.46$ $P = 0.16$	$F_{2,4} = 1.56$ $P = 0.31$

Means in a column with the same letter are not significantly different (LSD, $P = 0.05$).

No-choice test

T-test analysis showed that at the first two days post-treatment the females laid significantly more eggs in control leaflets than in kaolin-treated leaflets. But after that, they laid an equal number of eggs in both control and treated leaflets (fig. 3), indicating that there was habituation of females to the kaolin treatment. Daily number of eggs laid by females changed significantly in time within control ($F_{6,42} = 6.296$, $P < 0.001$) or treated groups ($F_{6,42} = 6.297$, $P < 0.001$), as it was a periodic pattern in control treatment and increased over time in kaolin (fig. 3).

Effects of kaolin and mineral oil on egg viability and hatching rate

Pre-treatment means \pm SEM (standard error of mean) of eggs were 20.55 ± 1.65 , 21 ± 2.05 and 19.4 ± 0.78 per leaflet on kaolin, mineral oil and control treatments, respectively. Results showed that treatments had a significant effect on percentage of egg hatched ($F_{2,6} = 23.86$, $P = 0.001$) and unhatched-unviable ($F_{2,6} = 25.53$, $P = 0.001$). The highest percentages of unhatched-unviable eggs were observed in mineral oil treatment. Percentage of unhatched-

viable eggs were not statistically different in all treatments ($F_{2,6} = 1.77$, $P = 0.25$). There was no significant difference between kaolin and control in respect of any egg conditions (fig. 4).

Discussion

The field results showed that the two biorational products (kaolin and mineral oil) and the conventional insecticide (diazinon) were statistically similar in decreasing the population density and damage of Dubas bug. Kaolin has been shown to be effective in suppression of many pests such as leafhoppers (Glenn *et al.*, 1999; Glenn & Puterka, 2005; Marko *et al.*, 2008), aphids (Karagounis *et al.*, 2006), psyllids (Puterka *et al.*, 2000, 2005; Hall *et al.*, 2007) and Lepidoptera (Unruh *et al.*, 2000; Sisterson *et al.*, 2003). It seems that kaolin effects on Dubas bug was more due to disrupted feeding than causing mortality because the amount of honeydew which is an index of feeding activity (Puterka *et al.*, 2005) was lower in kaolin treatment than others (but not statistically significant). While, in terms of reduction in pest density, kaolin treatment was ranked as medium

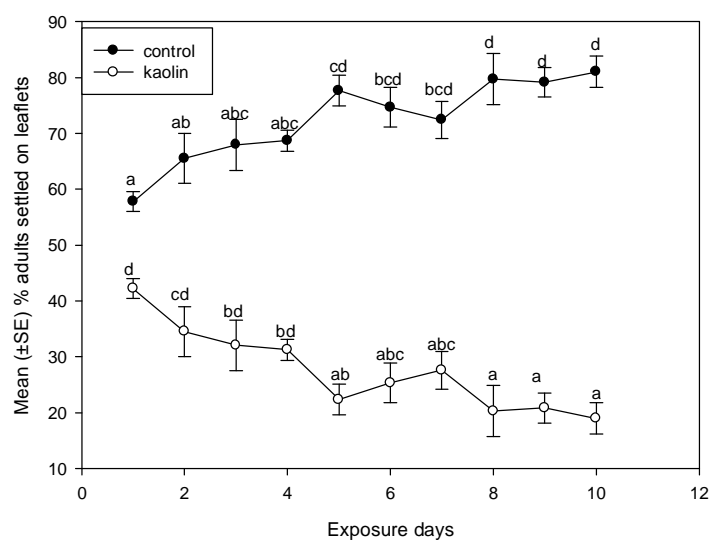


Fig. 1. Mean (\pm SE) percentage of adults *Ommatissus lybicus* settled daily on control or kaolin-treated leaflets in choice test. For each treatment, means followed by the same letter are not significantly different (LSD, $P = 0.05$).

compared with others. Feeding inhibition has also been documented for oblique banded leafroller, *Choristoneura rosaceana* (Harris) (Knight *et al.*, 2000) and potato psyllid, *Bactericerca cockerelli* Sulc (Liu & Trumble, 2004).

As it is evident in control plots, the density of Dubas bug on leaflets decreased in the last observation. This could be attributed to the changes in bug's distribution pattern within the trees as the season

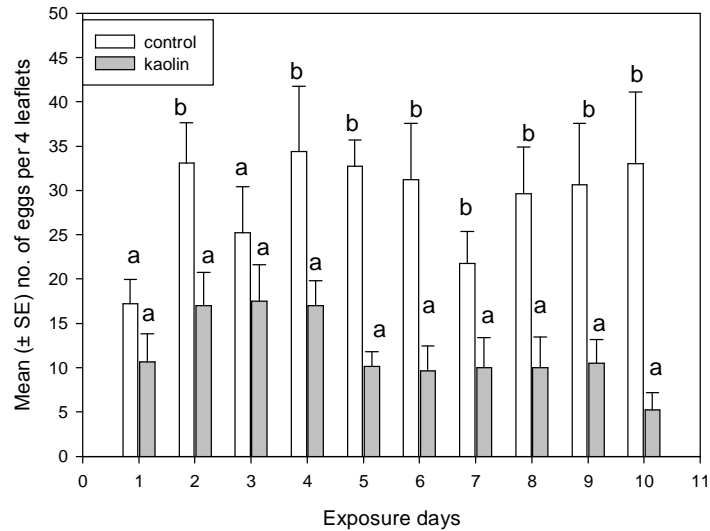


Fig. 2. Mean number of eggs (\pm SE) laid daily by females *Ommatissus lybicus* on control or kaolin-treated leaflets under choice conditions. For each day, means followed by the same letter are not significantly different (*t*-test, $P = 0.05$).

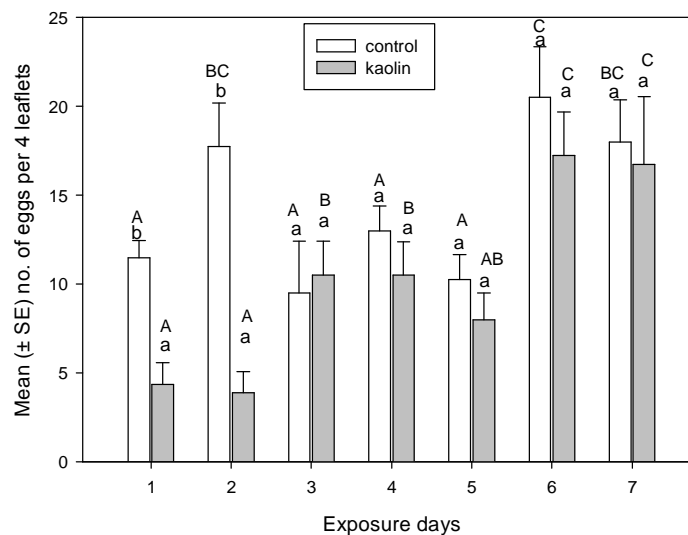


Fig. 3. Mean number of eggs (\pm SE) laid daily by females *Ommatissus lybicus* on four leaflets (either treated with kaolin or tap water) under no-choice condition. For each day, means followed by the same small letter are not significantly different (*t*-test, $P = 0.05$). For each treatment, means followed by the same capital letter are not significantly different (LSD, $P = 0.05$).

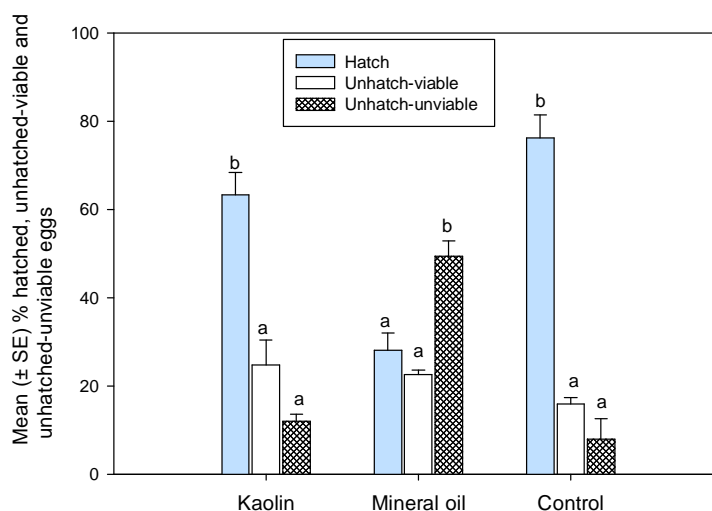


Fig. 4. Mean (\pm SE) percentage of hatched, unhatched-viable and unhatched-unviable eggs of *Ommatissus lybicus* in leaflets treated by kaolin, mineral oil or water. For each egg situation, means followed by the same letter are not significantly different (LSD, $P = 0.05$).

progresses. Hussain (1963) also noted that in order to escape the summer heat, the nymphs of the overwintering generation migrate towards the bases of the young fronds (the heart of the date palm) and complete the migration during the last week of June.

Table 2. Mean infestation scores (honeydew production) (\pm SE) of *Ommatissus lybicus* nymphs per tree in the field trials.

Treatments	17 days after treatment	25 days after treatment
Kaolin	0.22 (\pm 0.11) a	0.33 (\pm 0.19) a
Mineral oil	0.33 (\pm 0.19) a	0.66 (\pm 0.19) a
Diazinon	0.33 (\pm 0.19) a	0.89 (\pm 0.48) a
Control	1.22 (\pm 0.29) b	3.66 (\pm 0.19) b
	$F_{3,6} = 7.56$	$F_{3,6} = 24.74$
	$P = 0.018$	$P = 0.001$

Means in a column with the same letter are not significantly different (LSD, $P = 0.01$).

The effect of horticultural mineral oils is well known for several decades against many pests (Bradley *et al.*, 1966; Simons *et al.*, 1977; Awamleh *et al.*, 2009; Martoub *et al.*, 2011). The cost of the kaolin and mineral oil treatments are less than the cost of most conventional insecticide treatments. Further, with their low impact on the environment (Glenn *et al.*, 1999; Talebi-Jahromi, 2006), the use of kaolin and mineral

oil as crop protectants in organic farming is highly appealing. Karagounis *et al.* (2006) evaluated the efficacy of organic pesticides (kaolin, mineral oil and insecticidal soap) as alternatives to chemical synthetic insecticide (imidacloprid) for the control of *Myzus persicae* (Sulzer) in a peach orchard. They found that the three alternative products, especially kaolin are promising for aphid control in organic peach orchards.

Repellency of kaolin in choice tests has also been found in other insects, including *Anthonomus grandis* Boheman (Showler, 2002) and *Homalodisca coagulata* (Say) (Puterka *et al.*, 2003). In the choice assay, Dubas bug ability to discriminate between the kaolin-treated and control leaflets increased significantly over time for settling. This ability was also increased over time (but not statistically) for oviposition. Host discrimination over time in choice test has also been shown by Showler (2003) and Puterka *et al.* (2005).

Results of no-choice test were interesting as they revealed that confining in a dish with only treated leaflets, the leafhoppers will be adapted to the situation two days after treatment and lay eggs in treated leaflets as much as untreated leaflets. This result is consistent with the results observed in choice test. More field

investigation on the Dubas bug oviposition behavior is needed to clarify that to what extent the field situation is likely to reflect the choice or no-choice scenarios. However, it seems that one of the important problems in plant protection against pests is the potential of insects to get habituated rapidly to deterrent materials. This situation has been demonstrated by Bomford & Isman (1996) for tobacco cutworm, *Spodoptera litura* Fabricius, larvae feeding on cabbage discs treated with azadirachtin, and Gokce *et al.* (2006) for Colorado potato beetle, *Leptinotarsa decemlineata* Say, larvae feeding on potato leaves treated with five different plant extracts. In the available studies related to kaolin treatments under no-choice tests, little information is available which hints directly on the effect of time on insect habituation. For example, Puterka *et al.* (2005) noted that very few eggs were produced by pear psylla, *Cacopsylla pyricola* Förster under no-choice condition (particle film treatments) over the 4-day period. Reasons for the difference in results may be related to difference in host and insect species, material and environmental conditions. For example, the ovipositor of Dubas bug has two saw-like barbs that could use them to make hole (0.17-0.2 mm in diameter and 0.4-0.5 mm. deep) in the leaf tissues (Hussain, 1963). Having such a strong ovipositor may distance every barrier for oviposition. Habituation to deterrent materials may be mitigated in insects by presenting mixtures of different deterrents together. For example, Reitz (2008) reported that combination of essential oils and kaolin can significantly reduce the incidence of thrips-transmitted tomato spotted wilt virus. The possible combinations with insecticides and fungicides are important question to answer in the use of kaolin particle film technology (Marko *et al.*, 2008). Sisterson *et al.* (2003) found that combination of the pyrethroid lambda-cyhalothrin and kaolin provided the best control of pink bollworm, *Pectinophora gossypiella* (Saunders), followed by kaolin alone, and the pyrethroid alone.

Mineral oil that was applied directly to the leafhopper eggs on date palm leaflets had significant mortality effect on egg of the pest. One of the best strategies to control overwintering eggs of pests is application of mineral oils. This material has proven to be efficient in suppressing the eggs of several pests by reducing hatching frequency as for example in several aphid species (Lawson & Weires, 1991; Iversen & Harding, 2007). These results could also be seen in the case of Dubas bug which has a long incubation period (50 and 141 days for summer and overwintering generations, respectively) and overwinters as egg stage (Hussain, 1963). However, kaolin treatment did not affect the egg mortality. As Hall *et al.* (2007) observed no effect of kaolin application on egg viability on Asian citrus psyllid, *Diaphorina citri* (Kuwayama). Kaolin particle films have also been shown ineffective on egg viability of codling moth, *Cydia pomonella* (L.) (Unruh *et al.*, 2000), oblique banded leafroller, *C. rosaceana* (Knight *et al.*, 2000) and pear psylla (Puterka *et al.*, 2005).

In conclusion, results of the present study show that mineral oil and kaolin could be used as alternatives to diazinon for managing Dubas bug population. Mineral oil which is considered as an organic pesticide revealed to be effective on damage reduction and egg mortality. Kaolin was found to be an effective product to reduce pest damage. However, more field investigations are needed to observe the effect of kaolin on Dubas bug oviposition, natural enemies, pollinators, date palm horticulture and on some date palm diseases, especially, bunch fading which has decreased date production in recent years in Iran. It is also necessary to study the side effects of kaolin on human health in chronic toxicity studies. Further research to improve the efficacy of kaolin, particularly in combination with other materials (e.g. plant extracts or insecticides) is warranted to prove its usefulness as part of an IPM program in date palm orchards.

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