

Comparison of mineral spray oil, Confidor, Dursban, and Abamectin used for the control of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), and an evaluation of the activity of this pest in citrus orchards in northern Iran

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Abstract: The efficacy of three types of mineral spray oil (MSO), and Abamectin plus MSO to control the citrus leafminer *Phyllocnistis citrella* Stainton were compared with the conventional broad spectrum pesticides, Confidor, and Dursban, in two citrus orchards in northern Iran. Differences were found among the various concentrations of MSO in the number of mines and live larva per leaf, sampled 5 days after the application of 3 sprays at an interval of 5 days. Mineral spray oil applied at a rate of $\geq 0.65\%$ in water, showed no significant differences when compared with Confidor, and Dursban. Abamectin plus MSO at a rate of 0.02% plus 0.50% in water respectively, provided the highest level of control and reduced the population of larva up to 85%. Generally, a concentration of $\geq 0.65\%$ MSO in water caused a significant damage reduction. However, by reducing the MSO concentration, the rate of control was decreased. In another field experiment conducted in mature citrus orchards, the effect of two pest management types (applying just MSO vs. usage of synthetic pesticides) on the activity of *P. citrella*, was evaluated. The comparison results indicated that there is more *P. citrella* damage in orchards under pressure of synthetic pesticides than in orchards in which the synthetic pesticides were eliminated for years.

Key words: mineral oils, *Phyllocnistis citrella*, pesticides, sustainable agriculture

Introduction

Phyllocnistis citrella Stainton is a native pest of citrus in tropical and subtropical Asia (Neale *et al.* 1995). It has also been reported all over the world, including Africa, Australia, the Caribbean, America, and the Middle East (Heppner 1993; Heppner and Dixon 1995; Knapp *et al.* 1995; Pena *et al.* 1996; Hoy and Nguyen 1997; Legaspi *et al.* 2001; Diez *et al.* 2006). The activity of *P. citrella* was reported from the Khorasan and Fars Provinces by Farahbakhsh in 1961 (Besheli 2004) and the activity gradually spread throughout the northern provinces of Iran. *P. citrella* is a microlepidopteran. During the larval stages, it mines the adaxial and abaxial surfaces of newly formed leaves. Injured young leaf curls become chlorotic then necrotic (Pena *et al.* 1996). Larval damage is not limited to leaf mining, but also increases the susceptibility of plant leaves and trees to pathogens, including citrus canker bacterium *Xanthomonas axopodis* pv. *citri* (Sohi and Sandhu 1968; Cook 1988; Schubert and Sun 1996; Gottwald *et al.* 1997, 2002). According to Beattie and Smith (1993), only two mines in each leaf is enough to reduce the growth of trees to less than five years. Currently, the control of *P. citrella* is done by a variety of synthetic broad-spectrum pesticides (Beattie *et al.* 1995). Their long-term use may cause a non-satisfactory performance, and an outbreak of other pests

(Beattie *et al.* 1995). Natural enemies of *P. citrella* have been reported (Chen *et al.* 1989; Amalin *et al.* 1996; 2001a, 2001b, 2002; Hoy *et al.* 2007; Xiao *et al.* 2007). According to Xiao *et al.* (2007), mortality of *P. citrella* as a result of natural enemies can be as high as 89%. Environmental pollution results from the application of pesticides. Such applications cause a biological imbalance (Damavandian 2007). There is also an increasing demand for organic citrus orchards. Thus, some compounds must be found to replace the conventional synthetic insecticides originally used to control *P. citrella*. Mineral oils are currently regarded as more environmentally friendly than synthetic pesticides. Such oils are again becoming an essential part of many Integrated Pest Management (IPM) programs for agricultural crops worldwide. The integration of mineral oils is mainly because they are effectively non-toxic to vertebrates (Beattie and Smith 1996), degrade relatively quickly in the environment (Davidson *et al.* 1991; Beattie *et al.* 1995), and have never been associated with resistance or outbreaks of secondary pests (Beattie 1990; Beattie and Smith 1993). According to Kim *et al.* (2010), mineral oils can control a range of pests and can replace synthetic pesticides in organic orchards. The effectiveness of mineral oils for the control of *P. citrella* has also been demonstrated in Australia (Beattie *et al.* 1995) and China (Rae *et al.* 1996). The

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aim of this research was to improve the management of *P. citrella* while taking into account the weather conditions of the Mazandaran province. The goal is to reduce applications of conventional synthetic insecticides in the citrus orchards of Iran's northern province.

Materials and Methods

Field trials

Two experiments were conducted in adjunct citrus orchards on the outskirts of Babolsar (Latitude 36°43'N, Longitude 52°39'E, and Altitude -21m *asl*) in northern Iran. The citrus trees, cultivar *Citrus sinensis* (L.) var. *novel*, were five years old. The trees were closely planted with an inter-row distance of 5 m. The distance between trees within a row was 3 m. The trees were inspected individually and all unhealthy, stunted trees were excluded from the experiments. A completely randomized design was used for both experiments with the provision that no single row contained two replicates of the same treatment. There were three replicates per treatment and two adjacent trees per replicate. Three weeks before the experiments commenced, new foliar shoots were hand pruned from the trees, and 5 days later, urea (45% granules) was applied to the soil around each tree at a rate of 200 g per tree and then the trees were irrigated to induce a uniform flush growth. Specifications of the oils and the pesticides that were used are shown in table 1.

Spraying of trees was done using a 100-l engine Honda sprayer (GX120T1, 160T1, 200T) and it began with operators standing with their right shoulder facing a tree and the spray gun aimed at the foliage, at an angle of 45° above horizontal. Sprays were then applied until run-off, with operators walking slowly in a clockwise direction around the tree. When the starting point was reached, the procedure was repeated in the opposite direction. This method resulted in - 3 l of spray mix being applied to each tree. After each treatment, the Honda sprayer was thoroughly rinsed with water to avoid any effect on the next treatment.

Experiment 1

The block in which this experiment was conducted, consisted of eight rows. Each row included about 20 trees. Eleven treatments in this experiment were compared; with each other and with the control. At the first signs of damage, when the largest leaves of the branches were

about 10 cm, the mineral oil treatment of *P. citrella* was started on 16, 20 and 24 July 2010, and other treatments were used according to the company's recommendations, on 16 July 2010. Ten flushes of each tree (60 flushes per treatment) were sampled. When flushes of a tree were fewer than ten, all flushes were marked and sampled. Flushes were hand pruned 5 days after the last spray, placed in labelled plastic bags, stored in a cool room at 5°C, and assessed within 3 days. Assessments involved: the number of mines per leaf and the number of live larva of *P. citrella*. Foliage was also inspected for any symptoms of phytotoxicity such as necrotic spots. Treatments used included: Confidor, Dursban, Ghazal mineral oil (EC) with concentrations of 100, 500, and 900 ml, in 100 l of water, Ghazal, and Volk mineral oils with concentrations of 100, 500, and 900 ml, in 100 l of water, and only water as a control.

Experiment 2

The experimental block consisted of eight rows and each row includes 18 trees. Eleven treatments in this experiment were compared with each other and with the control. Dates, way of sampling, and evaluation methods were as described above. Treatments used in this experiment include: Confidor, Dursban, Ghazal mineral oil (EC) with concentrations of 125, 250, 375, 500, 650, 800, and 950 ml, in 100 l of water, and Ghazal mineral oil (EC) with concentrations of 350 and 500 ml, in 100 l of water plus Abamectin, and just spray water as the control.

Experiment 3

Comparison of two types of citrus pest management on the abundance of P. citrella

In order to evaluate the effects of synthetic pesticides on the activity of *P. citrella*, in Mazandaran citrus orchards, two regions, the Kallebast and Moghricola, were considered. In each region two orchards with more than one hectare in area were selected. In one orchard of each region, the common method of pest control (synthetic pesticides) was used. In another orchard for three consecutive years starting in 2007, only mineral oils were applied. Fifteen trees were randomly selected in each of the four studied orchards. In the early season before pests began to cause damage, in any main geographical direction, eight main branches were marked (a total of 32 branches on each tree). Two weeks after the first damage was ob-

Table 1. Specifications of the mineral oils and pesticides that were used

Common name	Trade name	Chemical group	Formulation	LD ₅₀ [mg/kg]	Manufacturer
Mineral oil	Ghazal	mineral oil	80% O	4,300	Ghazal shimi
Mineral oil	Ghazal (EC)	mineral oil	90% O	4,300	Ghazal shimi
Mineral oil	Volk	mineral oil	80% O	4,300	Arya shimi
Imidacloprid	Confidor	imidazol	35% SC	450	Moshkfam pars
Chlorpyrifos	Dursban	organophosphate	40.8% Ec	135-163	Shimi keshavarz
Vertimac	Abamectin	avermectin	1.8% Ec	10	Shimi keshavarz

O - oil; SC - suspension concentrates; Ec - emulsion concentrates

Table 2. The average number of mines per leaf and the average number of live larva per leaf in treatments

Treatment	Average no. of mines per leaf \pm SD	Average no. of live larva per leaf \pm SD
The control	3.88 \pm 3.02	0.73 \pm 0.68
Confidor*	0.71 \pm 0.57	0.007 \pm 0.11
Dursban	2.16 \pm 1.2	0.12 \pm 0.09
Mineral oil (EC) 100 ml	1.3 \pm 1.0	0.11 \pm 0.14
Mineral oil (EC) 500 ml*	1.0 \pm 0.90	0.50 \pm 0.12
Mineral oil (EC) 900 ml*	1.06 \pm 0.65	0.03 \pm 0.05
Mineral oil (Ghazal) 100 ml	2.0 \pm 1.0	0.20 \pm 0.27
Mineral oil (Ghazal) 500 ml	2.01 \pm 2.07	0.22 \pm 0.53
Mineral oil (Ghazal) 900 ml	1.33 \pm 0.61	0.06 \pm 0.07
Mineral oil (Volk) 100 ml	1.59 \pm 0.56	0.28 \pm 0.35
Mineral oil (Volk) 500 ml	2.05 \pm 2.04	0.13 \pm 0.16
Mineral oil (Volk) 900 ml	1.49 \pm 0.96	0.10 \pm 0.06

*significant difference when compared with the control; SD – standard deviation

served, sampling was started. In each step, two branches in any direction (from the eight previously marked branches) and a total of eight branches separated from each tree, were individually put in one plastic bag each, and transferred to the laboratory. Live larva numbers were counted by Stereomicroscope. Sampling was conducted during the two years; each year during three steps with 15 days interval (22 July, and 12, 17 August 2010) and (15, 29 July and 12 August 2011). The average number of live larva that was counted in each orchard was compared using Fisher's pairwise comparison.

Statistical analyses

In Experiments 1 and 2, dependent variables included in the analysis were the average number of mines and live larva on each sampled leaf of a flush. To stabilize variance and improve the fit of data to a normal distribution, an appropriate transformation was used for each variable. An appropriate transformation used for mines per leaf was the square root ($\sqrt{\cdot}$), and for the number of live larva, the appropriate transformation used was $\log(\sqrt{\cdot} + 1)$. The effect of the oil and insecticide treatments on the leaf miner was assessed using a general linear model analysis of variance (ANOVA) (SPSS 18). Considering that the results of the analysis of variance were significant, planned comparisons were used to compare dependent variable means with the control group. To control type 1 errors, the Sidak formula was used (Sokal and Rohlf 2012).

$$a_s = 1 - (1 - \alpha)^{1/c}$$

where: a_s – calculated Sidak probability, α – familywise (0.05), c – number of comparisons.

Results

Experiment 1: The average number of mines per leaf differed significantly among the treatments ($F = 2.025$; $df = 11, 70$; $p = 0.042$). This difference corresponds to Con-

fidor ($p < 0.000$), 500 ml mineral spray oil – MSO (EC) ($p < 0.002$), and 900 ml MSO (EC) ($p < 0.004$) treatments with the control. The number of mines per leaf and number of live larva in the other treatments was lower than that found in the control (Table 2). The number of live larvae per treatment compared with the control at a 5% level (p), indicates that most treatments significantly differed from the control with the exception of 100 ml Ghazal and Volk MSO (Table 3), however at the 0.0046 level (Sidak) only Confidor, 500, and 900 ml MSO (EC) differed significantly.

Experiment 2: The average number of mines per leaf differed significantly among the treatments ($F = 3.656$; $df = 17, 67$; $p = 0.001$). The average number of live larva in the treatments with the control also showed a significant difference ($F = 39.357$; $df = 17, 67$; $p = 0.000$). No difference was detected in the number of live larva per leaf treated with ≤ 650 ml MSO (EC) and those treated with synthetic pesticides (Table 4). Increased concentrations of MSO resulted in a significant decrease in the number of live larva per leaf (Fig. 1). Reduction of pest larva caused by treatments used in this experiment was shown on figure 2.

Experiment 3: This experiment was a comparison of two citrus pest management methods on pest activities. As indicated in figures 3 and 4, the greatest number of live larva in the Mirbazar and Moghricola regions, in 2010 and 2011, were observed in orchards which had used the synthetic pesticides to control other pests. Generally, there were many more live citrus leafminer larva during the 24 sampling steps in citrus orchards contaminated with synthetic pesticides, than in orchards without synthetic pesticides. The exceptions were on 22 July 2010 and 15 July 2011 in the Mirbazar orchard, and 29 July 2011 and 12 August 2010 in the Moghricola orchard. It must be mentioned, that the above sampling dates took place a few days after the general insecticide spraying on that regions. The live-larva decrease in citrus orchards was likely due to the spraying of the insecticides.

The average data taken from these two regions for a two consecutive year period was compared. The data showed that the number of live larva per leaf in orchards where synthetic insecticides had been sprayed compared

Table 3. Comparison of the number of live *P. citrella* larvae per leaf in treatments with the control ($\alpha = 0.05$)

Treatments	Dose [ml/100 l of water]	No. of mines per leaf	
		p	t
Confidor	70	0.000	3.70
Dursban	200	0.033	2.17
Mineral oil (EC)	100	0.013	2.55
Mineral oil (EC)	500	0.001	3.49
Mineral oil (EC)	900	0.001	3.35
Mineral oil (Ghazal)	100	0.32	2.19
Mineral oil (Ghazal)	500	0.006	2.85
Mineral oil (Ghazal)	900	0.006	2.85
Mineral oil (Volk)	100	0.164	1.40
Mineral oil (Volk)	500	0.024	2.31
Mineral oil (Volk)	900	0.045	2.05

p = 0.05; t – sample T-test

Table 4. Comparison of the number of live *P. citrella* larva per leaf in treatments ($\alpha = 0.0018$)

Comparisons of the treatments	p	df	t
Confidor vs. Ec* 650	-2.341	56	0.023
Confidor vs. Ec 800	-1.320	56	0.192
Confidor vs. Ec 950	-0.093	56	0.926
Dursban vs. Ec 650	2.188	56	0.033
Dursban vs. Ec 800	3.259	56	0.002
Abamectin + Ec 350 vs. Ec 650	-2.285	56	0.026
Abamectin + Ec 350 vs. Ec 800	-1.214	56	0.230
Abamectin + Ec 350 vs. Ec 950	0.073	56	0.942
Abamectin + Ec 500 vs. Ec 800	-2.534	56	0.014
Abamectin + Ec 500 vs. Ec 950	-1.246	56	0.218

*mineral oil (EC); p = 0.05; t – sample T-test

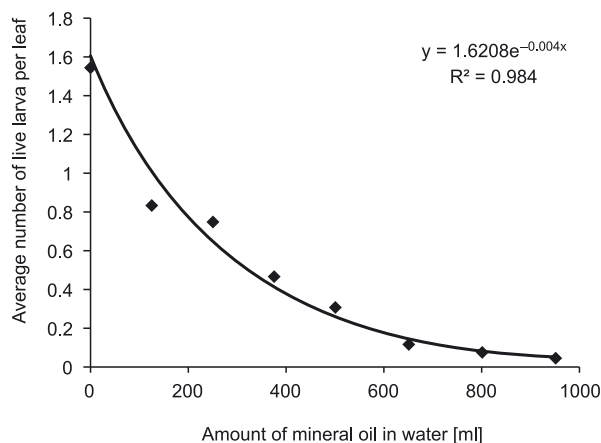


Fig. 1. The average number of *P. citrella* live larva per leaf in different mineral oil concentrations

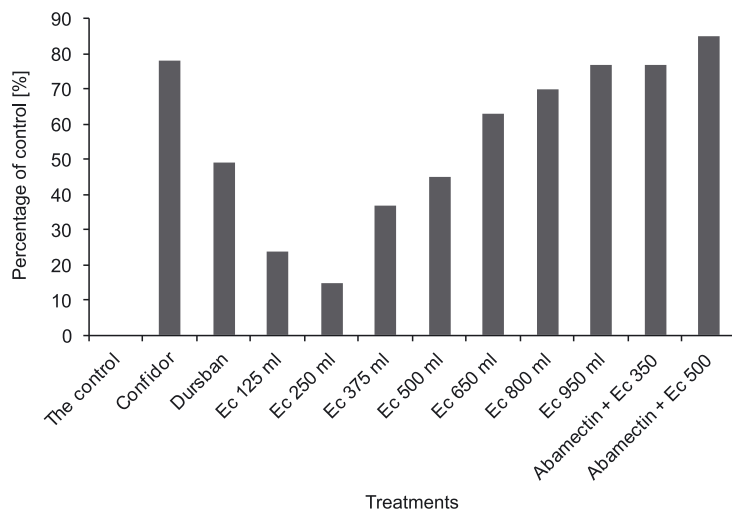


Fig. 2. Reduction of pest larva caused by treatments used in the second experiment

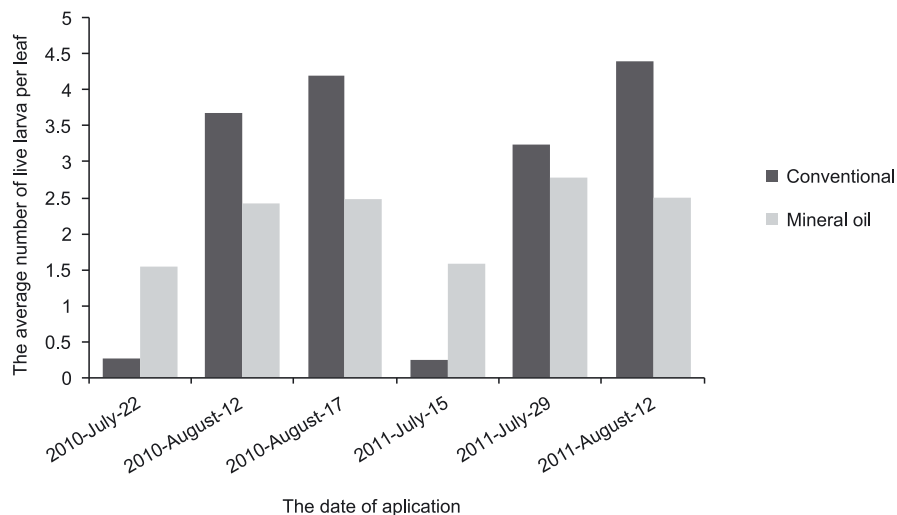


Fig. 3. Comparison of live larva numbers per leaf in two types of management in Mirbazar during 2010–2011

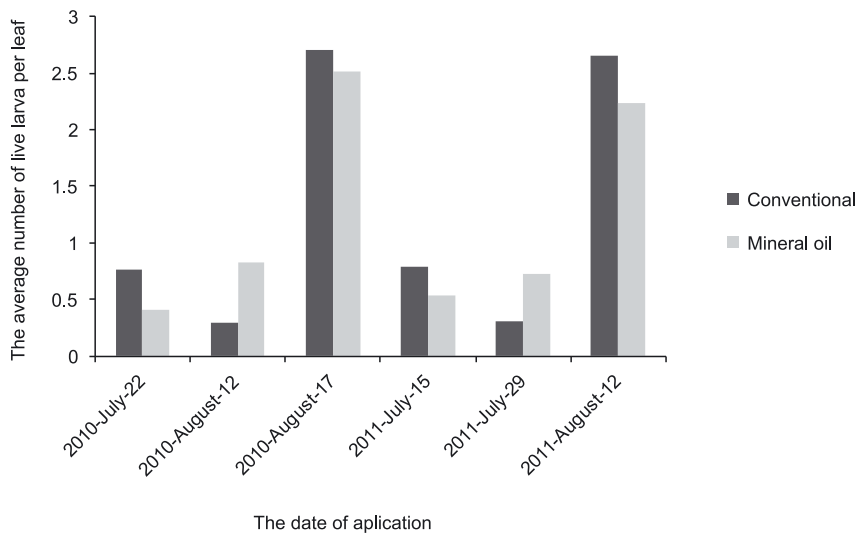


Fig. 4. Comparison of live larva numbers per leaf in two types of management in Moghricola during 2010–2011

Table 5. Comparison of the number of live *P. citrella* larva per leaf in treatments in Experiment 3 ($\alpha = 0.05$)

Comparisons of the treatments	Date	p
The Mirbazar region conventional orchard vs. just the mineral oil treatment	22-July-2010	0.000
	12-August-2010	0.000
	17-August-2010	0.000
	15-July-2011	0.000
	29-July-2011	0.151
	12-August-2011	0.000
The Moghricola region conventional orchard vs. just the mineral oil treatment	22-July-2010	0.009
	12-August-2010	0.007
	17-August-2010	0.522
	15-July-2011	0.194
	29-July-2011	0.099
	12-August-2011	0.316

to orchards without synthetic insecticides in eight comparison steps, was more. Their "p" values are shown in table 5.

Discussion

When mineral oil spray and synthetic pesticides were compared, there were no differences in their abilities to suppress the *P. citrella* infestation and the visible symptoms of phytotoxicity. The risk of acute phytotoxicity increases if oils are poorly mixed and applied when trees are stressed, and temperatures are extreme (Rae *et al.* 1996). The current study demonstrates that under favourable conditions, up to 3 sprays of 0.9% mineral oil can be used without causing acute phytotoxicity. To determine chronic phytotoxicity, which can result from long-term use of oils, a citrus orchard which is managed by using pesticide-free protocols was chosen in the county of Babolsar, of the Mazandaran Province. All pests in the orchard were controlled by a 0.7–1.5% mineral oil spraying, two or three times a year starting in 1993; no pesticides were used and no chronic phytotoxicity was reported (Damavandian 2007).

In California, mineral oils are recommended to be used on citrus for two or three major pests, the California red scale, *Aonidiella aurantii* Maskell, and the citrus red mite, *Panonychus citri* McGregore (Davidson *et al.* 1991). In France, mineral oil provided good control of *P. ulmi* Koch on fruit trees (Girantet *et al.* 1997). According to Rae *et al.* (1996), 3–4 sprays of mineral oil at an interval of 6–7 days will provide effective control of the citrus leafminer. In Iran, mineral oil on citrus controlled citrus brown scale, *Chrysomphalus dictyospermi* Morgan (Damavandian 1993), citrus wax scale, *Ceroplastes floridensis* comstock (Damavandian 2003), citrus rust mite, *Phyllocoptruta oleivora* Ashmed (Damavandian 2005), citrus red mite, *P. citri* McGregor (Damavandian 2007), and orange pulvinaria scale, *Pulvinaria aurantii* Cockerell (Damavandian 2010).

Field trails demonstrated that a dilute application of $\geq 0.65\%$ mineral oil controls *P. citrella* as effectively as synthetic pesticides (Table 4). When oils are used, they must be applied as thorough coverage flushes, namely high volume sprays requiring up to 3 l of water per 5-year-old Thomson novel tree.

Experiment 2 demonstrated that 0.95% mineral oil (EC) controlled *P. citrella* as effectively as Confidor, and 0.5% mineral oil plus Abamectin, with the population being reduced 77, 78, and 85%, respectively (Fig. 2). The most effective control of *P. citrella* was achieved with the use of 0.5% oil plus Abamectin. When Abamectin was mixed with 1% oil, 94–100% larva mortality was reached (Rae *et al.* 1996).

Abamectin has demonstrated nematicidal, acaricidal, and insecticidal activity (Lasota and Dybas 1991). Abamectin breaks down rapidly (< 1 days) when exposed to sunlight or when present as a thin film (Clark *et al.* 1995). Reservoirs of the chemical can remain within the mesophyll layer of leaves, particularly when this chemical is applied with oil (Lasota and Dybas 1991). Thus, Abamectin becomes much more accessible to pests such as the leafminer, than to their predators or parasites. Morse *et al.* (1987) found that field-weathered residues of Abamectin did not cause residual mortality to three selected beneficial arthropods of citrus (*Aphytis melinus* De Bach; mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant; and *Euseius stipulates* Athias-Henriot). These characteristics indicate that Abamectin could be used for integrated pest management in citrus. In contrast, current recommendations in Florida limit the use of Abamectin to three applications per year (Knapp 1995) so as to manage resistance. Among pesticides, Abamectin was ranked as a very dangerous pesticide (Metcalf and Luckmann 1994). It should be recommended only in very urgent cases. Meanwhile the use of Abamectin, Confidor, and Dursban led to a severe infestation of the citrus red mite *Panonychus citri* (personal observation). These pesticides were said to have eliminated the predatory mite, *Amblyseius oddoensis* Vander Merwe and Ryke (Keetch 1968), although the mentioned synthetic pesticides are registered and have been used for many years against the citrus leafminer in northern Iran. Therefore, use of these pesticides in integrated pest management programs does not seem logical.

Mineral spray oil has been used traditionally to control small, relatively immobile insects. The oil is said to suffocate the insects (Ebeling 1950; Davidson *et al.* 1991). Najar-Rodriges *et al.* (2008), though, observed no signs of oil accumulation within the trachea. They believed

Table 6. Cost of different treatments when applied at 1,000 l/ha (350 trees/ha)

Treatments	Concentration [AI/100 l of water]	Price [\$/kg or l AI]	Cost [\$/ha]
Confidor	80 ml	11.66	9.328
Dursban	200 ml	6.25	12.5
Mineral oil (EC)	650 ml	0.83	5.395
Abamectin + EC	20 + 350 ml	3.75 + 0.83	3.655
Abamectin + EC	20 + 500 ml	3.75 + 0.83	4.9

AI – Active ingredient

that rapid penetration of oil through the insect's body and accumulation in the nerve ganglia has the direct effect of suppressing synaptic transmission in the insect's ganglia. In both ways of controlling pests, mineral oil should contact the pests. Beattie *et al.* (1995) indicated that the proportion of dead larva in mines in which oil treatments were used, was similar to the control. Whereas in the Fenoxycarb and Methidation treatments, the proportion of dead larva was significantly higher than in the control. On the other hand, according to their study, mineral oil caused a significant reduction in *P. citrella* damage which is similar to our results (Fig. 2). This reduction may refer to mineral oil acting as an ovipositional deterrent to the pest. Mature citrus trees can sustain a *P. citrella* population up to 0.74% mines per susceptible leaf without incurring economic loss (Huang *et al.* 1989), with regard to control over 70% *P. citrella* by mineral oil (Fig. 2).

Based on results of this study, in the Mazandaran citrus orchards, a 0.65% concentration of mineral oil provided appropriate control, and cost the least compared to conventional insecticides (Table 6). In northern Iran, farmers sometimes use the above-mentioned insecticides every 12 days the during tree budding period, against the citrus leafminer. Many times, as we stated before, some pests, such as the citrus red mite *P. citri*, are exposed and the use of acaricides such as Nisoron (hexythiazox) caused the mentioned costs. Therefore, three steps of oil spraying in 5–6 days intervals at a cost of 16 (\$/ha) would be the more effective and economical method. Whereas the use of synthetic pesticides and these iteration numbers and conventional acaricide Nisoron cost about 53 (\$/ha).

In conclusion, mineral oil spraying should be able to provide adequate control under most circumstances and can replace the synthetic pesticides that are applied in the Mazandaran citrus orchards. In addition, applying just mineral oil in mature commercial citrus orchards for several successive years resulted in less damage from the citrus leaf miner. It is important to note, that farmers must thoroughly agitate the oil/water emulsion so that all the foliage is covered, and the temperature should be between 0°C and 35°C and > 20% relative humidity (RH) (Beattie 1990; Davidson *et al.* 1991). The timing of the spraying is also crucial, the spray must be applied as soon as the largest leaves of a flush are > 1 cm in length (Rae *et al.* 1996). Every 5–6 days, the spraying should be repeated until the flush hardens and is no longer susceptible to attack.

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