

Future studies should also investigate the toxicity of insecticide residues after exposure in the field for various intervals.

**3.5 Toxicity evaluation of Nissorun, Palizin, Sirinol and Tondexir on *Tetranychus urtica* Koch a pest of *Brassica napus* L**

There were significant differences among different treatments ( $P < 0.01$ ). The comparison between different post treatment times has shown that treatments significant differences ( $P < 0.01$ ); Table 19). These results showed that each factor has independent and separate effect on percentage of mortality.

The results of current experiment showed that among different treatments of the Nissorun with 3/1000 and tondexir with 3/1000 (95 %) were more effective than the others on citrus TSSM mortality (Table 20 Fig. 1).

In addition, among periods of the post spraying methods, 76 h was significantly different with 48 h and they were more effective than 24h on pest mortality of the TSSM (Table 3).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
treat	.023	4	.006	99.047	**
day	.001	2	.001	9.163	**
treat * day	.000	8	3.29E-005	.558	ns

\*\* showed significantly different ( $P < 0.01$ )

ns not significantly different

Table 19. The ANOVA of the different treatments

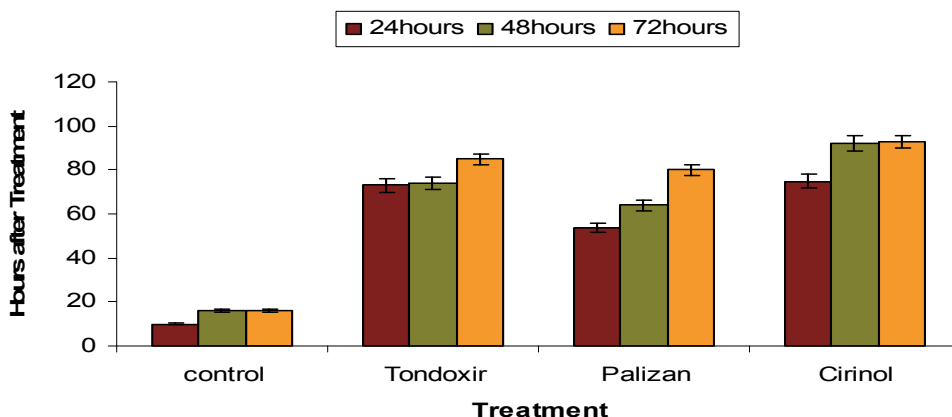


Fig. 1. Comparison of the effect of different toxins on TSSM

Treatment Time	Time Hours(h)		
	24	48	72
Control	11.67±4.41a	13.33±4.41a	13.33±4.41a
Cirinol(3/1000)	74.33 ± 3.18c	91.67± 3.71c	95.67 ± 3.33c
Palizan(3/1000)	47.33±5.48b	60.33 ± 2.60b	75.33 ± 8.19b
Nisiron(3/1000)	71.67±7.68d	97.33± 1.66d	97.33 ± 1.66d
Tondoxir(3/1000)	75.33±14.17c	87.33±6.64c	95±4c

Means followed by the same letter are not significantly different

Table 20. The comparison of the mean of different treatments on percentage of mortality of TSSM consist of Tukay 's test.

#### 4. Discussions

The goal of control program is to protect the main growth flushes particularly the summer shoots grown in greenhouses which are important as fruit-bearing branches. The CLM larvae are protected by a cuticle layer of the leaves in the serpentine mine and the pupal stage is also protected by the rolled leaf margins (Raga *et al.*, 2001). The use of chemical and synthetic insecticides in developing countries and its concomitant problems is necessary to test the alternative traditionally oriented methods for pest control. The present work revealed the effect of the biorational insecticides including Insecticidal Emulsion (IE), Insecticidal Gel (IG), *Bacillus thuringiensis* (Bt) and the mineral oils (MO) on CLM. These insecticides had significant insecticidal activity against CLM larvae, but the IE and IG were more effective than the Bt and MO. The IE and IG are used against a wide rang of pest. However, they have very low toxicity against vertebrate and mammals (With LD<sub>50</sub> more than 10000 mg/kg). These results suggest that there may be different compounds in IE and IG possessing different bioactivities.

Pesticides may be applied to protect new flushes of growth when the leaves are most vulnerable to CLM damage. However, the best foliar insecticides confer only 2 weeks of leaf miner infestations (Michaud and Grant. 2003). Recently, Mafi and Ohbayashi (2006) found that the percentage corrected mortality of eggs of the citrus leafminer exposed to insecticides (dipping method bioassay) ranged from 3 to 44%, but all the insecticides tested showed almost over 90% mortality to the first instar larvae of citrus leafminer. It is important to select less toxic chemicals against the natural enemies in order to expect both the activity of natural enemies and control effect of insecticides for suppressing the infestation of CLM.

The results of this study has shown that the IE, IG, BT, Reldan, Runner, Tracer, Sireinol, Palizin and MO are active against the leafminer demonstrating that these biopesticides penetrate into leaf mines, killing the larvae as observed by Shapiro et al (1998) and the oil of the IE, IG and MO might be reduced the infestation by acting as an oviposition deterrent in the field (Liu et al 1999). The IE, IG, Reldan, Runner, Tracer, Sireinol, Palizin and MO solution that tested in current experiment had two different effects on CLM mortality. Firstly, they had insecticidal activity when applied these biopesticides against the CLM at the recommended dose. Secondly, they increased the efficacy of the commercial formulation

of IE, IG, Bt and MO by helping them to penetrate the plant cuticle and enhanced the activity of the active ingredient of these biorational insecticides when applied to the pest, probably due to increased penetration through the mine stomata into the mines. Since CLM preferentially mine the abaxial surface of leaves, enhanced stomatal infiltration is especially useful against CLM.

The petroleum oil spray residues reduced infestations of CLM by preventing oviposition and its effects depended on concentration of oil and time of spraying (Beattie *et al.*, 1995, Smith *et al.* 1997). Raga *et al.* (2001) reported that both of Abamectin and Lufenuron pesticides along with petroleum oil provided a significant increase in CLM larval activity. However, the efficacy of petroleum-derived spray oils used as oviposition deterrents to control citrus leafminer is related to time of spraying, the amount of oil dose and the persistence of oil molecules on sprayed surfaces or efficacy is also related to increasing molecular weight of oil molecules as reflected by  $nC_{17}$  values and, therefore, persistence of oil molecules on sprayed surfaces (Liu *et al.*, 2001). Therefore, the petroleum oils alone or combine with microbial agent as emulsifier which has synergist and less harmful effect for the environment recommended for using in IPM program (Khyami and Ateyyat, 2002).

Plant allelochemical may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack (Murugan *et al.*, 1996; Senthil Nathan *et al.*, 2005a).

It has been shown that neonicotinoid, pyrethroid and growth regulator insecticides have a significant, negative impact on some predators which are appearing to be the most important biological control agents of leafminers. Thus, it is necessary to be aware about the effect of these pesticides on beneficial insects, therefore, the usage of biorational insecticides, such as BT, are recommended (Grafton and Gu, 2003; Villanueva-Jiménez *et al.*, 2000). In addition, the toxicity of pesticides such as Avant was higher than Pyriproxyfen and Buprofezin (Amiri (b), 2006). IE, IG, BT and MO are relatively much safer compounds than the conventional organophosphorus insecticides, Buprofezin, Avant and Pyriproxifen (Maha and Abdalla, 1999). Chemistry of natural compounds is a very complex subject and screening for activity will have to face, among other factors, isolation and identification of the products variability due to the plants or the environment, a synergism due to the mixtures of compounds in crude extracts (Chiue, 1989). The high toxicity of the IE and IG may be due to penetration through mine stomata into the mines. Since CLM preferentially mine the abaxial surface of leaves, enhanced stomatal infiltration is especially useful against these pests. The low toxicity of the mineral oils in this study may be due to different factors including cuticle properties, ambient temperature, and the molecular size and the volume of oil molecules. According to Cole (1994), the choice of insect and bioassay can greatly influence the outcome of a screening. However, to develop a useful commercial product, testing against agricultural pests is important. Amonkar and Reeves (1970) found that garlic killed an insecticide resistance strain of *Aedes nigromaculis* as well as susceptible *Aedes* species. However, the neem formulations can be used as follow-up sprays under heavy infestation and as prophylactic sprays during new flush emergence (Verghese and Jayanthi, 2004). Howard (1993) has shown that Azadiractin and abamectin both as a biorational insecticide were potentially useful for controlling CLM.

The results of this study indicated that the plant-based compounds such as IE and IG may be effective alternative to conventional synthetic insecticides for the control of CLM. For further understanding it is necessary to investigate the third generation pesticides such as growth regulators (IGRs) and Biorational insecticides in combination with mineral oil, to get

much more suitable results in the field conditions. Since the spring population density of CLM is very low, it is not necessary to control CLM before late June in most parts of the citrus growing regions in north of Iran. On the other hand, it is so important to protect the new shoots of the young or top grafting citrus trees from the infestation of summer generations of CLM.

The effect of insecticides in citrus orchards against the CLM is difficult to achieve the maximum CLM larval mortality and it is not very sufficient because several generations of CLM are usually overlapping and the CLM larvae are protected by a cuticular layer of the leaves in the serpentine mine and the pupal stage is also protected by the rolled leaf margins (Raga *et al.*, 2001). The results of present study clearly demonstrated that the efficacy of *Bt* and *Bt* plus MO against CLM increased with the increasing *Bt* concentration. It has been shown that the larval mortality vary with spray volume suggesting that the oil reduced the infestation by acting as an oviposition deterrent (Liu *et al.*, 2001).

In our experiment, by comparing the activity of the commercial formulation between *Bt* and *Bt* plus MO against the CLM, we observed that the CLM larval mortality was higher (not statistically) in *Bt* plus MO treated groups than the *Bt* alone. Several research groups have shown that, the application of Abamectin in combination with petroleum oil provides the most synergistic effect to control of the *Helicoverpa armigera* and CLM (Wang *et al.*, 2005).

Sometimes the indirect damage of CLM is very important. Mining of immature foliage by the larvae can lead to reduced growth rates, yield and mined surfaces serve as foci for the establishment of diseases such as citrus canker, *Xanthomonas citri*. In the absence of citrus canker, citrus leafminer is a serious pest of rapidly growing immature or pruned trees. But in presence of citrus canker, it is a major pest of both immature and mature trees (Liu *et al.*, 2001). Therefore, it is important to select less toxic chemicals against the natural enemies in order to expect both the activity of natural enemies and control effect of insecticides for suppressing the infestation of CLM. The higher activity of *Bt* in *Bt* plus MO treated groups at the present study may be due to increased penetration of *Bt* through the mine by helping of MO.

For better understanding it is necessary to investigate the third generation pesticides such as growth regulators in combination with mineral oil, microbial and fungi insecticides to get much more suitable results in the field conditions. However, more field studies will need to be performed to understand the effect of *Bt* and *Bt* plus MO against *P. citrella* and to determine the optimum timing of the multiple application.

The CLM is one of the key pests in citrus growing, especially in nurseries, top-grafted trees and newly planted trees in north of Iran. Therefore, it is so important to protect new shoots of young or top-grafted trees from the damage caused by summer and autumn generations of CLM. The goal of cultural, chemical and other control programs is to protect the main growth flushes. The results obtained at the present study indicated that insecticidal control is difficult to achieve the maximum CLM larval mortality. Because the larvae of the CLM are shielded within the mines by the leaf epidermis and the pupal stage is also protected by the rolled leaf margins (Raga *et al.*, 2001). Foliar sprays may be applied to protected new flushes of growth when the leaves are most vulnerable to CLM damage. However, the best foliar insecticides confer only 2 weeks of leaf miner infestations (Michaud and Grant. 2003).

Recently, the toxicity of different insecticides to the citrus leafminer and its parasitoids was evaluated under laboratory conditions in Japan (Mafi and Ohbayashi, 2006). They found that the percentage corrected mortality of eggs of the citrus leafminer exposed to insecticides (dipping method bioassay) ranged from 3 to 44%, but all the insecticides tested showed almost over 90% mortality to the first instar larvae of citrus leafminer. Comparison between two spray methods at the present study leaf-dipping method was more effective than topical spray method on pest mortality. According to several authors, the application of Abamectin in combination with petroleum oil provides the most effective control of the CLM. It has been reported that in the absence of citrus canker, citrus leafminer is a serious pest of rapidly growing immature or pruned trees. But in presence of citrus canker, it is a major pest of both immature and mature trees (Liu *et al.*, 1999). In our study, it was shown that the toxicity effect of Avant was higher than Pyriproxyfen and Buprofezin pesticides. Previously, it has been shown that pesticides such as Buprofezin and Pyriproxyfen decreased the number of laying eggs, hatched eggs and also short life cycle in *Bemisia tabaci* (Yasui *et al.*, 1987; Ishaaya *et al.*, 1994). They concluded that Buprofezin and Pyriproxyfen affects on reproductive system of *Bemisia tabaci* at immature stage of life cycle (Ishaaya *et al.*, 1988). It has been shown that the two pest control agents, Buprofezin and Super Royal are relatively much safer compounds than the conventional organophosphorus insecticides (Maha and Abdalla, 1990). Exposure of adult beetles species (*Circellium bacchus*) to Pyriproxyfen did not affect egg production or the viability of eggs, nor did the compound have adverse effects on immature development, indicating that Pyriproxyfen is unlikely to be the cause of the observed population depression of *Circellium bacchus* (Kruger and Scholtz, 1997). In our study, no significant difference in effectiveness was found between periods of post spraying in citrus leafminer larval mortality. The results obtained at the present study suggest that the Avant chemical pesticide can be account as an effective tools in controlling the spreading of citrus leafminer in citrus growing regions in north of Iran. Since the spring population density of CLM is very low, it is not necessary to control CLM before late June in most parts of the citrus growing regions in north of Iran. For further understanding it is necessary to investigate the third generation pesticides such as growth regulators in combination with mineral oil, microbial and fungi insecticides to get much more suitable results.

These data represent some of the first published information on the effects of Reldan, Runner, Tracer, Sireinol, Palizin and oil on CLM. It is very difficult to protect the new shoots of young trees from CLM damage, especially in nurseries and newly planted orchards in north Iran. The goal of any CLM control program is to protect the main growth flushes, particularly the summer shoots of young trees. The insecticides studied here had significant activity against CLM larvae, but Reldan, Runner and Tracer were more effective than Sireinol (IE), Palizin (IS) and oil. The above pesticides are used against a wide range of pests. However, they have very low toxicity to vertebrates and mammals. The results from this study suggest that there may be different compounds in IE and IG which have different bioactivities.

This study has shown that Reldan, Runner, Tracer, Sireinol, Palizin and oil are active against CLM, demonstrating that dips of these pesticides penetrate into leaf mines. , and the adjuvant ingredient of Reldan, Runner, Tracer, Sireinol, Palizin and MO might reduce the infestation by acting as an oviposition deterrent in the field (Liu *et al.*, 1999).

The results of this study will contribute to a significant reduction in the application of synthetic insecticides, which in turn will increase the opportunity for natural control of

various important horticultural pests by botanical pesticides. Since these are often active against a limited number of species including specific target insects, are easily biodegradable, non-toxic products, and potentially suitable for use in CLM control programs (Alkofahi *et al.*, 1989), they could lead to the development of new safer classes of insect control agents. Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds which increase their resistance to insect attack (Senthil Nathan *et al.*, 2005a). In addition, the translocation and translaminar properties of the above insecticides make them available in the host plant tissues to control leaf feeders, however, surface residues disappear quickly, thus making them safe for parasitoids and most natural enemies (Brunner *et al.*, 2001).

Reldan has a lower mammalian toxicity, LD<sub>50</sub> (oral, rat) is 3000 mg/kg, than Dursban with an LD<sub>50</sub> (oral, rat) of 135 mg/kg (Kalyanasundaram *et al.*, 2003). Reldan is mainly effective against rice stem borer, aphids, cutworms, plant and leaf hoppers, mole rickets, some moths and stored grain pests. Reldan also has high toxicity to CLM, as high as Runner and Tracer in these experiments.

Methoxyfenozide is a dibenzoylhydrazine insect growth regulator, similar to tebufenozide in its mode of action, its ability to induce a lethal molt and its specificity for Lepidoptera (Carlson *et al.*, 2001). Methoxyfenozide has a much lower ability to bind with receptors in non-lepidopteran species, making it a highly selective insecticide and useful in a number of crops. Low levels of resistance to methoxyfenozide in codling moth, beet armyworm and oblique banded leaf roller have been found, necessitating precautions similar to those for tebufenozide. In our study, methoxyfenozide (Runner), although considerably less toxic than the other insecticides based on LC<sub>50</sub> levels, still resulted in substantial (83%) mortality of CLM after 96 h.

A significant advantage of spinosad is that it is effective against strains of *R. dominica* which are resistant to pyrethroids and methoprene (Nayak *et al.*, 2005). Some of the newer insecticides, such as spinosad, indoxacarb, and emamectin benzoate, have been shown to be relatively safe on predacious hemipterans, mites, coccinellids, lacewings and some parasitoids. Relatively rapid degradation of surface residues in the field would definitely improve the compatibility potential with natural enemies. This would likely be the case with spinosad (Williams *et al.*, 2003).

This study indicated that plant-based compounds such as IE and IS may be effective alternatives to conventional insecticides for the control of CLM. For further understanding it is necessary to investigate the third generation pesticides such as growth regulators (IGRs) and bio-rational insecticides in combination with mineral oil, to ensure that they work in field conditions.

A number of novel insecticides with unique modes of action were registered during the late 1990s and early 2000s for insect control in agriculture. These new insecticides have several advantages over older insecticides. Firstly, low mammalian toxicity allows for short re-entry and pre-harvest intervals, allowing the insecticides to be easily incorporated into pest control programs. Many also have greater selectivity and so are less likely to harm natural enemies than the broad-spectrum organophosphate, carbamate, neonicotinoid and pyrethroid insecticides. As such, they are less likely to cause outbreaks of secondary pests, and may be used as "clean-up" sprays to manage outbreaks of such pests.

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