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The Effect of Some Botanical Pesticides Against Citrus Leafminer (CLM) and Two Spotted Mite (TSM)

Behnam Amiri-Besheli
Sari Agricultural Sciences and Natural Resurces College of Agriculture Science
Iran

1. Introduction

The novel natural extracts of the higher plants have a variety characteristics including insecticidal, antifungal, antiviral and antibacterial activity, repellence to pests, antifeedant effects, insect growth regulation, toxicity to nematodes, mites, agricultural pests, (Prakash and Rao, 1997). Insecticidal activity of many plants has been demonstrated (Carlini and Grossi-de-Sá, 2002). The deleterious effects of plant extracts on insects can be manifested in several manners including toxicity, mortality, and reduction of reproduction, fecundity and fertility. The plants that used for pest insect control have strongly correlated to medicinal and pesticidal plants (Yang and Tang, 1988). The plant substances contain components that are toxic to insects through a novel mode of action. In addition to the obvious implication of discovering a new target site against which to design insecticides, existing mechanisms of resistance may not confer cross-resistance to these plant extracts.

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidptera: Gracillariidae), is an important pest of citrus and related rutaceae and ornamental plants almost worldwide (Achor *et a.,* 1996). The CLM mines leaves, surface tissue of young shoots and stems, and less frequently the fruit (Sponagel and Diaz, 1994). Although citrus leafminer causes indirect damage to young leaves, which predisposes them to infection by canker so, controlling citrus leafminer is a vital component of canker management (Pena *et al.,* 1996; Belasque *et al,* 2005). The first record of CLM from southern and northern Iran, with a dramatic increase and widespread dispersal, was noted in 1961 and 1994, respectively. The pest has about 5-9 generations over year, with peak periods in early summer and early autumn. Preliminary field trials with selected insecticides indicate the superiority of Dimilin (diflubenzuron) over Diazinon, Zolone (Phosalone) and Ekamet (Etrimfos) in controlling CLM in the northern Iran, but it is not totally effective (Jafari, 1996). There is no biological control plan of CLM, but some studies are underway for planned extension activities using biorational insecticides. Several different insecticides, such as Avant, Buprofezin and Pyriproxifen was used (Amiri, 2006 (a)), but these may involve interference in control of the pest by natural enemies (Guerra *et al*., 1997). However, biological control is the best option for control (Pena, 1997), but the effective control of CLM is very complicated because of its high migration ability from outside of orchards, high fertility, present epidermis of citrus leaf as substantial protection and the difficulty of direct contact of chemical to the larval body. However, CLM has a long history of resistance to many insecticides and development of resistance against the chemicals sometimes makes it difficult to obtain enough control effect (Tan and Huang, 1996, Mafi and Ohbayashi, 2006).

Recently, the use of biorational insecticides (any type of natural or synthetic material active against pest populations) has been extremely increased. *Bacillus thuringiencis* sub sp *Krustaki* is the soil bacterial insecticide most widely used for controlling Lepidoptera larvae population (Broderick et al., 2000; Lacey *et al*., 2001) that is safe for many non-target insects with a minimal environmental impact (Jyoti, J and Brewer 1999). Because CLM is protected inside the mine it is suggested that the mineral oils would be as a surfactant and reduce the surface tension and increase the penetration of the *Bacillus thuringiencis* suspension through the epidermis of the citrus leaf (Dias *et al*., 2005). *Bacillus thuringiencis* and *Bt* plus MO are active against the leafminer, demonstrating that these biopesticiedes penetrate into leaf mines, thereby killing the larvae (Amiri, 2007).

Chlorpyrifos-methyl (Reldan) is a wide-spectrum insecticide which belongs to the organophosphate group. It is effective against rice stem borer, aphids, cutworms, plant and leaf hoppers, mole crickets, some moths and stored grain pests. Currently, its main use in Iran is in stored grain. The mode of action of Reldan is cholinesterase inhibition. Methoxyfenozide (Runner 240 SC), a moult accelerating compound and an ecdysone agonist which is currently submitted for registration in Iran, is a compound compatible with IPM (Integrated Pest Management) that has strong, activity against lepidopterous pests.

Spinosad is a mixture of two macrolide lactones, Spino- syn A and D, produced by fermentation of the soil actionomycete *Saccharopolyspora spinosa* (Mertz and Yao, 1993). Spinosad is classified by the U.S. Environmental Protection Agency as a reduced-risk material due to its low environmental persistence and very low toxicity to most vertebrates (Thompson et al., 2000).

There is no biological control plan for CLM, but studies are underway using bio-rational insecticides. Several different insecticides, such as indoxacarb (Avaunt) and pyriproxifen (Esteem), have been used4, 5, but these may interfere in the control by natural enemies13. Biological control is the best option for long-term control, however the effective control of CLM is complicated by its high migration ability from orchards, high fertility, and protection of the larvae inside the citrus leaf making contact with the chemical difficult. As well, CLM has a history of developing resistance to insecticides making it difficult to achieve sustainable control27, 18.

Biopesticides, including botanicals, can offer a safe and effective alternative to conventional insecticides for con- trolling major insect pests within an integrated pest mana- gement program. The use of biopesticides such as Tondexir which is extracts from hot pepper, discourage insect pests from laying eggs on leaves and pose lower risk to humans and the environment than other pestici- des. Pepper worked better when insects are soft-bodied during the larval stage because the chemical is able to penetrate. It worked fast.

The citrus leaf-miner (CLM), *Phyllocnistis citrella* Stain-ton (Lepidoptera: Gracillariidae), is an important pest of citrus and related Rutaceae and ornamental plants world- wide (Achor et al., 1996). The CLM mines leaves, the surface tissue of young shoots and stems, and less fre- quently the fruit (Sponagel and Diaz, 1994). Although CLM causes indirect damage to young leaves, which pre- disposes them to infection by canker, controlling CLM is a vital component in 1996; Belasque et al., 2005). The first record of CLM from southern and northern Iran, with a dramatic increase and widespread dispersal, was noted in 1961 and 1994, respectively. There is no biological control plan for CLM, but some studies are underway for planned extension activities using biorational insecticides. Several different insecticides, such as Avant, Buprofezin and Pyriproxifen have been used (Amiri, 2006a, b, 2007), but these may cause interference in the control of the pest by natural enemies (Guerra et al., 1997). CLM has a long history of resistance to many insecticides and the development of such resistance makes it difficult to achieve control (Mafi and Ohbayashi, 2006).

Bacillus thuringiencis (*Bt*) subsp *Krustaki* is the bacterial insecticide most widely used for controlling Lepidoptera larvae population (Broderick *et al.,* 2000). It is safe for many non-target insects and has a minimal impact on the environment (Jyoti & Brewer, 1999). Since CLM is protected inside the mine it is suggested that the mineral oils used as a surfactant would reduce the surface tension and increase the penetration of the *Bt* suspension through the leaf epidermis. Petroleum oil reduced infestation by preventing oviposition and there is a negative effect between the number of mines/leaf and concentration of oil (Dias *et al.,* 2005).

Botanical insecticides are important products for pest management in industrialized countries (Isman, 1997). The pesticide resistance and negative effects on non-target organisms, for example, man and environment, are the main problems (Franzen, 1993). The natural insecticides are relatively inactive against the nontarget organism. The pests mainly controlled by synthetic pesticides in the last fifty years, but today the main strategy in aim of the plant protection is reduction of synthetic pesticides and use of botanically insecticides.

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch is widely distributed in the world and a common pest of many plant species in greenhouses, nurseries and field crops. A population of TSSM can increase rapidly especially during hot and dry periods. It infests many crops including tomatoes, beans, peepers, eggplants and ornamental plants (Cagle, 1949).

Many new acaricides are now available in the market but they have a high cost associated with their use and application restrictions listed on the label to prevent the development of resistance.

The aim of this study was to evaluate the toxicity of the commercial formulation of *Bacillus thuringiencis* as a biorational insecticide, mineral oils as a selective pesticide, Methoxyfenozide (Runner), Spinosyn A and D (Tracer), Insecticidal Gel (Palizin, IG), Insecticidal Emulsion (Sirinol, IE), Tonexir with/without Mineral Oils (MO), chlorpyrifos-methyl (Reldan), methoxyfenozide (Runner), spinosad (Tracer), an insecticidal soap (Palizin, IS), an insecticidal emulsion (Sirenol, IE), plant secondary metabolites on second and third instars larvae of the CLM in laboratory and field conditions and also toxicity evaluation of Nissorun, Palizin, Sirinol and Tondexir on *Tetranychus urtica* Koch a pest of *Brassica napus* L

2. Materials and methods

2.1 Laboratory bioassay and experimental design

The toxicity of commercial and biorational insecticides (table.1) to the citrus leafminer was tested in the laboratory of toxicology at Sari Agricultural and Natural Resources University in 2005, 2006, 2007, 2008, 2009, 2010. Newly growth leaves were sampled from citrus Thomson tree of different orchards at Sari district in North of Iran. The bioassay method (Leaf-dip method) was devised to test toxicities of above biorational insecticides. Leaves were examined in a laboratory with the aid of a stereo-microscope. The numbers of larvae, pre pupae, pupae and adult leaf miners on leaves ≥10 mm in length were recorded.

Common name	Trade name	Chemical group	Formulation company	LD50 for rats (mg/kg)	Dose (ml/l)
Tondexir	Tondexir	Pepper extract	EC Kimia Sabzaver	> 5000	2
chlorpyrifos-methyl	Reldan	organophosphate	50 EC	> 3000	1
Methoxyfenozide	Runner	Diacylhydrazin(IGR)	Dow AgroSciences 24% SC	> 5000	1
		Spinosyn A & D			
Spinosad	Tracer		240sc	>5000	0.75
		Biopesticides			
Bacillus thuringiensis	Bitiran		LD 3.6%	NT	4
sub sp morrison (BT			(liquid dispersal)		
MO		MO			
	PO		O 80%	4300	0.05
Insecticidal emulsion		Garlic extract			
(IE)	SIRENOL	insecticide	EC Kimia Sabzaver	>5000	2 & 4
Insecticidal Gel (IG)		Insecticidal and			
	PALIZIN	miticidal soap	Kimia Sabzaver WS	>5000	2 &4
Avant					
		Oxadiazon			
Buprofezin	Indoxicarp		SC150	2198	2
		IGR (Chitin			
Pyriproxyfen	Aplaud	Synthesis Inhibitors)	EC40%	>5000	0.5
		IGR			
	Admiral	(Juvenile Hormone	EpC 10%	>5000	0.5
		Mimic)			

Table 1. Pesticide used in experiments.

In assay, only leaves with actively feeding second or third instars leaf miner larvae were completely excised with petioles from citrus Thomson trees and used for bioassays. To keep the leaves turgescent during the bioassay, each petiole was covered by wet cotton. Leaves were dipped separately for approximately 10 seconds into each of three different pesticides (Table 1). After dipping, the leaves were air-dried for approximately 2 hours and placed at the bottom of the plastic Petri dishes (9 cm diameter × 2.5 cm high) which was lined with a wet filter paper and covered with a plastic lid. The experiment for each treatment was replicated four times along with distilled water treated as a control group. After 24, 48, 72 and 96 hours of post-treatments the numbers of live and dead larvae for each replicate were counted in the laboratory under a stereomicroscope.

2.2 Semi field assay

The toxicity of the six different pesticides (Table 1) to CLM was examined in the nursery and Laboratory at Sari Agricultural Science and Natural Resources University in 2007. 72 young trees (4 years) of the citrus variety of Thomson navel (*Citrus sinensis*) nursery within Sari agricultural University and spray method bioassay were used [7].

Experiments for each treatment were replicated four times, and distilled water was used as a control. At 24, 48, 72 and 96 hours post-treatment, the numbers of live and dead larvae in each replicate were counted in the laboratory under a stereomicroscope.

2.3 Topical spray toxicity assay

In topical spray assay, leaves containing actively feeding second and third instars larvae of citrus leafminer were placed into plastic Petri dishes (9cm diameter × 2.5 cm high). The leaves were sprayed with one ml aliquots of each chemical pesticide (Table 1) in a Potter Precision Spray Tower (Burkard Manufacturing Co. Ltd., Rickmansworth Herts, UK). Each treatment was replicated four times along with distilled water treated as a control group. The leaves in the control group were sprayed with 1 ml of distilled water. Following these treatments, the leaves were individually transferred to clean plastic Petri dishes. After 24 and 48 hours of post-treatment the numbers of live and dead larvaes for each replicate were counted in the laboratory (as above).

2.4 Measurements and statistical analysis

This experiment was conducted in a completely randomised design using factorial arrangment of treatments. Variables measured per replicate of each treatment were the average number of mines per leaf, larval mortality (the proportion of larvae that were dead). Normality was assessed using probability plots. The normal distributed was approximated for the number of dead larvae per leaf when these data were reciprocally transformed using $ArcSin\sqrt{\frac{y}{100}}$. Mortality data were corrected using Abbott's formula (Abbott, 1925). The analysis of data was performed on each dependent variable using the ANOVA procedure (SPSS, 1993). If a significance effect of variables was calculated, means were contrasted by Duncan's multiple range test and Tukey's test.

The insecticides and respectively concentrations used were *Bt* (0.5, 1, 3, and 6 g L^{-1} of water) in experiment 1, different percentage (0.1, 0.2, 0.3, 0.5) of mineral oil (MO) in experiment 2 and BT (0.5, 1, 3, and 6 g L^{-1}) + 0.5 % MO in experiment 3. In each experiment a control group was run using sterile water. The leaf-dip bioassays were devised to test the toxicities of *Bt* pesticide. In assay, only leaves with actively feeding second or third instar leafminer larvae were completely excised with petioles from citrus Thomson trees and used for bioassays. To keep the leaves turgescent during the bioassay, each petiole was covered by wet cotton. Leaves were dipped separately for approximately 10 seconds into each treatment. Air-dried for approximately 2 hours and placed at the bottom of the plastic petridishes (9 cm diameter × 2.5 cm high). These dishes were lined with a wet filter paper and covered with a plastic lid. The experiment for each treatment was replicated four times along with distilled water treated as a control group. After 24, 48, 72 and 96 hours of post-treatment the numbers of live and dead larvae for each replicate were counted under a stereo-microscope. Variable measured per replicate of each treatment were the average number of mines per leaf larval mortality (the proportion of larvae that were dead).

Tondexir, Sirinol and Palizin with 3, 1, 0.5, 0.1 mlL^{-1}dose from Kimia Sabz co. were used in this experiment. One bioassay methods (Leaf-dip method) were devised to test toxicities of these pesticides at five concentration (3, 1, 0.5, 0.1 mlL^{-1}) in distilled water. Leaves with actively feeding adult TSSM excised with petioles from *Brassica napus* L and used for bioassays. This experiment was conducted in a completely randomized design using factorial arrangements of treatments with two factors (TONDEXIR, SIRINOL and PALIZIN with 4 dose plus control and post spraying period with 4 times) in four replicates. The average number of mortality (the proportion of larvae that were dead) was detected. Normality was assessed using probability plots. The normal distributed was approximated for the number of dead larvae per leaf when these data were reciprocally transformed using $ArcSin\sqrt{\frac{y}{100}}$ (Sato et al., 2005). The analysis of data was performed on each dependent variable using the ANOVA procedure (SPSS, 1993). If a significance effect of variables was calculated, means were contrasted by Duncan's multiple range and LSD tests. The lethal concentrations (LC_{50} and LC_{90}) were calculated using probit analysis (Finney, 1971). The percentage mortality was calculated and corrections for mortality when necessary were done by using Abbot's (1925) formula.

3. Results

3.1 Effect of pyriproxifen, avant and buprfezin on CLM

Analysis of variance indicated that there were significant differences (P<%1) among methods of bioassays and type of chemical insecticides used at the present study (Table 2). The interaction effect of methods of bioassays and type of chemical insecticides was significant (P<%5), but no significant differences were found between post spraying methods**.**

Sources of variance	*df*	*Sum of squire*	*Mean of squire*	*Significance*
Type of bioassays (A)	1	0.905	0.905	**
Type of pesticides (B)	3	1.67	0.557	**
Post spraying period (C)	1	0.144	0.144	ns
A×B	3	0.550	0.183	*
A×C	1	0.008	0.008	ns
B×C	3	0.0470	.015	ns
A×B×C	3	0.0088	0.0029	ns

** Significant at the 1% level, * Significant at the 5% level, ns – nonsignificant.

Table 2. The ANOVA of effects of different factors on citrus leafminer larval mortality.

Interaction effect of post spraying methods on treatment was also not significant (Table 2).

Among the used chemical insecticides at the present study, Avant pesticide was more effective (35.58%) than Buprofezin (21.25%) and Pyriproxifen (19.31%) on citrus leafminer larval mortality (Table 3).

Treatment	Subset for Alpha =0.01		
	c	b	a
Control	7.96±3.05		
Pyriproxyfen		19.31±3.5	
Avant			35.58±5.7
Buprofezin		21.25±4.8	

[a, b, c] Means followed by different letters are significantly different.

Table 3. Effects of fixed factors (three different commercial chemical insecticides) used in the model on citrus leafminer larval mortality (%).

Multiple slope of Duncan's test showed that between two types of spray methods, leaf-dipping method was more effective (29.5%) than topical spray method (12.20%) on pest mortality (Table 4).

Spray methods	Pesticides			
	Control	Pyriproxyfen	Avant	Buprofezin
Leaf-dipping	10.94±3.5[dc]	22.07±5.3[bcd]	49.75±8.09[a]	36.67±5.3[ab]
Topical Spray	5.0±2.67[e]	19.55±2.97[cde]	21.42±4.08[bc]	5.0±2.3[de]

[a] Means followed by the same letter are not significantly different

Table 4. Comparison between two bioassay methods on citrus leafminer larval mortality (%).

No significantly difference in citrus leafminer larval mortality was detected between periods of post spraying in pest mortality (Table 5).

Post spraying	Pesticides			
	Control	Pyriproxyfen	Avant	Buprofezin
24 h post treatment	6.25±2.25	19.57±7.07	42.96±7.30	35.11±9.15
48 h post treatment	15.62±9.37	24.57±8.74	56.53±14.88	38.23±6.81

Table 5. Comparison between two post spraying treatments on citrus leafminer larval mortality (%).

3.2 Effect of Bt and Mineral Oils (MO) on CLM

A significant reduction in the number of larval mortality per leaf in all chemical treated groups (19.31-35.58%) compared to untreated leaves as a control group (7.96%) was achieved (Table 7).

Analyses of variance indicated that there were significant differences among *Bt* treatments (P<0.01). The results clearly demonstrated that the efficacy of *Bt* against CLM increased with increasing *Bt* concentration (Table 6).

Treatment	Mean comparison		
	c	b	a
Control	8.49±1.5		
0.5	35.40±7.5	35.40±7.5	
1		40.07±5.3	40.07±5.3
3		46.96±5.7	46.96±5.7
6			61.16±7.5

a, b, c, Means did not followed by the same letters in rows are significantly different (P<0.01).

Table 6. The effect of different concentrations of *Bt* (0.5, 1, 3 and 6 gram per liter of water) on percentage of CLM larvae mortality (Mean±sd).

The comparison between different post treatment times showed significant differences (P<0.05) among the periods of the post treatments (Table 7).

Post treatment time (h)	Mean comparison	
	b	a
24	16.81±3.8	
48	35.37±4.64	35.37±4.64
72		47.45±6.70
96		54.10±7.15

a, b, Means did not followed by the same letters in rows are significantly different (P<0.01).

Table 7. The effect of different post treatment time of *Bt* on percentage of CLM larvae mortality (Mean±sd).

Post-treatment for 96, 72 and 48 h were more effective than 24 hour on pest mortality. There was no significant difference between interaction of *Bt* and post treatment time on CLM larvae mortality. Results for different concentrations of *Bt* plus MO indicated significant differences among treatments. The results showed that the treatment of *Bt* plus MO increased the mortality of CLM larvae at higher concentration of *Bt* (Table 8).

Treatments	Mean comparison		
	c	b	a
control	8.48±1.5		
0.5		53.38±3.4	
1		54.72±5.6	54.72±5.6
3		56.25±4.8	56.25±4.8
6			63.13±5.5

a, b, c, Means did not followed by the same letters in rows are significantly different (P<0.01).

Table 8. The effect of different concentrations of *Bt* (0.5, 1, 3 and 6 gram per liter of water) plus MO (0.5%) on percentage of CLM larvae mortality (Mean±sd).

The comparison between different post treatment times showed significant differences (P<0.05) among the periods of the post treatments on CLM larvae mortality (Table 9).

Post treatment time (h)	Mean comparison	
	b	a
24	42.6±10	
48	59.27±9.6	59.27±9.6
72	64.82±8.3	64.82±8.3
96		85.82±5.9

a, b, Means did not followed by the same letters in rows are significantly different (P<0.05).

Table 9. The effect of different post treatment time of *Bt* plus MO (0.5%) on percentage of CLM larvae mortality (Mean±sd).

No statistically differences were observed in CLM larvae mortality between *Bt* treated groups in comparison with their counterparts *Bt* plus MO groups (Table 10).

Treatments(dose) mg/l	BT	BT+MO
0	8.49±1.51	8.49±1.51
0.5	35.40±7.60	56.04±3.46
1	38.22±5.62	53.73±5.65
3	46.96±5.72	54.24±4.91
6	61.16±7.56	63.21±5.61

Table 10. Mean comparison between the effect of *Bt* and *Bt* plus MO (0.5%) on percentage of CLM larvae mortality (Mean±sd).

3.3 Effect of Bt, MO, IG (Palizin) IE (Sirinol) on CLM

There were significant differences (p<0.001) among different biorational insecticides and post spraying methods that used at the present study (Table 11 & 12), but the interaction effect of insecticides and time was not significant. These results showed that each insecticides and time have independent and separate effect on percentage of larval mortality.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment(A)	.017	4	.004	10.706	**
Day(B)	.016	3	.005	13.872	**
treat * day	.002	12	.000	.524	ns
Error	.016	40	.000		

**showed significantly different (P<0.01)
^{ns} not significantly different

Table 11. The ANOVA of different biorational insecticides on citrus leaf miner larval mortality

The percentage of larvae mortality in IE, IG, BT and MO was 67.83±9.10, 62.45±8.10, 49.08±6.70 and 37.70±8.50, respectively (Table 12). There were significant differences in larvae mortality between control and treatment groups ($p<0.0001$).

treatment	Subset for alpha = .05		
	1a	2	3
control	19.16 ± 5.25		
MO	37.75 ± 8.47	37.75 ± 8.47	
BT		49.08 ± 6.68	49.08 ± 6.68
IG			62.50 ± 8.10
IE			67.83 ± 9.09

[a] Means followed by the same number are not significantly different

Table 12. The comparison of the mean of different Biorational insecticides on percentage of larval morality of CLM consist of Tukay ´s test.

A significantly differences in CLM mortality was found among post spraying period (Table 4). The percentage of larvae mortality among 96 and 72 hours of post treatments (71.9±5.9 and 54.4±6.3) were more effective than 24 and 48 hours post treatments (25.2±6.8 and 37.4±7.8). The results have shown that 96 and 72 hours post spraying period were more effective than 48 and 24 hours post spraying period on mortality of the CLM (Table 13).

Time Hours(h)	Subset for alpha = .05		
	1	2	3
24	25.2±6.8		
48	37.4±7.8	37.4±7.8	
72		54.4±6.3	54.4±6.3
96			71.9±5.9

[a] Means followed by the same letter are not significantly different

Table 13. The comparison of the mean of different post spraying period on percentage of larval morality of CLM consist of Tukay ´s test ($P < 0.01$) .

Among different biorational insecticides at the present study, IE with 67.83±9.10 and IG with 62.45±8.10 were more effective than Bt with 49.08±6.70 and MO with 37.70±8.50 percentage on citrus leafminer larval mortality (Table 14).

Treatment	Mean
Bacteria	49.08 ± 6.68*
Mineral oil	37.75 ± 8.47**
.Insecticide gel	62.50 ± 8.10**
Insecticide emultion	67.83 ± 9.09**
Control	19.16 ± 5.25
Tukay(0.1)	17
Tukay(0.5)	22

**showed significantly different (P<0.01)
*showed significantly different (P<0.05)

Table 14. Mean comparison between the effects of biorational insecticide on percentage of CLM larval morality (Mean ± sd).

3.4 Effect of Reldan, Runner, Tracer, Sirinol, Palizin and MO on CLM

The toxicity of Reldan, Runner, Tracer, Sirenol, Palizin and oil (Table 1) on CLM was investigated using a leaf-dip bioassay. There were significant differences (p<0.001) among the different treatments and also among the intervals before assessment (Table 15).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	0.072	6	0.012	104.192	**
Time	0.010	3	0.003	28.043	**
Treatment * time	0.006	18	0.000	2.855	**
Error	0.006	56	0.000		

** Significantly different (p<0.01)

Table 15. ANOVA details for different insecticides and times to assessment.

The percent of larvae dead with Reldan, Runner, Tracer, oil, Palizin and Sirenol was 89.33 %, 86.66 %, 100 %, 84.66 %, 83.66 % and 50 %, respectively, at 96 hours after treatment (Table 16). The difference between the control and all other treatments was significant at p<0.0001.

Treatment	Time after treatment			
	24 h	48 h	72 h	96 h
Control	8.33 ± 0.8 a[1]	8.33 ± 1.55 a	8.33 ± 1.66 a	8.33 ± 1.66 a
Tracer	41 ± 4.9 d	93.33 ± 6.66 d	93.33 ± 6.66 d	100 d
Reldan	79 ± 10.59 d	89.33 ± 5.36 d	89.33 ± 5.36 d	89.33 ± 5.36 d
Runner	54.33 ± 1.73 cd	61 ± 20.1 cd	73.66 ± 1.54 cd	86.66 ± 6.88 cd
Palizin(IS)	41 ± 4.93 c	52 ± 15.63 c	66 ± 8.62 c	83.66 ± 2.33 c
Sirenol(IE)	24.33 ± 4.33 b	31 ± 5.85 b	43.33 ± 3.33 b	50 ± 5.77 b
Oil	14.66 ± 8.11 c	60 ± 3 c	60 ± 3 c	84.66 ± 8.41 c

Means followed by the same letter are not significantly different

Table 16. Comparison of the mean time on Tracer, Reldan, Runner, IS, IE and oil on the percentage mortality of CLM larvae using Tukey´s test.

Reldan, Runner and Tracer, with 96-h mortalities of 89.33 %, 86.66 % and 100 %, respectively, were more effective than IS, oil and IE with mortalities of 84.66 %, 83.66 % and 50 % at the rates used (Table 3). CLM mortality also varied significantly with the post-spraying interval tested, increasing with time (Table 5).
The total percentage of larvae mortality achieved by Reldan, Tracer, Runner, MO, IG and IE were 86.75 ± 3.2, 81.91 ± 7.5, 68.91 ± 7.16, 54.83 ± 8.07, 60.66 ± 6.24 and 37.16 ± 3.69, respectively (Table 17). Significant differences in larvae mortality between the control and the various treatments were observed ($P < 0.0001$).

treatment	Subset for alpha = .05[a]			
	a	b	c	D
Control	8.33+_0.0			
IE		37.16 ± 3.69		
IG			60.66 ± 6.24	
MO			54.83 ± 8.07	
Runner			68.91 ± 7.16	68.91 ± 7.16
Tracer				81.91 ± 7.5
Reldan				86.75 ± 3.2

Means followed by the same letter are not significantly different

Table 17. Comparison of the different insecticides using the overall mean percentage mortality of CLM larvae and Tukey´s test.

Significant differences in the mortality of CLM larvae were also found between the lengths of time after treatment (Table 18).

Time Hours (h)	Subset for alpha = .05 [a]		
	a	b	c
24	37.52 ± 7.8		
48		56.42 ± 6.8	
72		62 ± 6.3	62 ± 6.3
96			71.80 ± 5.9

[a] Means followed by the same letter are not significantly different

Table 18. Comparing the mean effect of different post-spray assessment periods on percentage mortality of CLM larvae using Tukey´s test (P < 0.01).

The percentage mortality at 96 and 72 h post-treatment (71.80 ± 5.9 and 62.1 ± 6.3, respectively) was considerably higher than the mortality at 48 and 24 h post-treatment (56.42 ± 6.8 and 37.4 ± 7.8, respectively). Thus, the 96 and 72 h post-treatment periods were more effective than the 48 and 24 h treatments on CLM mortality. Among the different pesticides tested, Reldan (86075 ± 3.2) and Tracer (81.91 ± 7.5) were more effective than the others on percentage CLM larvae mortality.

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 Nissuron 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

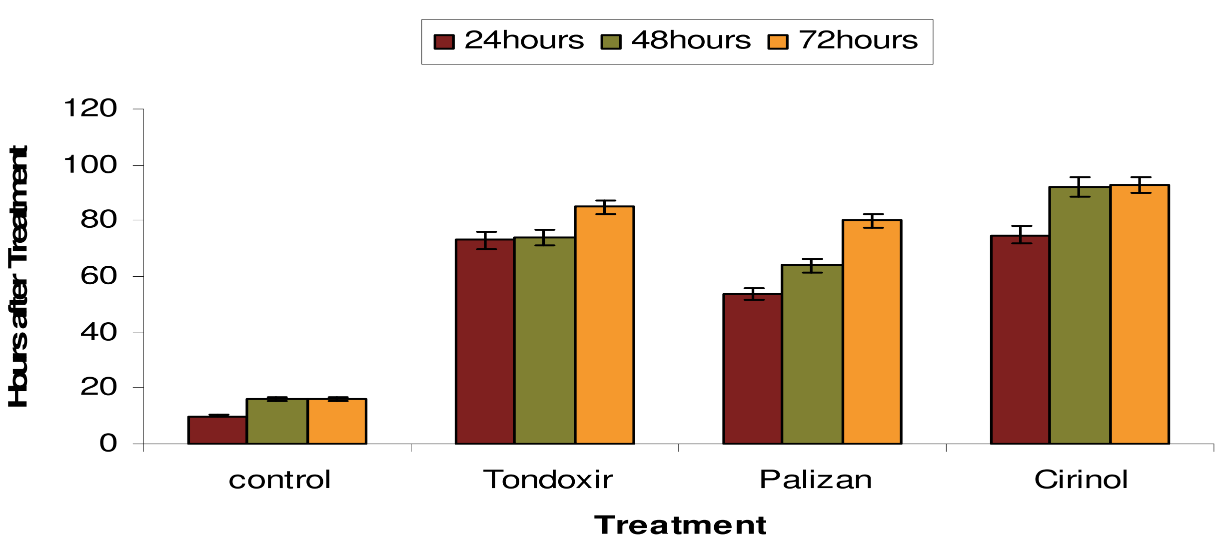

Fig. 1. Comparison of the effect of different toxins on TSSM

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 morality 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 serpintine 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 turingiensis (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_{50} 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, Sirenol, Palizin and MO are active against the leafminer demonstrating that these biopesticiedes 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, Sirenol, 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 *nCy* 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, therfore, 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 serpintine 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 Pyriproxifen decreased the number of laying eggs, hatched eggs and also short life cycle in *Bemizia tabaci* (Yasui *et al.*, 1987; Ishaaya *et al.*, 1994). They concluded that Buprofezin and Pyriproxifen affects on reproductive system of *Bemizia 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, Sirenol, 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 Sirenol (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, Sirenol, 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, Sirenol, 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_{50} (oral, rat) is 3000 mg/kg, than Dursban with an LD_{50} (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_{50} 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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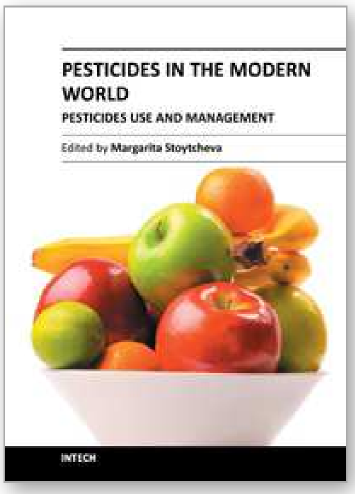

Pesticides in the Modern World - Pesticides Use and Management

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This book brings together issues on pesticides and biopesticides use with the related subjects of pesticides management and sustainable development. It contains 24 chapters organized in three sections. The first book section supplies an overview on the current use of pesticides, on the regulatory status, on the levels of contamination, on the pesticides management options, and on some techniques of pesticides application, reporting data collected from all over the world. Second section is devoted to the advances in the evolving field of biopesticides, providing actual information on the regulation of the plant protection products from natural origin in the European Union. It reports data associated with the application of neem pesticides, wood pyrolysis liquids and bacillus-based products. The third book section covers various aspects of pesticides management practices in concert with pesticides degradation and contaminated sites remediation technologies, supporting the environmental sustainability.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
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Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821