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Full Length Research Paper

Toxicity evaluation of Tracer, Palizin, Sirinol, Runner and Tondexir with and without mineral oils on *Phylocnistis citrella* Stainton

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The bioinsecticides are considered to be the best and effective pesticides. The main aim of this experiment was to determine the efficacy of Tracer (Spinosyn), Insecticidal Gel (Palizin), Insecticidal Emulsion (Sirinol), Runner (Methoxifenozide) and Tondoxir with and without mineral oils (MO) on the control of citrus leaf-miner (CLM), *phylocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in laboratory condition at 24, 48, 72 and 96 h post treatments. Leaves of citrus with second and third instars of leaf-miner larvae were used in all tests and the larval mortality were monitored. Analysis of variance showed that there were significant differences between treatments and control, and also significant differences were found among treatments. Tukay-Test among above treatments has shown that Tracer + oil, Runner and Runner + oil and Tondxir + oil with 98 ± 3. 2, 98 ± 8.1 and 93 ± 5% mortality were more effective than MO, Tondxir and Sirinol + oil with 85 ± 8.3 and 81±7.2 and 78.25 ± 8.2% mortality and significant different with control (P< %1), respectively. Tukay-Test among 96 and 72 h post treatments with 77 ± 3.82 and 70.65 ± 7.5% of total mortality are more effective than 48 and 24 h post treatments with 52.27 ± 4 and 35.90 ± 3.8% of total mortality, respectively.

Key words: Tracer, Runner, Tondxir, Sirinol, mineral oils, Palizin, *Phylocnistis citrella* Stainton.

INTRODUCTION

Biopesticides, including botanicals, can offer a safe and effective alternative to conventional insecticides for controlling major insect pests within an integrated pest management 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 pesticides. Pepper worked better when insects are soft-bodied during the larval stage because the chemical is able to penetrate. It worked fast.

Methoxyfenozide (Runner 240 SC), a Moulting Accelerating Compound (MAC) and an ecdysone agonist, currently submitted for registration, is an IPM (Integrated Pest Management) compatible compound with strong, broad-spectrum activity against lepidopterous pests.

Spinosad is a mixture of two macrolide lactones, Spinosyn A and D, produced by fermentation of the soil actionmycete *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).

The novel natural extracts of higher plants have a variety of characteristics including insecticidal, antifungal, antiviral and antibacterial activity, repellence to pests, antifeedant effects, insect growth regulation, and toxicity to nematodes, mites and agricultural pests. Insecticidal activity has been demonstrated in many plants (Carlini and Grossi-de-Sá, 2002). The plant substances contain components which 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 leaf-miner (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important pest of citrus and related Rutaceae and ornamental plants worldwide (Achor et al*.,* 1996). The CLM mines leaves, the surface tissue of young shoots and stems, and less frequently the fruit (Sponagel and Diaz, 1994). Although

Table 1. Pesticide used in this experiment.

Common name	Trade name	Chemical group	Formulation company	LD50 for rats (mg/kg)	Dose (ml/l)
Tondexir	Tondexir	pepper extract insecticide	ECKimia Sabzaver	> 5000	2
Methoxyfenozide	Runner	Diacylhydrazin(IGR)	Dow AgroSciences 24% SC	> 5000	1
Spinosad	Tracer	Spinosyn A & D	240sc	>5000	0.75
MO	PO	MO	O 80%	4300	0.05
Insecticidal emulsion (IE)	SIRENOL	Garlic extract insecticide	EC Kimia Sabzaver	>5000	2
Insecticidal Gel (IG)	PALIZIN	Insecticidal and miticidal soap	WSKimia Sabzaver	>5000	2

CLM causes indirect damage to young leaves, which predisposes them to infection by canker, controlling CLM is a vital component in 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. 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).

Because CLM is protected inside the mine it is suggested that the use of mineral oils would act as a surfactant to reduce the surface tension and increase the penetration of insecticides through the epidermis of the citrus leaf (Dias et al., 2005). The aim of this study was to evaluate the efficacy of Methoxyfenozide (Runner), Spinosyn A and D (Tracer), Insecticidal Gel (Palizin, IG), Insecticidal Emulsion (Sirinol, IE), Tonexir with/without Mineral Oils (MO) against *P. citrella* Stainton (Lepidoptera: Gracillariidae).

MATERIALS AND METHODS

Semi field bioassay and experimental design

The toxicity of the six different pesticides (Table 1) on CLM was examined in the Laboratory of Toxicology at Sari Agricultural and Natural Resources University in 2008. The bioassay method was used as described by Amiri 2008 with some modification. The young citrus Thomson (*Citrus sinensis*) trees at nursery were used. 72 young citrus trees (4 year-old) were randomly selected at Sari Agricultural and Natural Resources University.

The experiment comprised of 12 treatments (T1, T2, T3, T4,…….. and T12) allocated in a randomised block design and each treatment consisted of 4 replicates. Leaves of the trees of T1, T2, T3,……and T11 were treated with 10 ml of Table 1 pesticides with and without mineral oils (using a hand sprayer) respectively. Trees of T12 were treated with distilled water (control). During 4 days the number of dead larvae and pu-pae from attacked leaves from T1, T2, T3……. and T12 were re-corded at 4-day interval.

Statistical analysis

The data of experiment were analyzed by a completely randomized design using factorial arrangements of treatments (four replicates for each treatment). 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 distribution was approximated for the number of dead larvae per leaf when these data were reciprocally transformed using *ArcSin* $\sqrt{y}/100$. Mortality data were corrected using Abbott's formula (Abbott 1925). The analysis of data was performed on each dependent variable using the treatments were compared for significance with ANOVA. Mean separation was determined using the Tukey's test.

RESULTS

The toxicity of Tondexir, Runner, Tracer, Sirenol, Palizin with/without MO (Table 1) on *P. citrella* Stainton (Lepidoptera: Gracillariidae) was investigated under laboratory conditions using the leaf-dip assay. There were significant differences ($p<0.001$) among the different insecticides and the post-spraying methods used in the present study (Table 2).

The results showed that each insecticide and each time point had an independent and separate effect on the percentage of larvae mortality. The percentage of larvae mortality with Tondexir, Runner, Tracer, Palizin and Sirinol without MO were 81 ± 7.29, 98, 76.25 ± 3.40, 63.5 ± 9.39 and 71.25 ± 11.42, respectively, and the only MO was 85 ± 8.35 at 96 h post-treatment (Table 3). When the MO was added to the above pesticides, the mortality was slightly increased but not significantly (Table 3). There were also significant differences in larval mortality between the control and the treatments ($p<0.0001$). Among the different insecticides, Tondexir + Oil, Runner and Tracer + Oil with 93 ± 5, 98 and 98% mortalities, respectively, were more effective than others after 96 h post treatment (Table 3).

The total higher percentage mortality was obtained with

Table 2. ANOVA of different biorational insecticides o citrus leat miner larval mortality.

Source	Type III sum of squares	Df	Mean square	F	Sig.
Treat	.073	11	.007	27-975	**
Day	/0.30	3	.010	41.986	**
Treat*day	.007	33	.000	.938	Ns
Error	.034	144	.000		

**Significant at the 1% level; ns: non significant.

Table 3. The comparison of the mean of different toxins on percentage of larval morality of CLM consist of Tukay ´s test.

Treatment	Time (h)			
	24	48	72	96
Control	5±1.1a*	5±1.1a	11.25±6.57a	11.25±6.57a
Tondoxir(2/1000)	12.25 ± 6.25bc	34.25± 3.86bc	69 ± 6.25bc	81 ± 7.29bc
Tondoxir +Oil	31.75±7cdef	59.75 ± 9.98cdef	75 ± 8.88cdef	93 ± 5cdef
Tracer0.75/1000	51.50±18.17cde	67 ± 11.45cde	76.25 ± 13.40cde	76.25 ± 3.40cde
Tracer(0.75/1000)+Oil	76±12.70cde	90.75 ± 7.25cde	98cde	98cde
Runner(1/1000)	56.75±4.25f	87.25 ± 6.47f	98f	98f
R+Oil	52 ± 11.35ef	71±18.41ef	94.25±3.75ef	98ef
Palizin(2/1000)	9± 3.87b	33.50 ± 10.94b	56.75 ± 7.65b	63.50 ± 9.39b
Palizin +Oil	35.75 ± 8.03bcd	46.25 ± 13.85bcd	72.50 ± 4.09bcd	72.50 ± 4.09bcd
Sirinol (2/1000)	22 ±11.78bc	35.75 ± 8.03bc	56.75 ± 13.75bc	71.75± 11.42bc
Sirinol +Oil	45±8.61bcde	45±8.61 bcde	63.75 ± 11.70 bcde	78.25 ± 8.62 bcde
Oil(5/1000)	33.75±9.97cde	51.75±9.97 cde	76.25±8.40 cde	85±8.35 cde

*Means followed by the same letter are not significantly different.

Runner, Runner + Oil and Tracer + Oil with 78.81±6.67, 85±4.69 and 90.69±4 after 96 h post treatment respecttively (Table 4). Significant differences in larvae mortality between the control and the various treatments were observed ($P < 0.0001$).

Significant differences in CLM total mortality were found among the post-spraying periods (Table 5), which showed that 96 and 72 h post-spraying periods with 77.21±3.82 and 70.65±3.90% mortality were more effective than 48 and 24 h post-spraying periods. Thus, the 96 and 72 h post-treatment periods were more effective than the 48 and 24 h treatments on CLM mortality.

DISCUSSION

The above biopesticides (Table 1) caused different degrees of mortality and offered various level of protection to the leaves of citrus. One of the new plant extracts used in this experiment, was pepper extract (Tondexir). These products can control the CLM in a very good way (Table 2). These results can corroborate the studies of pepper extracts and formulation on other insect species showed that pepper has indeed insecticidal properties (Gbewonyo et al., 1993).

These data represent some of the first published information on the effects of Tondexir, Runner, Tracer, Sirenol, and Palizin with or without MO on CLM. It is very difficult to protect the new shoots of young trees from the damage caused by CLM, which is one of the main and complex pests affecting growing citrus, especially in nurseries and newly planted orchards in north Iran. The CLM larvae are protected by the 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 insecticides studied here had significant insecticidal activity against CLM larvae, but Tondexir + Oil, Runner and Tracer + Oil were more effective than the other treatments (Table 2). The results from this study suggest that there may be different compounds in Tondexir, Runner, Tracer, IE and IG which have different bioactivities.

This study has shown that Tondexir, Runner, Tracer, Sirinol, Palizin and MO are active against the CLM, demonstrating that these pesticides penetrate into leaf mines, killing the larvae as observed by Shapiro et al. (1998), and the adjuvant ingredient of Tondexir, Runner, Tracer, Sirinol, Palizin in combining with MO might reduce the infestation by acting as an oviposition deterrent in the field (Liu et al., 2001). The Tonexir, Runner, Tracer, Sirenol, Palizin with MO solutions tested in the current study had two different effects on CLM mortality. Firstly, they had insecticidal activity when applied against CLM at

Table 4. Larval mortality (%) of CLM after treatment (means) with different biorational insecticides (Tukey's test).

Treatment	Subset for alpha = 0.05					
	a*	b	c	d	e	f
Control	8.13±2.73					
Palizin		40.69±6.68				
Sirinol		46.56±7.10	46.56±7.10			
Tondoxir		49.13±7.56	49.13±7.56			
Palizin +Oil		56.75±5.65	56.75±5.65	56.75±5.65		
Sirinol +Oil			58±5.56	58±5.56	58±5.56	
Oil			61.69±6.65	61.69±6.65	61.69±6.65	
Tondoxir+Oil			64.88±6.80	64.88±6.80	64.88±6.80	64.88±6.80
Tracer(0.75/1000)			67.75±6.91	67.75±6.91	67.75±6.91	67.75±6.91
Runner+Oil					78.81±6.87	78.81±6.87
Runner					85±4.69	85±4.69
Tracer+oil						90.69±4

*Means followed by the same letter are not significantly different.

Table 5. Effect (means) of different times of evaluation after treatment on percentage of larval mortality of CLM (Tukey's test; $P < 0.01$).

Day	Subset for alpha = 0.05		
	a*	b	c
1	35.90±3.88		
2		52.27±4.28	
3			70.65±3.90
4			

*Means followed by the same letter are not significantly different.

the recommended dose. Secondly, they increased the efficacy of the commercial formulation of Tondexir, Runner, Tracer, Sirenol and Palizin by helping to penetrate the plant cuticle, and enhanced the activity of the active ingredient of these insecticides when applied to the pest, probably due to increased penetration through the stomata into the mines. Since CLM preferentially mines the abaxial surface of leaves, enhanced stomatal infiltration is especially useful against CLM.

Undoubtedly, plant-derived toxicants and other insecticides may play a more prominent role in CLM control programs in the future (Mordue and Blackwell, 1993). Since the active ingredient of these biopesticides are often active against a limited number of species including specific target insects, are less expensive, 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. Natural products 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).

Methoxyfenozide (Runner) is a dibenzoylhydrazine IGR, and is similar to tebufenozide in its mode of action, its ability to induce a lethal molt and its specificity for Lepidoptera (Carlson et al., 2001). In our study, however, the IGR methoxyfenozide (Runner), although considerably more toxic than the other insecticides, still resulted in substantial (98%) mortality of CLM after 96 h. Similar results found by Schneider et al. (2003) that halfenozide, a similar ecdysteroid agonist to methoxyfenozide, was slightly toxic to adult *Hyposoter didymator* (Hymenoptera: Ichneumonidae).

Spinosad will be good promising pesticide for controlling the CLM A significant advantage of Spinosad is that it is effective against strains of *Rhyzopertha 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 (Elzen, 2001). 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). In insects, the mode of action of Spinosad is associated with excitation of the insect's nervous system (Salgado, 1998). Once ingested, Spinosad kills the target pest by over-stimulating its nervous system in a manner that is unique to insects. DOW asserts that this mode of action helps prevent insects from building up resistance to the compound (Brank-Glenn, 2005)

The efficacy of petroleum-derived spray oils used as oviposition deterrents to control CLM is related to time of spraying, the dose of oil and the persistence of oil molecules on sprayed surfaces (Liu et al., 2001). Therefore, the petroleum oils alone or combined with other pesticides such as Tondexir, Palizin, Sirinol may have a synergistic and less harmful effect on the environment is recommended for use in IPM programs (Khyami and Ateyyat, 2002).

Amonkar and Reeves (1970) found that garlic, similar to IE, killed an insecticide resistant strain of *Aedes nigromaculis* as well as susceptible *Aedes* species which is similar results has been obtained with Sirinol in this test.

This study indicated that plant-based compounds such as Sirinol, Tondexir and Plizan alone or combination with MO may be effective alternatives 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, in the field and semi field condition to obtain suitable results.

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