http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (13): 2483-2487, 2006
ISSN 1028-8880

Toxicity Evaluation of Avant, Buprofezin and Pyriproxifen Pesticides Against the *Phyllocnistis citrella* Stainton (Lepidptera: Gracillariidae)

B. Amiri Besheli
Department of Plant Protection, Laboratory of Toxicology,
Faculty of Agricultural Sciences, Mazandaran University, Sari, Iran

Abstract: Different methods have been used to control the citrus leafminer (*Phyllocnistis citrella*) but the chemical and the microbial controls are realized to be the best and effective methods. The purpose of the present study was to evaluate the efficacy of three different commercial chemical insecticides (Avant, Buprofezin and Pyriproxifen) with 2 spray methods (leaf-dip and topical spray) at 24 and 48 h of post treatment to citrus leafminer. Analysis of variance showed that there were significant differences between treatment and untreated control groups. Among the treatments, Avant pesticide was more effective (35.58%) than Buprofezin (21.25%) and Pyriproxifen (19.31%) on citrus leafminer larval mortality. The results indicated that there were significant differences between two spray methods and leaf-dip was more effective than topical spray on citrus leafminer larval mortality. No significant difference in effectiveness of 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. As there are no previous reports concerning the effect of these commercial chemical insecticides on natural enemies of citrus leafminer, further research in this area and also a field analysis to confirm their suitability for the pest management is necessary.

Key words: Avant, buprofezin, pyriproxifen, bioassay

INTRODUCTION

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidptera: Gracillariidae) is a serious pest of citrus and related species of Rutaceae family and also some ornamentals in many parts of the world (Beattie *et al*., 1995). CLM larvae form mines predominantly in leaves, but also in succulent stems and sometimes fruit. The larvae bore through the leaf epidermis, ingesting the sap and producing a chlorotic leaf patch. CLM may prevent young leaves from expanding, causing them to remain twisted and curled. After the CLM have finished feeding, other insects such as aphids and mealybugs often continue feeding on the damaged area (Michaud and Grant, 2003). The CLM is believed to have originated in India and southern China, where it is a major constraint to citrus production. In the last century it has spread to the Philippines, Japan, the Middle East, Australia and parts of Western Africa. The movement into Northern Africa, Central America and the United States is very recent. Today, it is found in nearly all citrus growing regions of the world (Beattie *et al*., 1995). Sometimes much more important for the crop is the indirect damage of the pest. In this particular case, CLM can help spread citrus canker *Xanthomonas axenopoids* (Pena *et al*., 1996). Several different insecticides are used against this pest, but these may involve undesirable effects on the environment, including interference in control of the pest by natural enemies (Gottwald *et al*., 1997).

Enhancement of natural enemies, especially parasitic wasps, is considered the most effective means of managing infestations of the citrus leafminer on older fruit bearing citrus. Many of the parasites that attack citrus peelminer also attack citrus leafminer. The native eulophid wasp, *Cirrospilus coachellae*, which attacks the citrus peelminer, is expected to shift to the leafminer (Pena *et al*., 1996). Although, the biological control is considered to be the most suitable method for the management of CLM, but it hasn't been the total answer. So far, chemical control, mainly practiced as foliar applications of pesticides, has been the major strategy used against CLM. Effective chemical control of CLM is difficult because the larva is protected by leaf cuticle and the pupa is protected by rolled leaf margins (Raga *et al*., 2001). The first record of CLM from southern parts of Iran dates back to 1961, but in northern Iran its presence was noted for the first time in September 1994. Since then, it has shown a dramatic increase and widespread dispersal (Jafari, 1996). Almost all commercial varieties are affected but data on economic losses are not available. The pest has about 5-9 generations during the 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). No information on other pesticides, indigenous parasitoids and methods for controlling of CLM in Iran is available. A number of products, singly or in combination, has been tested and recommended for controlling the CLM. In this research we describe and compare results from bioassays applied against leafminers in isolated citrus leaves in laboratory conditions.

MATERIALS AND METHODS

Laboratory bioassay: The toxicity of some chemical insecticides to the citrus leafminer was examined in the laboratory of toxicology at Mazandaran University on summer 2005. Immature growth flushes of leaves were sampled from citrus Thomson trees of different farms at Sari district in north of Iran. The leaves were placed in a moistened labeled paper bag, then, with other samples, in a large plastic bag and stored at 4°C until assayed. Two bioassays were devised to test toxicities of 3 commercial chemical insecticides (Avant, Buprofezin and Pyriproxifen).

Leaf-dip assay: In both assays only leaves that contained actively feeding second or third instars 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 5 sec into each of three different pesticides (Table 1). After dipping, the leaves were air-dried for approximately 2 h 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 and 48 h of post-treatment the numbers of live and dead larvae for each replicate were counted in the laboratory under a stereomicroscope.

Topical spray assay: In topical spray assay, leaves containing actively feeding second and third instar larvae of citrus leafminer were placed into plastic petri dishes (9 cm diameter×2.5 cm high). The leaves were sprayed with 1 mL aliquots of each chemical pesticide (Table 1) in a Potter Precision Spray Tower (Burkard Manufacturing Co. Ltd., Rickmansworth Herts, UK) at 55 kPa pressure. Each treatment was replicated 4 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 h of post-treatment the numbers of live and dead larvae for each replicate were counted in the laboratory (as above).

Measurements and statistical analysis: This experiment was conducted in a completely randomized design using factorial arrangement 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:

$$\mathrm{ArcSin}\sqrt{\frac{y}{100}}$$

Mortality data were corrected using Abbott's formula (Abbott, 1925). The analysis of data was performed on each dependent variable using the ANOVA procedure. If a significance effect of variables was calculated, means were contrasted by Duncan's multiple range test.

Table 1: List of compounds, chemical group, formulations and dose rates of chemical insecticides tested for citrus leafminer

Material	Trade name	Chemical group	Formulation	Dose	LD_{50}
Avant	Indoxicarp	Oxadiazon	SC150	0.5/1000	3619 mg kg
Buprofezin	Aplaud	IGR (Chitin Synthesis Inhibitors)	EC40%	2/1000	2198 mg kg
Pyriproxyfen	Admiral	IGR (Juvenile Hormone Mimic)	EC 10%	0.5/1000	>5000 mg kg

RESULTS

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. The 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). Multiple slope of Duncan's test showed that between two types of spray methods, leaf-dip method was more effective (29.5%) than topical spray method (12.20%) on pest mortality (Table 4). No significantly difference in citrus leafminer larval mortality was detected between periods of post spraying in pest mortality (Table 5). 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 3).

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

Sources of variance	df	Sum of square	Mean of square	Significance
Type of bioassays (A)	1	0.9050	0.9050	**
Type of pesticides (B)	3	1.6700	0.5570	**
Post spraying period (C)	1	0.1440	0.1440	ns
A×B	3	0.5500	0.1830	*
A×C	1	0.0080	0.0080	ns
B×C	3	0.0470	0.0150	ns
A×B×C	3	0.0088	0.0029	ns

**Significant at the 1% level, *Significant at the 5% level, ns- non significant

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

	Subset for alpha = 0.01		
Treatments	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 letter(s) are significantly different

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

	Pesticides			
Spray methods	Control	Pyriproxyfen	Avant	Buprofezin
Leaf-dip	10.94±3.5[de]	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,b,c,d,e Means followed by the same letter(s) are not significantly different

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

	Pesticides			
Post spraying	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

DISCUSSION

The CLM is one of the key pest 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-dip 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 demonstrated that using petroleum oil spray residues reduced infestations of CLM by preventing oviposition (Beattie *et al*., 1995). It has been reported that both of Abamectin and Lufenuron pesticides along with petroleum oil provided a significant reduction in CLM larval activity (Raga *et al*., 2001). However, the efficacy of petroleum-derived spray oils used as oviposition deterrents to control citrus leafminer is related to timing of spray application, the amount of oil deposited on sprayed surfaces (dose) and the 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).

Sometimes the indirect damage of CLM is very important. Mining of immature foliage by the larvae can lead to reduced growth rates and yield (Zhang *et al*., 1994) and mined surfaces serve as foci for the establishment of diseases such as citrus canker, *Xanthomonas citri* (Sohi and Sandhu, 1968). 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). 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. Depending on the rate of insecticide used, the number and timing of applications and the level of coverage of the tree. Thus, it is necessary to be aware about the effect of these pesticides on beneficial insects including parasitoid and predators (Grafton and Gu, 2003; Villanueva-Jiménez *et al*., 2000). In this 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 2 pest control agents, Buprofezin and Super Royal are relatively much safer compounds than the conventional organophosphorus insecticides (Fahmy and Abdalla, 1998). Exposure of adult beetles species (*C. 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 this 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. 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. 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.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Beattie, G.A.C., Z.M. liu, D.M. Watson, A.D. Clift and L. Jiang, 1995. Evaluation of petroleum spray oils and polysaccharides for control of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). J. Aust. Entomol. Soc., 34: 349-353.

Fahmy, M.A. and E.F. Abdalla, 1998. Genotoxicity evaluation of buprofezin, petroleum oil and profenofos in somatic and germ cells of male mice. J. Applied Toxicol., 18: 301-305.

Gottwald, T.R., J.H. Graham and T.S. Schubert, 1997. An epidemiological analysis of the spread of citrus canker in urban Miami, Florida and synergistic interaction with the Asian citrus leafminer. Fruits, 52: 383-390.

Grafton, C.E. and P. Gu, 2003. Conserving Vedalia Beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), in Citrus: A continuing challenge as new insecticides gain registration. J. Econ. Entomol., 96: 1388-1398.

Ishaaya, I., Z. Mendelson and V.M. Melamed, 1988. Effects of Buprofezin on embryogenesis and progeny formation of sweet potato white fly (Homoptera: Aleyrodidae). J. Econ. Entomol., 81: 781-784.

Ishaaya, I., A. Decock and A. Degheele, 1994. Pyriproxifen, a potent suppressor of egg hatch and adult formation of the greenhouse whitefly (Homoptera: Aleyrodidae). J. Econ. Entomol., 87: 1185-1189.

Jafari, M.I., 1996. Report of the workshop on citrus leaf-miner (*Phyllocnistis citrella*) and its control in the near East. Safita (Tartous), Syria, 30 September-3 October, 1996, pp: 33.

Khyami, H. and M. Ateyyat, 2002. Efficacy of Jordanian isolates of *bacillus thurigiensis* against the citrus leafminer *Phylocnistis citrella.* Intl. J. Pest Manage., 48: 297-300.

Kruger, K. and C.H. Scholtz, 1997. Possible risk posed by drift of the insect growth regulator pyriproxyfen to the rare dung beetle *Circellium bacchus* in a national park. J. Insect Conser., 1: 215-220.

Liu, Z., G. Beatie, L. Jiang and D.M. Watson, 1999. Volumes of petroleum spray oil required for control of Phyllocnistis citrella Stainton (Lepidoptera: Gracillariidae) in mature citrus orchards. J. Aust. Entomol. Soc., 38: 141-146.

Liu, Z., G. Beatie, M. Hodgkinson and L. Jiang, 2001. Influence of petroleum derived spray oil aromaticity, equivalent n-paraffin carbon number and emulsifiers concentration on oviposition of citrus leafminer *Phyllocnistis citrella* Stainton. J. Aust. Entomol. Soc., 40: 193-196.

Mafi, S.A. and N. Ohbayashi, 2006. Toxicity of insecticides to the citrus leafminer, *Phyllocnistis citrella* and its parasitoids, *Chrysocharis pentheus* and *Sympiesis striatipes* (Hymenoptera: Eulophidae). Applied Entomol. Zool., 41: 33-39.

Michaud, J.P. and A.K. Grant, 2003. IPM-compatibility of foliar insecticides for citrus: Indices derived from toxicity to beneficial insects from four orders. J. Insect. Sci., 3: 1-8.

Pena, J.E, R. Duncan and H. Browning, 1996. Seasonal abundance of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in south Florida citrus. Environ. Entomol., 25: 698-702.

Raga, A., M.E. Sato, M.F. de Souza Filho and R.C. Siloto, 2001. Comparison of spray insecticides against citrus leafminer. Arq. Inst. Biol. Sao Paulo, 68: 77-82.

Sohi, G.S. and M.S. Sandhu, 1968. Relationship between citrus leafminer (*Phyllocnistis citrella* Stainton) injury and citrus canker (*Xanthomonas citri* (Hasses) Dowson) incidence on citrus leaves. J. Res., Punjab Agric. Univ., 5: 66-69.

Villanueva-Jiménez, J.A., M.A. Hoy and F.S. Davies, 2000. Field evaluation of integrated pest management-compatible pesticides for the citrus leafminer *P. citrella* and its parasitoid *Ageniaspis citrocola.* J. Econ. Entomol., 93: 357-67.

Yasui, M., M. Fukada and S. Maekawa, 1987. Effect of buprofezin on reproduction of the greenhouse whitefly, *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). Applied Entomol. Zool., 22: 266-271.

Zhang, A., C. O'Leary and W. Quarles, 1994. Chinese IPM for citrus leafminer. IPM Practitioner, 16: 10-13.