Residual Toxicity of Abamectin, Chlorpyrifos, Cyromazine, Indoxacarb and Spinosad on *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) in Greenhouse Conditions

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SUMMARY

*Liriomyza trifolii* is an important pest of vegetable crops in many parts of the world including Iran. In this study potted bean plants were sprayed with recommended field rates of abamectin, chlorpyrifos, cyromazine, indoxacarb and spinosad. To assess the residual activities of these insecticides, the plants were infested with *L. trifolii* adults 2 hours; 1, 3, 5, 7, 10, 15, 20, 25 and 35 days after insecticidal treatments. The adults were allowed to stay on treated plants for eight hours. The treated plants were kept in a greenhouse. Number of feeding stipples and larval mines on leaves, as well as pupation and adult eclosion rates were assessed. Two-way ANOVA procedure of SAS was used for statistical analysis and the treatment means were separated using Duncan’s multiple range test. Abamectin and spinosad severely affected egg hatching and embryonic development. Eggs oviposited in leaves with residues of chlorpyrifos up to 5 days old, had reduced hatching. Larval development was also, affected by residues of chlorpyrifos up to four weeks old. Indoxacarb reduced larval development and adult eclosion in treatments with up to 20 days old residues. Cyromazine had no effect on the number of larval mines, but, pupation was severely hampered and adult eclosion was completely ceased even in treatments with five weeks old residues. Determining the residual activity of insecticides used for controlling this pest is useful in avoiding unnecessary treatments.

Keywords: *Liriomyza trifolii*; Insecticides; Toxicity; Residues
INTRODUCTION

Serpentine leafminer *Liriomyza trifolii* (Burgess) is a polyphagous insect and attacks a wide range of vegetables and ornamentals all over the world (Schuster and Everett, 1983; Parrella, 1987). Both larvae and adults damage the host plants. The larvae feed on leaf mesophyll and reduce chlorophyll content. The adults also damage the leaves by puncturing them for feeding and oviposition (Parrella et al., 1985). In recent years, this insect has become one of the major pests of greenhouse crops in Iran. Biological control of the leaf miner has had limited success so far and at present, chemical control is the main tactic in managing these pests (Parrella et al., 2006). *Liriomyza* has a potential to develop a resistance to insecticides (Parrella, 1987). This potential affects effective control of leafminers (Parrella and Keil, 1984). The strong capability of *L. trifolii* to develop the resistance to insecticides also made a displacement of other species on several crops possible (Schuster and Everett, 1983). It was also demonstrated that *L. trifolii* is more tolerant to insecticides than other agromyzid species (Parrella and Keil, 1985).

Cyromazine, abamectin and spinosad are known as effective compounds for the control of leafminers (Ferguson, 2004; Prijono et al., 2004). Abamectin provides effective control of mites, thrips and leafminers (Lasota and Dybas, 1991). Spinosad has a unique mode of action against a variety of target pests, leaf and fruit worms, thrips, flies and mites; and expresses relatively low toxicity to mammals and birds (Eger and Lindenberg, 1998; Van Leeuwen et al., 2006). Cyromazine is a triazine insect growth regulator with good activity against many dipteran species. This insecticide has proved to be effective against leafminer larvae in many studies (Roo et al., 1996; Prijono et al., 2004). Indoxacarb is a broad-spectrum, highly effective and relatively new insecticide registered for use in orchards and vegetable crops for the control of lepidopteran pests and some sucking insects. It acts by blocking sodium channels in insect neurons (Lapied et al., 2000) and is designated as a reduced risk product by EPA (Liu et al., 2002). It has been used for controlling insect pests of cotton, fruits and vegetables in many countries. Some researchers have reported effective control of leafminers with indoxacarb (Seal et al., 2002; Martin et al., 2006). Chlorpyrifos is a broad spectrum organophosphorus insecticide with fairly low mammalian toxicity. It is used for controlling flies, storage insects, soil and foliar insects (Rigterink and Kenaga, 1966). It is also commonly recommended insecticide for controlling leafminers in Iran (Mosallanejad et al., 2003).

The growers apply insecticides when larval mines are visible on leaves and usually make several applications during each growing season. The intention of integrated pest management is to reduce environmental contamination, decrease and delay the development of resistance of pests against pesticides and to reduce pest management costs and increase growers’ benefits (Dent, 1995). Determining the residual activity of insecticides used for controlling pests will be useful in avoiding unnecessary chemical treatments. In this study the residual effect of three biorational insecticides, namely abamectin, cyromazine and spinosad and two conventional compounds indoxacarb and chlorpyrifos on *L. trifolii* was assessed.

MATERIAL AND METHODS

The experiments were conducted on bean plants – *Phaseolus vulgaris* cv Khomein. The bean plants were grown in pots (7 cm in diameter and 12 cm high) containing sand, clay and organic matter mixture (40, 40 and 20% respectively). The pots were watered daily with tap water.

Insect rearing

Different growth stages of serpentine leafminer were collected from greenhouses in Yazd (located in the centre of Iran) in January 2007. The insects were reared on common bean plants (*Phaseolus vulgaris*) for several generations in greenhouse conditions at 26 ± 2°C, 60 ± 10% RH and 16:8h (L:D) photoperiod. The adults were fed with 10% diluted honey solution. Synchronization was done according to method used by Cox et al. (1995). Nine to 10-day old bean plants were infested with 1-2-day old adult leaf miners. The infestations were carried out in wooden framed cages (140 × 70 × 70 cm) covered with 80 mesh organdie cloth. The flies were allowed to stay and oviposit on plants for six hours, after which they were removed. This short-term infestation allowed obtaining fairly uniformly aged eggs, larvae and adults. Synchronization was performed continuously during the study since the uniform insects were needed for the experiments.

Insecticides

The insecticides and concentrations used in the experiments are shown in Table 1. The concentrations used were based on recommended field rates of these products.
Ten-day old potted bean plants (one plant per pot) were used in bioassays. Cotyledon leaves of plants were dipped in insecticide solutions for three seconds. To ensure a complete wetting of the treated leaves, Tween®–80 was added to insecticide solutions (500 mg/l) as a surfactant. The control treatment consisted of distilled water and Tween®–80.

Two hours, 1, 3, 5, 10, 15, 20, 25 and 35 days after the treatments (DAT), four plants treated with each of the compounds and 24 pots in total (including control plants) were transferred to infestation cage. Five hundred (± 40) 1-2-day old adult flies (ca. 1:1 sex ratio) already fed with 10% diluted honey solution were released in the infestation cage and were allowed to stay and oviposit for eight hours. The flies were then removed from the plants by shaking them and using hand aspirator. The plants were then transferred to a fly free cage. Two days after infestation, fly feeding stipples were counted on each plant. Four days after the infestation, the leaves were clipped and the number of larval mines was recorded. Then the leaf petiole was wrapped in a wet cotton wool. Each leaf was transferred into a plastic Petri dish lined with tissue paper. The Petri dishes were wrapped with Parafilm to slow evaporation and drying of the leaves, and were transferred in a growth chamber with a photoperiod of 16:8h (L:D), relative humidity of 60 ± 5% and temperature of 26 ± 2°C. Five days later, the number of nymphs was recorded. The healthy nymphs were then transferred into glass vials (10 cm long and 1 cm in diameter). The openings of the vials were covered with 80 mesh organici cloth and the number of adults was recorded as they emerged.

Larval mortality was calculated using the formula described by Leibee (1988):

\[
\text{% larval mortality} = \frac{(m-p)}{m} \times 100
\]

Where \( m \) was the number of mines or larvae in each treatment and \( p \) was the number of pupae reared from the treatment with each compound.

Pupal mortality was assessed using the same formula with some modification:

\[
\text{% pupal mortality} = \frac{(p-a)}{p} \times 100
\]

Where \( p \) was the number of pupae in each insecticide and \( a \) was the number of adults emerged from treatment with each compound.

Analysis of data

The data were inspected for homogeneity of variance and normality. In the case of non-homogeneity, percent values were transformed by arcsine-square-root transformation and the number of feeding stipples and larval mines were transformed using square-root transformation before data analysis. Where significant F values were obtained (\( P<0.05 \)), the treatment means were separated using Duncan’s test. Two-way ANOVA procedure of SAS was used for all statistical analysis (SAS Institute, 2004).

RESULTS AND DISCUSSION

Feeding stipples

The interaction of age of residue*insecticide treatment on feeding stipples was not significant (\( F = 0.52; \text{df} = 45, 660; P = 0.996 \)). Hence the data were pooled for assessment. Feeding stipples on bean leaves were affected significantly by DAT (\( F = 17.49; \text{df} = 9, 660; P = 0.0001 \)). The number of feeding stipples was low in all treatments where infestation was carried out up to 3 DAT. It seems that the colour, firmness and the size of leaves were important factors in adult feeding, where 15-35-old day plants had suitable quality for adult feeding. Beside leaf quality, repellency and antifeedant the effect of used insecticides may have also affected adult feeding (resulting in the number of feeding stipples). Higher toxicity of insecticides in earlier days after treatment is possibly another reason for lower feeding.

Table 1. Insecticides used in residual bioassay

| Concentration (mg a.i./l) | Field rate | Manufacturer | Active ingredient | Product |
|--------------------------|------------|--------------|------------------|--------|
| 21.6                     | 1.2 (ml/l) | Partonar, Iran | Abamectin | Abamectin 1.8 EC |
| 1224                     | 3.0 (ml/l) | Ariashimi, Iran | Chlorpyrifos-ethyl | Chlorpyrifos 40.8 EC |
| 72                       | 0.3 (ml/l) | Dow Agrosciences, UK | Spinosad | Tracer’ 240 SC |
activity. Schuster and Taylor (1987) also related the decrease in the number of feeding punctures of *L. trifoli* on leaves with 5-day old residues of abamectin to higher contact toxicity of this insecticide. In all treatments, the number of feeding stipples was the highest at 5 DAT or when the plants were 15 days old. Cyromazine had the least effect and chlorpyriphos was the most effective in reducing the density of feeding stipples, but the difference between the different insecticides tested was not statistically significant ($F = 2.21; \text{df } 5, 660; P = 0.061$). In spite of expectation for a lower toxicity of older insecticide residues, the density of feeding stipples was lower in 35-day old residues, probably due to unattractiveness of leaves for adult feeding.

**Larval mines**

In abamectin treatment, no observable larval mines were formed in any of infestation periods. In spinosad treatment, no larval mines were formed on leaves with residues up to 25 days old. Only in treatments with 35-day old residues, a few larval canals were recorded on leaves. No pupae or adults were formed in any of these treatments. Therefore, no analysis of variance could be performed on the data obtained for these compounds. The ovicidal and/or larvicidal activity of these compounds had an inverse relationship with the number of visible larval mines on the treated leaves. Based on the results obtained, abamectin was the most toxic of all tested insecticides followed by spinosad. These results are in agreement with the findings of Schuster and Taylor (1987) and Hossain and Poehling (2009) pointing out to the strong ovicidal activity of abamectin against leaf miners. Ovicidal and larvicidal activities of abamectin lasted up to 35 DAT. This indicates at a considerably long-term activity of abamectin with a single treatment. Long residual activity of abamectin is also reported by other researchers (Lasota and Daybas, 1991; Van de Veire et al., 2002).

The review of literature did not reveal any ovicidal activity of spinosad against flies. But, Charmillot et al. (2007) reported a considerable ovicidal activity of spinosad on small fruit tortrix (*Grapholita lobarzewskii*) eggs. It is possible that in the present study as well, the ovicidal activity of abamectin and spinosad or their highly toxic effect on neonate larvae have resulted in absence of larval mines development in treatments with up to 35 days old residues in the case of abamectin and up to 25 days old residues in the case of spinosad. Hossain and Poehling (2009) reported the absence of ovicidal activity of spinosad on *L. sativae*, but a high toxicity of spinosad for larvae during egg hatch and early larval instars, which supports our findings. They also documented that 14-day old residue of spinosad had considerable effect on larvae. A similar result was obtained in the studies done by Adan et al. (2002) on Mediterranean fruit fly.

The interaction between other treatments and time of infestation was significant for larval density ($F = 2.32; \text{df } 27, 440; P = 0.0003$). In cyromazine, indoxacarb...
and chlorpyrifos treatments, the number of larval mines was significantly lower compared to the control at the shorter aged residues (Table 2). However, as the age of the residues increased, these differences were less considerable. In chlorpyrifos treatments, no larval mines were formed on 2 h and one-day old residues and on the average one larva per plant in 5-day old residues. On residues up to 10 days old there were significant differences in the number of larval mines compared with the control. However, on older residues, the number of larval mines was not significantly different compared to the control. Although there has been no report concerning the ovicidal effect of chlorpyrifos on flies, El-Guindy et al. (1983) reported toxic effect of this insecticide on beet armyworm eggs. There were no significant differences between cyromazine and indoxacarb treatments and the control in residues beyond three days of aging. No considerable ovicidal activity was reported for indoxacarb (Liu et al., 2003) but ovicidal activity of cyromazine was reported on flies (Robinson and Scott, 1995). Also, the reduction in larval mines in cyromazine treatments was reported in literature (Smith, 1986) and is in agreement with the results obtained in this research.

### Table 2. Residual effects on larval density (number of larval mines) under greenhouse condition

| Age of residues before leafminer infestation | Chlorpyrifos | Cyromazine | Indoxacarb | Control |
|---------------------------------------------|-------------|------------|------------|---------|
| 2 hours                                    | 0±0 Dc      | 2.33±0.89 Bbc | 8.83±1.52 Aa | 8.83±1.52 Aa |
| 1 day                                      | 1.08±0.60 Dc | 5.25±1.66 BCh | 10.91±2.62 Aa | 10.91±2.62 Aa |
| 3 days                                     | 5.08±2.12 Ch | 3.66±0.9 Ch  | 19.91±7.2 Aa  | 19.91±7.2 Aa |
| 5 days                                     | 7.33±1.81 BCh | 3.33±1.29 BCb | 15.00±11.9 Aa | 15.00±11.9 Aa |
| 7 days                                     | 10.08±3.03 Ab | 2.75±2.36 AAb | 14.66±2.66 AA | 14.66±2.66 AA |
| 10 days                                    | 10.08±3.03 Ab | 1.50±1.55 AAb | 12.41±4.39 A | 12.41±4.39 A |
| 15 days                                    | 12.00±1.45 Aa | 1.25±1.5 AAb  | 14.08±3.33 Aa | 14.08±3.33 Aa |
| 20 days                                    | 12.58±2.12 Aa | 1.41±1.88 A    | 14.66±1.72 Aa | 14.66±1.72 Aa |
| 25 days                                    | 13.83±1.78 Aa | 1.15±1.54 A   | 7.00±1.08 B   | 7.00±1.08 B |
| 35 days                                    | 10.00±1.54 Aa | 8.58±1.73 A   | 8.58±1.73 A   | 8.58±1.73 A |

Mean (±SE) numbers followed by same letter are not significantly different (upper case letters within rows, lower case letters within columns p < 0.05 (Duncan’s test)).

### Larval mortality and residual activity

Regarding larval mortality, interaction between the age of residues and treatments was significant ($F = 15.55$; $df = 25$, $P = 0.0001$). In abamectin and spinosad treatments, no larva reached pupal stage. Beside ovicidal effect, larvicidal activity of abamectin should be considered as an important factor in preventing the formation of larval mines in residues of all ages. This indicates at a long-term residual activity of this compound. Several researchers have also reported highly toxic effects of this insecticide against leaf miner larvac (Leibee, 1988; Cox et al., 1995; Ferguson, 2004). The results obtained by other researchers regarding spinosad also support the findings of the current study (Hossain and Poehling, 2009; Adan et al., 2002).

The mean percentage of larval mortality in treatments with differently aged residues of cyromazine, chlorpyrifos and indoxacarb are shown in Table 3. In chlorpyrifos treatment, only on residues up to 3 days old the larvae did not reach pupal stage. Our other finding (Askari et al., 2009) did not reveal a very high toxicity of chlorpyrifos against *L. trifolii* larvae either. Low toxicity of this compound against larvae was also reported by Parrella et al. (1982). Walia et al. (1988) also reported a short-term residual activity of chlorpyrifos which is in compliance with our finding.
In cyromazine treatment, no pupa was formed on residues up to 25 days old; which indicates at high larvicidal activity of this compound. Strong larvicidal activity of cyromazine against *L. trifolii* was also reported in literature (Smith, 1986; Ferguson, 2004; Askari Saryazdi et al., 2009). According to Weintraub (2001) residual activity of cyromazine on potato plants against *L. huidobrensis* was more than 40 days in field conditions. Root et al. (1996) reported half-life of >20 days for this insecticide on tomato leaves in greenhouse conditions. These are in agreement with findings of this study.

In indoxacarb treatment, with residues up to 24 h old, no pupa was formed. However larval mortality in all indoxacarb treatments was significantly higher compared to the control (Table 3). A significant decrease in pupal formation on all residues has a strong connotation concerning population increase in future generations. Even longer persistence and efficacy of indoxacarb in field conditions are reported in the literature. Liu et al. (2002, 2003) reported an acceptable control of two cabbage leafminers (*L. xylostella* and *L. trigula*) in tomato leaves by indoxacarb treatments. According to Weintraub (2001) field trials were more than 40 days in field conditions. These are in agreement with findings of this study. Liu et al. (2002, 2003) reported an acceptable control of two cabbage leafminers (*L. xylostella* and *L. trigula*) in tomato leaves by indoxacarb treatments. According to Weintraub (2001) field trials were more than 40 days in field conditions. These are in agreement with findings of this study.

### Table 3. Residual effects on mean % larval mortality under greenhouse condition

| Treatments     | Age of residues before leafminer infestation (Days) | 2 hours | 1 day | 3 days | 5 days | 7 days | 10 days | 15 days | 20 days | 25 days | 35 days |
|----------------|----------------------------------------------------|---------|-------|--------|--------|--------|--------|--------|--------|--------|--------|
| **Chlorpyriphos** |                                                    |         |       |        |        |        |        |        |        |        |        |
|                | * *                                               | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 92.97±1.95 Ba | 83.97±5.42 Ca |
| **Cyromazine**  |                                                    | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa |
|                | 2 hours                                           | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa | 100±0 Aa |
| **Indoxacarb** | 100±0 Aa                                           | 100±0 Aa | 100±0 Aa | 81.90±5.22 ABb | 77.97±7.97 Bb | 65.85±9.36 CDb | 55.45±6.88 CDb | 52.91±10.96 CDb | 37.30±4.74 DEb | 25.42±3.74 EFb | 13.98±4.99 Fb |
| **Control**    |                                                  | 1.80±1.34 Ab | 4.64±2.15 Ab | 3.10±1.74 Ac | 1.25±0.65 Ac | 3.58±1.68 Ac | 4.86±1.88 Ac | 4.22±1.42 Ac | 2.62±1.29 Ad | 3.49±1.36 Ad | 1.81±1.81 Ac |

Mean (±SE) numbers followed by same letter are not significantly different (upper case letters within rows, lower case letters within columns p < 0.05 (Duncan's test)).

* No data was available due to 100% mortality in previous developmental stage.

As was mentioned earlier, no pupa was formed and no adults emerged in abamectin and spinosad treatments. This was the case in cyromazine treatment with residues up to 25 days old. The mean number of adults emerged from pupae was significantly affected by the age of residues in cyromazine, chlorpyrifos and indoxacarb treatments (Askari Saryazdi et al., 2009). The delayed effects of cyromazine on pupae and the lack of adult emergence as a result of cyromazine treatment (Askari Saryazdi et al., 2009) have also been reported by other researchers (Schuster and Everett, 1983; Ferguson, 2004).

Although no report of delayed effects of chlorpyrifos and indoxacarb on leaf miner was found in the literature, in this study the use of relatively higher doses (recommended rates) of these compounds resulted in some delayed effects and decrease in adult emergence.
Our results showed that abamectin, spinosad and cyromazine had a significant impact on *L. trifolii* developmental stages. However chlorpyriphos and indoxacarb showed a partial control of the leafminer. The higher efficacy of abamectin, spinosad and cyromazine against larvae coupled with their longer persistence, may control *L. trifolii* successfully for a fairly long period. Abamectin, spinosad and cyromazine are considered outstanding chemicals for leafminer control (Weintraub, 2001; Ferguson, 2004; Askari Sar yazdi et al., 2009; Hossain and Poehling, 2009). Since these biorational compounds are quite compatible with many biological control agents (Thompson et al., 2000; Bjorksten and Robinson, 2005; Hidrayani et al., 2005), they may be used in integrated management of greenhouse pests. If the longer persistence of these three insecticides on bean plants also remains valid on greenhouse grown crops, it will help avoid untimely and frequent application of these compounds in greenhouses. Greenhouse owners use insecticides, including abamectin, on 10-12-day intervals. If the results obtained from testing three biorational insecticides in this study remains valid in larger scale, spraying on monthly intervals would provide acceptable control levels in greenhouse conditions. This has considerable connotations in terms of economics and environmental aspect. In contrast, higher concentrations and more frequent applications of indoxacarb and chlorpyriphos may be required over time to ensure a sufficient efficacy. Persistence of abamectin and spinosad has been studied by other researchers regarding leafminers (Schuster and Taylor, 1987; Hossain and Poehling, 2009) but the residual efficacy of cyromazine, indoxacarb and chlorpyriphos on *Liriomyza* are new findings. Furthermore, it should be mentioned that we used rates of abamectin, spinosad and cyromazine recommended for other greenhouse pests. Due to the strong effects obtained against *L. trifolii*, lower dosages may be sufficient, but this will require further investigation. Based on the efficacy and relative safety of biorational insecticides tested in this study, it seems that these compounds would be suitable candidates to be used in leafminer management of greenhouse crops on rotational basis.

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Rezidualna toksičnost abamektina, hlorpirifosa, ciromazina, indoksakarba i spinosada za *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) u uslovima stakleničke proizvodnje

**REZIME**

*Liriomyza trifolii* je značajna štetoca povrtarskih biljaka u mnogim delovima sveta uključujući i Iran. U ovom radu, biljke pasulja uzgojene u saksijama su prskane preporučenim dozama abamektina, hlorpirifosa, ciromazina, indoksakarba i spinosada. U cilju procene rezidualne toksičnosti ovih insekticida biljke su infestirane imagom *L. trifolii* u trajanju od 2 časa; 1, 3, 5, 7, 10, 15, 20, 25 i 35 dana nakon tretmana insekticidima. Imagu je bilo dozvoljeno da se zadrži na tretiranim biljkama osam časova. Tretirane biljke su čuvane u stakleniku. U radu je ocenjen broj tačkastih oštećenja usled ishrane i broj mina na listovima, kao i stepen ulutkavanja i eklozije imaga. Podaci su obrađeni dvofaktorijskom analizom varijanse u SAS programu, a primenjen je i Dankanov test višestrukih poređenja. Abamektin i spinosad su značajno uticali na piljenje i embrionalni razvoj. Piljenje iz jaja položenih u listove sa reziduama hlorpirifosa starim do pet dana je bilo smanjeno. Razvoj larvi je takođe bio pod uticajem rezidua hlorpirifosa starosti do četiri nedelje. Indoksakarb je uticao na smanjenje razvoja larvi i ekloziju imaga u tretmanima sa reziduama starim do 20 dana. Ciromazin nije uticao na broj mina, ali je utuktavanje bilo značajno ometeno, a eklozija imaga u potpunosti zaustavljena čak i u tretmanima sa pet nedelja starim reziduama. Određivanje rezidualne aktivnosti insekticida koji se koriste za kontrolu ove štetocene je korisno sa aspekta izostavljanja nepotrebних tretmana.

**Ključne reči:** *Liriomyza trifolii; insekticidi; toksičnost; rezidue*