ABAMECTIN, PYMETROZINE AND AZADIRACHTIN SEQUENCE AS A UNIQUE SOLUTION TO CONTROL THE LEAFMINER LIROMYZA TRIFOLII (BURGESS) (DIPTERA: AGROMYZIDAE) INFesting GARDEN BEANS (PHASEOLUS VULGARIS L.) IN EGYPT

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SUMMARY

Field trails were conducted to determine the performance of three different sequences as a unique solution for the control of the leaf miner Liriomyza trifolii (Burgess) infesting garden beans (Phaseolus vulgaris L.) during two successive seasons of 2004 and 2005. Furthermore, during the evaluation period, the side effect against the ectoparasite Diglyphus isaea (Walker) was put into consideration. Meanwhile, the comparative evaluation of the pesticides alone showed that abamectin and azadirachtin were highly effective against Liriomyza trifolii, while carbosulfan, pymetrozine and thiamethoxam provided a moderate effect. Moreover, carbosulfan showed harmful effect to the larvae of the ectoparasite Diglyphus isaea (Walker), while abamectin and azadirachtin gave a moderate effect. Thiamethoxam and the detergent (Masrol 410) had slight effects in this respect. The highly effective sequence among the sequences was the abamectin, pymetrozine and azadirachtin, against Liriomyza trifolii (Burgess), with slight harmful effect on Diglyphus isaea (Walker). However the sequence of azadirachtin, pymetrozine and abamectin had a moderate effect on Liriomyza trifolii (Burgess) and exhibited a slight toxic effect on Diglyphus isaea (Walker). In contrast, the sequence of carbosulfan, thiamethoxam and pymetrozine was the least effective one and represented a slight effect on Diglyphus isaea (Walker). From this study, it was concluded that abamectin, pymetrozine and azadirachtin sequence has proved to be a unique solution for the control of the leaf miner Liriomyza trifolii (Burgess) infesting garden beans (Phaseolus vulgaris L.) in Egypt.

INTRODUCTION

The garden bean (Phaseolus vulgaris L.) is not considered only one of the important vegetable crops in Egypt for both local as well as export markets, but also valuable for its richness in the amino acids as lysine and tryptophane that are lacking in cereals and other foods (Karel and Mghogho, 1985) and Tokumaru., (2005). The leaf miner (Liriomyza trifolii Burg.) is considered of the major pests
attacking *Phaseolus vulgaris*. This destructive and serious pest is attacking vegetable crops and ornamental plants worldwide as well. Injury is occurred when adult females puncture leaves for feeding, or oviposition and also when offspring larvae form serpentine mines (Paula, et al. 2003). The leafminer begins to damage the host plant as soon as its eggs hatch. Bean garden's leafminer may prevent young leaves from expanding and causing a curled and twisted damage. Meanwhile, this destructive pest may also attack succulent stems. After the miner has finished feeding, other insects such as the aphids; e.g., *Aphis gossypii* appeared (Madanjid et al. 2002). The chemical control is one of the effective ones to minimize the risk and harmful effect of this pest population. Careless or excessive use of pesticides, however, can result in poor control, crop damage, higher expenses and hazards to health and environment. Moreover the extensive use of these pesticides has seriously affected the population densities of its ectoparasite *Diglyphus isaea*. Furthermore the repeated application of certain pesticides upon some garden beans pests could develop resistance to these classic pesticides. A new line of agrochemical and biological agents are being employed and tested to be an important tool in the Integrated Pest Management (IPM) programs. Taking into consideration that *Diglyphus isaea* as an effective biological control agent of *Liriomyza trifolii* on *Phaseolus vulgaris*, therefore, this study looking forward to evaluating and determining certain sequences which could be also followed in the IPM programs to control *Liriomyza trifolii* and to maintain ectoparasite *Diglyphus isaea* Walk as well.

**MATERIALS AND METHODS**

**Experimental design and field trials**

The experimental area was divided into plots [12 m\(^2\) (3 × 4 m) for each]. The plants were grown along distance of 25-30 cm apart and in rows of 70 cm width. In summer and neely cultivations of the seasons 2004 and 2005, seeds were sown on 23\(^{rd}\) March and 3\(^{rd}\) September, respectively. Treatments were arranged in complete randomized block design with three replicates for each treatment.

**Chemicals and biological agents used**

Thiamethoxam (Actara 25 % WG), pymetrozine (Chess 25% WG), azadirachtin (Achook 0.15% EC), abamectin (Vertemic1.8 % EC), carbosulfan (Marshal 25% WP) and detergent (Masrol 410) were used in this study. These compounds were used according to the recommended dose by Egyptian Ministry of Agriculture to be applied against the targeted pests. Two applications of each tested compound were evaluated.
Sequences used

Three sequences of the compounds were implemented and their effects were studied in the neely cultivation of the season 2005. For selecting the best and effective sequence of insecticidal application against the *Liriomyza trifolii*, each compound was followed by the other in a sequential application with interval period of 14 days as follows:

**Sequence (1):** Abamectin → Pymetrozine → Azadirachtin, consequently.

**Sequence (2):** Azadirachtin → Pymetrozine → Abamectin, consequently.

**Sequence (3):** Carbosulfan→ Thiamethoxam→ Pymetrozine, consequently

**Sampling technique and leafminer *Liriomyza trifolii* inspection**

10 leaflets from the plants were taken at random way from each plot before and after treatment at 0, 2, 5, 7 and 10 days. The leaves samples were transferred to the laboratory to be examined under binocular microscope.

**The side effect of the tested compounds on Leaf miner parasitoids *Diglyphus isaea* Walk**

Samples of 30 leaves from the garden beans plants were collected randomly from each treatment after 2, 5, 7 and 10 days post-spraying. Samples were inspected under stereoscopic binocular microscope for counting number immature stages larvae and pupae of *Diglyphus isaea* Walk. inside the tunnel between the upper and lower leaflets surface.

The reduction percentage of the infestation calculation (% R) for each treatment was calculated by using the formula of Henderson and Tilton (1955) as follows:

\[
\text{Reduction} \% = 1 - \left(\frac{a}{b} \times \frac{c}{d}\right)
\]

**Where:**
a = Population in treatment after spraying
b = Population in treatment before spraying
c = Population in check untreated (control) before spraying
d = Population in check untreated after spraying

**Statistical analysis**

Data were subjected to the analysis of variance test (ANOVA) and Randomized Complete Block Design (F. test). The least significant differences (LSD) at the 5% level were determined according to the method described by Steel and Torrie (1981).
RESULTS AND DISCUSSION

Biological performance of the used agrochemical and natural biological agents against the leaf miner *Liriomyza trifolii* Burg. in the neely cultivation of the season 2004

In the 1st application, the results in indicated that, pymetrozine was the most effective among the tested ones against larval stages of the leaf miner (*Liriomyza trifolii*) inducing 52.14% reduction percentage followed by azadirachtin, carbosulfan, thiamethoxam, and detergent (Table 1). After five days post treatment, all treatments reduced the population density of the leaf miner whereas, azadirachtin was the superior compound giving 82.31% reduction percentage followed by detergent, carbosulfan, pymetrozine. Thiamethoxam was the least effective compound in this respect giving 41.04% reduction percentage. After one week post treatment, the residual reduction percentage showed again that the superior compound was azadirachtin recording 87.26% reduction percentage followed by detergent, carbosulfan and thiamethoxam. Pymetrozine was the least effective compound giving 32.87% reduction percentage. After ten days post treatment, azadirachtin also came in the 1st rank giving 74.39% followed by detergent, pymetrozine, carbosulfan and thiamethoxam recording < 50% reduction percentage.

According to the mean number larvae / leaflet, the lowest number was observed when the treatment was carried out using azadirachtin giving 0.37 larva / leaflet. The general mean of reduction percentage throughout the whole infestation periods of 5, 7, and 10 days showed significant differences between azadirachtin and other tested compounds, while there were no significant differences among thiamethoxam, pymetrozine, carbosulfan, and detergent. According to the general mean of residual reduction, the superior compound in this respect was azadirachtin which gave 81.50% reduction percentage followed by detergent, carbosulfan, pymetrozine, and thiamethoxam, giving 49.70%, 46.20%, 41,805 and 38.79%, respectively, Figure (1). In the agreement with Oatman and Kennedy (1986), that found that the leafminer outbreak may be due to the highly toxic effect of insecticides applications to the parasite of the leafminer. These may give a reasonable explanation of the low efficacy of pymetrozine, and thiamethoxam against *Liriomyza trifolii* Burg. Moreover, this may also be due to resistance development to the used insecticides that has made their control difficult for many years.

In the 2nd application, Table (2), exhibited that abamectin treatment was the superior compound giving 58.48% reduction percentage. Moreover, there were no significant differences between azadirachtin and thiamethoxam inducing reduction of infestation percentage that recorded 56.24%, and 55.57%, respectively. Carbosulfan, pymetrozine, and detergent recorded < 50% reduction percentage in this respect. It was clear that azadirachtin was the superior compound after five
days post treatment of application giving residual reduction percentage of 80.29% followed by abamectin, carbosulfan, thiamethoxam, pymetrozine, and detergent giving 70.13%, 50.94%, 46.93%, 37.30% and 24.19%, respectively. Although abamectin proved its efficacy in controlling this destructive pest, it showed its satisfactory effect on keeping its ectoparasite *Diglyphus isaea* in reasonable population as well. Similar case was found out by Elizabeth and Marjorie (1983) who found that abamectin could successfully control phytophagous mite *E. orientalis* without affecting the population density of the predatory mite *E. scutali*. Beitia *et al.* (1999), reported that the chemical control of leaf miner *Liriomyza trifolii* Burg. was not satisfactory because of the biology of this insect that could develop resistance to various pesticides. It could be concluded from the described results that, azadirachtin and abamectin were the most suitable compounds for integration into a pest management program for leafminer *Liriomyza trifolii* Burg. on garden beans *Phaseolus vulgaris*.

Also, this natural products could be applied as needed in either greenhouse or field situations without inducing undesirable fluctuations in this respect. In response to the low effectiveness of many present agricultural chemicals, and the tendency to a rapid development of resistance, natural compounds have represented a badly need in the implementation of IPM programs.

**Biological performance of the used agrochemical and natural biological agents against the ectoparasite of the leaf miner *Diglyphus isaea* Walk. in the neely cultivation of the season 2004**

In the 1st application, azadirachtin was highly toxic to the larvae of the ectoparasite *Diglyphus isaea* giving 28.43% as an initial reduction percentage (Table 2). Thiamethoxam was in the second rank giving 21.40%, followed by detergent 18.57%, pymetrozine giving 9.94%, and carbosulfan 6.43%. From the previous results, it could be observed that azadirachtin was the most effective and toxic compound against the ectoparasite *Diglyphus isaea*. This fluctuation of *Diglyphus isaea* population might be as a result of the reduction occurring in the rate of the leaf miners' population as the main host affected by azadirachtin. Hence, the reduction and vanishing of the main host may lead to reduction of the parasite numbers.

Moreover, these findings were in full agreement with Mori and Gotoh, 2001, that found that the occurrence of low population fluctuation of the larvae of the ectoparasite *Diglyphus isaea* could be explained by unfavorable climate or lack of suitable host. Although it was not possible to quantitatively evaluate the mortality produced by predators or parasites, their presence was a sign of a good biological control and coincided with low target pest populations. To more effective utilize these natural enemies as biological control agents; we should acquire information on their susceptibility. Also, efforts should therefore be directed toward multi-generational studies in this extent. After 5 days post treatment, the reduction of the larvae of the ectoparasite *Diglyphus isaea* was different from the first 2 days.
Commutations in Agricultural and Applied Biological Science. Gehnt, Belgium. 72(3): 583-593

Carbosulfan was more effective than the other tested compounds giving 67.28%, followed by azadirachtin, detergent, thiamethoxam and pymetrozine. After one week post treatment, carbosulfan kept the same effective trend against the larvae of the ectoparasite *Diglyphus isaea* giving 67.06%, followed by detergent, pymetrozine, azadirachtin, and thiamethoxam. After 10 days post treatment, it was clear that, carbosulfan was still highly toxic against the larvae of the ectoparasite *Diglyphus isaea* giving 79.87%, followed by pymetrozine, detergent, azadirachtin, and thiamethoxam giving 51.56%, 47.63%, 32.08%, and 8.40%, respectively.

The general mean of the biological performance percentages after 5, 7, and 10 days from application confirmed the variation of the toxic effect of such tested compounds against the larvae of the ectoparasite *Diglyphus isaea* (Figures 2). The data indicated that carbosulfan was highly toxic to the larvae of the ectoparasite *Diglyphus isaea*. Moreover, there were no significant differences among these treatments represented in detergent, pymetrozine, azadirachtin, and thiamethoxam which gave 48.21%, 39.59%, 38.25%, and 17.79%, respectively. Therefore, the present results showed that thiamethoxam, pymetrozine, azadirachtin, and detergent were less toxic than carbosulfan. It was remarkable to mention that, carbosulfan was toxic to both larval stages of leaf miner (*Liriomyza trifolii* Burg.) and the larvae of the ectoparasite *Diglyphus isaea* as well.

In the 2nd application, there were significant differences among all treatments (Table 2). Azadirachtin reduced the parasitoid population of the ectoparasite *Diglyphus isaea* by 33.50%, followed by abamectin, detergent, pymetrozine, thiamethoxam, and carbosulfan that they gave initial reduction percentage 29.46%, 22.52%, 20.65%, 19.39%, and 14.30%, respectively. After 5 days post treatment, carbosulfan had a sever toxic effect giving 82.31% reduction percentage, followed by pymetrozine, azadirachtin, thiamethoxam, and abamectin, which the gave 56.34%, 52.63%, 44.79%, 41.62%, and 30.15%, respectively. Having a closer look again to Table (2), it could be shown that after one week post treatment, the larvae of the ectoparasite *Diglyphus isaea* were highly susceptible to abamectin, that gave 73.13% residual reduction percentage followed by carbosulfan, azadirachtin, pymetrozine and thiamethoxam.

In contrast, detergent had no effect on the larvae of the ectoparasite *Diglyphus isaea* in this extent. After ten days post treatment, the reduction percentages indicated that carbosulfan was harmful to the larvae giving 66.01% reduction percentage followed by abamectin, azadirachtin, pymetrozine, thiamethoxam and detergent inducing 51.38%, 41.66%, 31.94%, 11.61%, -1.94%, respectively. The general mean of the biological performance percentages among the whole period of inspection indicated that carbosulfan was harmful to the larvae of the ectoparasite *Diglyphus isaea* followed by abamectin, azadirachtin, pymetrozine, thiamethoxam and detergent which they gave 51.55%, 47.29%, 41.76%, 21.60%, and 11.99%, respectively (Figure 2). The previous results...
indicated that the larvae of the ectoparasite *Diglyphus isaea* were not highly susceptible to detergent and thiamethoxam. In fact, an understanding of the effectiveness of natural enemies is essential to avoid applying pesticides when biological control is adequate. In fact, the impact of pesticide treatments on natural enemies of secondary pests should also be considered when decisions are made to treat for primary pests. Once the decision has been made to apply pesticides, several parameters must be considered so that the pesticide could be used in the most efficient and least disruptive manner. In agreement with Iskandar and Aida, (2000), that pesticide selectivity should be divided into physiological selectivity and ecological selectivity based on the mechanism by which a pesticide treatment is preferentially toxic to pests versus their natural enemies.

Such differences in the toxicity among those tested compounds used may relate to the difference in susceptibility of the responded insects. Hence, physiological selectivity results from physiological differences in susceptibilities of pests and associated natural enemies to pesticides should be considered. Thus, many of the used agrochemicals could be considered as candidates for use in field of IPM programs. In a full agreement with Mori and Gotoh, (2001) and Zhao, and Kang (2003) who found that some tested synthetic pesticides have been intensively and extensively used to control agricultural pests for half century. These chemicals have side effects on non-target organisms including the natural enemies of target pests. The notion has contributed to increased interest in the use of selective pesticides that are compatible with natural enemies and present minimal risk to environment and human health. These support the findings reported by Johnson et al. (1999) that occurrence of the secondary pest outbreaks are the foremost concern and the primary pest lacks effective natural enemies. Also, survival of the primary pest after treatment is less important than for a potential resurgence problem. In situations where the secondary pest exhibits pesticide resistance to a wide range of compounds, it will generally be preferable to use compounds with high specificity to control the primary pest. This tactic avoids destruction of the secondary pest's natural enemies and reduces further development of resistance in the secondary pest.

**Effect of different sequences of agrochemical and biological agents against the leaf miner *Liriomyza trifolii* Burg. and its ectoparasite *Diglyphus isaea* Walk.**

The exhibited data in Table (3) demonstrated the calculated reduction percentages of leafminers during the application of the three suggested sequence of the used compounds. The 1st sequence of abamectin, pymetrozine, and azadirachtin achieved high efficacy during the 1st, 2nd and 3rd applications except for the 1st week in the 2nd application that gave 66.60%, 12.89%, -0.48%, 38.17% and 58.94%, as reduction percentage. The 2nd sequence came in the 2nd rank giving 20.08%, -41.83%, -7.82%, 38.77% and 60.65%. Meanwhile, the 3rd sequence was the least effective one that gave the lowest reduction percentage amounted by -
57.69%,-79.27%, 42.82%, 4.80% and 92.85%, respectively. It could be concluded that the results revealed the non toxic effect on the ectoparasite larvae *Diglyphus isaea* when the 1st sequence was used. It was noticeable that this non toxic effect was after the 1st and 2nd week of the 1st application and this reduce the number of parasite estimated by 75.60% and 71.75%, respectively.

In fact, the previous elucidated data showed the variation between the weekly calculated reductions of leafminer parasitoids when different insecticides and agrochemicals sequences were used. The data indicated that the 1st sequence was toxic to the larvae of the parasite even though the recommended dose of leaf miner was used (Figure 3). On the other hand, the 1st sequence gave general mean of reduction percentage estimated by 7.46%. The 2nd sequence had low toxicity against the larvae of the parasite *Diglyphus isaea* estimated by 2.50% reduction percentages as general mean.

After five weeks, the 3rd sequence became the least one in general reduction percentage which gave -90.78%. This non toxic effect was after the 1st in the 2nd week of the 1st application and this reduced the number of parasite. On the other hand, the 2nd sequence had low toxicity against the larvae of the parasite *Diglyphus isaea* in this respect. It is concluded that abamectin, pymetrozine and azadirachtin sequence has proved to be a unique solution for the controlling of the leaf miner *Liriomyza trifolii* infesting garden beans (*Phaseolus vulgaris* L.,) in Egypt and could be applied on IPM programs to control this economic important pest. This sequence has high efficacy in controlling of the leaf miner *Liriomyza trifolii* Burg. with little side effects on its ectoparasite *Diglyphus isaea*.

REFERENCES


Commutations in Agricultural and Applied Biological Science. Gehnt, Belgium. 72(3): 583-593

Table (1): Biological performance of the used agrochemical and natural biological agents against the leaf miner *Liriomyza trifolii* Burg. in the 1st and 2nd applications of the neely cultivation of the season 2004

<table>
<thead>
<tr>
<th>Agrochemicals or natural biological agents</th>
<th>A</th>
<th>% Initial reduction after 48 hrs.</th>
<th>% of residual reduction</th>
<th>After 5 days</th>
<th>After 7 days</th>
<th>After 10 days</th>
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☐ refers to the 1<sup>st</sup> application  ☐ refers to the 2<sup>nd</sup> application  
A: mean number of insects  B: % insect reduction

Figure (1): General mean of the biological performance of the used agrochemical and natural biological agents against the leaf miner *Liriomyza trifolii* Burg. in the neely cultivation season of the 2004
Table (2): Biological performance of the used agrochemical and natural biological agents against ectoparasite of the leaf miner *Diglyphus isaea* Walk. in 1{superscript}st and 2{superscript}nd applications of the neely cultivation of the season 2004

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<th>Agrochemicals or natural biological agents</th>
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<td>-</td>
<td>0.22</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1.10</td>
<td>-</td>
<td>0.73</td>
<td>-</td>
<td>0.66</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> refers to the 1<sup>st</sup> application  <sup>b</sup> refers to the 2<sup>nd</sup> application

A: mean number of insects  B: % % insect reduction

Figure (2): General mean of the biological performance of the used agrochemical and natural biological agents against the ectoparasites of leaf miner *Diglyphus isaea* Walk. in the neely cultivation of the season 2004
Table (3): Effect of different sequences of agrochemical and biological agents against the leaf miner *Liriomyza trifolii* Burg. and *Diglyphus isaea* Walk. in neely cultivation of the season 2005.

![Table (3): Effect of different sequences of agrochemical and biological agents against the leaf miner *Liriomyza trifolii* Burg. and *Diglyphus isaea* Walk. in neely cultivation of the season 2005.](image-url)

**Table (3):**

<table>
<thead>
<tr>
<th>Sequeces</th>
<th>% Reduction percentage per week</th>
<th>1st application</th>
<th>2nd application</th>
<th>3rd application</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st week</td>
<td>2nd week</td>
<td>1st week</td>
<td>2nd week</td>
</tr>
<tr>
<td>Sequence (1)</td>
<td>66.60</td>
<td>12.89</td>
<td>-0.48</td>
<td>38.17</td>
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<td></td>
<td>75.60</td>
<td>71.75</td>
<td>26.90</td>
<td>5.95</td>
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<tr>
<td>Sequence (2)</td>
<td>20.08</td>
<td>-41.83</td>
<td>-7.82</td>
<td>38.77</td>
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<tr>
<td></td>
<td>39.63</td>
<td>11.93</td>
<td>41.66</td>
<td>52.97</td>
</tr>
<tr>
<td>Sequence (3)</td>
<td>-57.69</td>
<td>-79.27</td>
<td>42.82</td>
<td>4.80</td>
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<tr>
<td></td>
<td>91.71</td>
<td>97.35</td>
<td>-248.48</td>
<td>-373.09</td>
</tr>
</tbody>
</table>

![Figure (3): Effect of different sequences of agrochemical and biological agents against ectoparasite of leaf miner *Diglyphus isaea* Walk. in neely cultivation of the season 2005.](image-url)

**Figure (3):** Effect of different sequences of agrochemical and biological agents against ectoparasite of leaf miner *Diglyphus isaea* Walk. in neely cultivation of the season 2005.
Figure (4): General mean of the effect of different sequences of agrochemical and biological agents against ectoparasites of leaf miner *Diglyphus isaea* Walk. in neely cultivation of the season 2005.