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Comparative Study on the Effectiveness of Chemical Versus Biological Control Against *Liriomyza trifolii* (Diptera: Agromyzidae) under Field and Greenhouse Conditions

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Abstract: Influences of certain insecticides, i.e. abamectin, Primiphos methyl Bemistop, Trigard and pyriproxyfen versus releasing the ectoparasitoid, *Diglyphus isaea* were evaluated to estimate their effectiveness for controlling the leaf miner *liriomyza trifolii* (Burgess) infesting Phaseolus vulgaris grown under field and glass house conditions. Results indicated that all treatments caused increased in the tested pest's and Diglyphus isaea populations in both applications. Highest reduction values in L. trifolii population occurred after 1st and 2nd applications of Trigard (87.2 & 81.0%) followed by Actellic (68.6 &75.8%), Vertimec (67.5 &75.3 %) and Admiral (60.5 & 66.2 %). While the low effect was shown after treatment by Bemistop (54.9 &57.1 %). In case of releasing *Diglyphus isaea* in the green house, data showed that in order to obtain satisfactory control of *Phaseolus vulgaris*, weekly releases of the parasitoid are needed and that the rates of 4/30 and 2/30 (parasite / larvae) proved as best rates for release. Therefore, *D. isaea* can be considered a good promised control element especially in successful release of some IPM programs of Phaseolus vulgaris grown under glass house conditions.

Key words: Abamectin · Primiphos methyl · IGR · *Liriomyza trifolii* · *Phaseolus vulgaris* · *Diglyphus isaea*

INTRODUCTION

Intensive chemical applications for the control of arthropod pests may result in population explosions of the targeted organisms due to various reasons including hormoligosis [1, 2], pesticide resistance to many active ingredients such as those found in pyrethroids, methamidophos, avermectins, cyromazine and spinosyn and the destruction of natural enemies [3, 5]. Pest outbreaks resulting from the destruction of natural enemies are classified as pest resurgences and secondary pest outbreaks (pest upsets) [6]. Numerous arthropod species including scale insects, aphids, whiteflies, leaf miners, lepidopterans, tetranychid mites and eriophyid mites often undergo resurgences and secondary pest outbreaks [7].

Common bean is widely consumed as vegetables and also as dry seeds. The annually cultivated area ranges between 9-18 thousand hectares, yielding 15,000 tons of dry seeds and 150,000 tons of green pods (data from the Central Administration of Agriculture in Egypt CAAE, 1994). Different *Rhizobium* spp. are able to nodulate and fix nitrogen with the common bean (*Phaseolus vulgaris*), including *Rhizobium leguminosarum* bv. *phaseoli* [8]. The percentage of biological nitrogen fixation on the N assimilation in *Phaseolus vulgaris* compared to other legumes, is rather low and with 40-50% compared to 75% with faba beans, 70% with peas and up to 95% with lupines [9]. More than 20 species of *Liriomyza* have been reported as being economically important and at least six species are polyphagous: *Liriomyza sativae* (Branchard), *Liriomyza trifolii* (Burgess), *L. huidobrensis* (Branchard), *L. bryoniae* (Kaltenbach), *L. strigata* (Meigen) and *L. longei* (Frick) [10, 11].

The aim of the present research was to evaluate the efficacies of different insecticides commonly used for control of *Liriomyza*, compared to the effectiveness of releasing the ectoparasitoid, *Diglyphus isaea* to manage the leaf miner, *L. trifolii*.

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MATERIALS AND METHODS

Tested Compounds:

- Insect growth regulator Trigard 10% SL. produced by Syngenta, Egypt, used at rate of 830cm^3/100L.
- An organophosphorus compound, Primiphos methyl (Actellic) 50% EC. (0-2, diethyl amino 6 methyl pyrimidin 4-yl 0, 0 dimethy phosphorothioate) recommended at rate of 375 ml/100 liters water.
- Microbial insecticide Vertimec 1.8% EC used at 40ml/100 liters of water, produced by Merck co., Rahway, NJ, USA). The active ingredient of this compound is abamectin which is a mixture of 80% avermectin B1a and 20% avermectin B1b.
- An insect growth regulator, pyriproxyfen (Admiral 10% EC), produced by Sumitomo chemical Co. Ltd Osaka, Japan, applied at rate of 300cm^3/100L.
- A botanical insecticide, Bemistop 21.1% EC, a new product of NM Agro Egypt Company coded 98402, at rate of 500 cm^3/100L.

Procedure: Experiments were carried out at the experimental station of Agriculture Research Center Giza, Egypt. An area of 1/4 feddan was sown of common bean seeds (*Phaseolus vulgaris* L.). Seeds were sown in rows at the rate of 12 rows/2 poles; the distance between the hills was 20 cm^2 apart on one side of the ridge. Treatments were arranged in a complete randomized block design with three replicates/treatment in the first and second applications, also three replicates were kept without treatment as control. Each replicate was 1/100 feddan. First application for tested compounds was in February, 14th and the second spray was applied on March 16th, 2019, using knapsack sprayer (20 liters). A number of 100 leaflets were picked at random from common bean plants from each plot before the treatment and at 2, 5, 7, 10 and 15 days after spraying. Every sample was placed to be taken to the laboratory where those were examined under a binocular stereoscopic microscope for counting the number of *L.trifolii* larvae inside the tunnels between upper and lower leaf surfaces, also to count the number of immature stages of the ectoparasite *D. isaea*. The reduction percentage (R %) for each treatment, compared to the control, was calculated and corrected according to Henderson Tilton's formula [12].

Percentage of Parasitism by the Larval Ectoparasitoid, *Diglyphus isaea*: This study was conducted in a greenhouse during season of 2019. Random samples of 20 leaflets of common bean were picked one month after sowing and continued weekly until the end of season (Four replicates were inspected). The leaves were randomly, picked, placed in paper bags and then taken to the laboratory to be examined by the aid of a binocular stereomicroscope to determine all a live instars of the leaf miner and parasitoid. Then the average percentage of parasitism was calculated according to the following formula:

% Parasitism = parasitized larvae/ No. of *Liriomyzae* larvae x100

Evaluation of the Efficiency of the Larval Ectoparasitoid, *Diglyphus isaea* for Controlling *Liriomyza trifolii*

Rearing of the Parasitoid: To establish the culture of *D.isaea* needed for this study, infested common bean leaves with *Liriomyza trifolii* larvae were collected from a common bean field at Qalyubia and Giza Government. Leaves were placed in big jars covered with pieces of thin cloth kept in position by means of rubber band until emergence of adult parasitoids. Thereafter collected adults were transferred to glass jars using an aspirator. These parasites were placed in rearing cage with honey droplets (60 cm^2 high, 50 cm^2 wide, 50 cm^2 long), potted common bean plants infested with late 2nd and early 3rd instar of the leaf miner were exposed to the parasites in the rearing cages for 24 h. and changed daily with new pots. The exposed pots were maintained for 10-12 days (just before parasites emergence from leaflets), then leaflets were cut and inspected under a stereomicroscope to collect the parasite pupae (pupae green then black with red eyes). Parasitoid pupae were placed singly in small glass vials with filter papers and held for adult emergence and then pairs of parasitoid (male and female) were placed in glass vials sealed with cotton for 24 h. to allow mating. Therefore, the parasitoid was ready to release against *Liriomyza trifolii*.

Treatment Procedures: The glasshouse area was divided into 4 equal parts (10m^2/each), separated by fine-mish screen to prevent parasitoids or *L. trifolii* adults from moving through the glass house whole area. Each of the four parts contained 50 pots of common bean plants 30 days old (three plants/ pot). Adults of *Liriomyza* (3 days old) were released into the four parts at rate of 3 females/10 plants.

Release of Parasitoid: One week after releasing the pests (*Liriomyza trifolii*), the parasitoid was released twice with 10 days intervals as follow:

- Part A- received 4 mated females of parasitoid/30 *Liriomyza* larvae.
- Part B - received 2 mated females of parasitoid/30 *Liriomyza* larvae.
- Part C - received one mated female of parasitoid/30 *Liriomyza* larvae.
- Part D - did not receive any parasitoid release as control.

Sampling: Before parasitoid release, the total number of *Liriomyza* larvae was counted by the first and second release in each glass house part to assure the parasitoid number needed for each part. Six plants per part were randomly sampled (plant as replicate) and the following parameters were counted, total leaves, infested leaves, parasitized larvae, total larvae and consequently, the percentage of parasitism were calculated. Sampling was carried out before release and after 3, 5, 7 and 10 days from the releasing time. The same procedure was repeated with the second release and continued weekly after second release until the end of 2nd *Liriomyza* generation.

Statistical Analysis: The statistical analysis was carried out using ANOVA with two factors under significance level of 0.05 for the whole results using SPSS (ver.19) and data were treated as complete randomization design according to Steel *et al*. [13].

RESULTS AND DISCUSSION

Toxicity of Tested Treatments Against Leaf Miner *Liriomyza trifolii*: The reduction percentages in *L. trifolii* population after 2, 3, 5, 7,10 and 15 days after 1st and 2nd applications with the tested compounds were tabulated in Table (1). Data revealed that the insect growth regulator, Trigard was significantly the highest effective against *L. trifolii* larvae, the reduction percentages after 2, 3, 5, 7, 10 and 15 days after the second application were 89.9, 89.8, 84.0, 78.5, 72.0 and 71.7 %, respectively, the mean reduction of residue from 2 to 15 days after treatment by Trigard was 87.2 % in the 1st application. The results agree with Yu [14] who mentioned that the insect growth regulators are chemicals or substances that disturb normal growth and development of target insects and finally can kill them.

The organophosphorus compound Actellic gave an unsatisfactory result against the leaf miner *L. trifolii* larval, the reduction of *L. trifolii* after 2, 3, 5, 7, 10 and 15 days from treatments were 72.7, 80.8, 81.7, 67.8, 67.3 and 41.5 %, respectively. Although Actellic is a chemical insecticide, the mean reduction for it after 15 days from application was 68.6 % after the 1st application.

Actellic and Bemistop are chemical insecticides, but, had unsatisfactory results against the leaf miner. These results are correspondent to those of Schuster and Everett [15]; Ferguson [4] and Reitz *et al.* [16] who reported that all the tested pesticides, including those that have systemic effects (Cyclone and Dynamec) and stomach poison effects (Bestox and Thunder) were not able to kill all the leaf miner larvae when applied at their recommended doses. *Liriomyza* leaf miners have high ability to develop resistance to pesticides. Cross-resistance to multiple classes of insecticides is also found in *Liriomyza* spp. Also, MacDonald [17] reported that resistance of *Liriomyza* leaf miner to most carbamate, organophosphate and pyrethroid insecticides has also been reported in the United Kingdom.

The microbial insecticide, vertimec had the same efficacy against *L. trifolii* as the organophosphorus compound Actellic against the leaf miner *L. trifolii* larvae, the reduction of *L. trifolii* larvae population reached 74.1, 73.5, 78.6, 71.7, 65.6 and 41.5 % respectively, the mean reduction for it after 15 days from application was 67.5% after 1st application.

Table 1: Reduction percentages in *Liriomyza trifolii* larval population in common bean plants after the first and second application.

	1st application							2nd application						
	Days after treatments							Days after treatments						
Treatments	2	3	5	7	10	15	Mean	2	3	5	7	10	15	Mean
Trigard	82.1	87.1	100	100	81.5	72.2	87.2	89.9	89.8	84.0	78.5	72.0	71.7	81.0
Actellic	72.7	80.8	81.7	67.8	67.3	41.5	68.6	66.5	72.3	73.7	76.2	82.3	84.0	75.8
Vertimec	74.1	73.5	78.6	71.7	65.6	41.5	67.5	71.5	78.4	80.5	76.0	74.5	71.1	75.3
Admiral	50.6	52.8	54.3	61.4	71.0	72.7	60.5	62.5	62.0	65.4	69.0	70.0	68.2	66.2
Bemistop	71.3	62.0	58.9	49.8	46.6	41.0	54.9	63.3	61.3	55.5	55.4	54.5	52.5	57.1

Table 2: Percentages reduction of treatments against larva and pupa of the parasitoid *Diglyphus isaea* in common bean plants in the first and second application.

	1st application							2nd application						
	Days after treatments							Days after treatments						
Treatments	2	3	5	7	10	15	Mean	2	3	5	7	10	15	Mean
Trigard	87.5	100	100	100	100	78.1	94.3	100	100	100	100	100	48.1	91.4
Actellic	67.0	74.7	89.5	77.0	71.5	70.8	75.1	79.0	76.6	72.6	66.0	64.0	41.5	66.6
Vertimec	44.4	76.7	72.0	68.2	51.0	50.0	60.4	37.0	50.8	61.7	61.0	48.1	45.0	50.6
Admiral	52.8	45.6	31.0	24.3	18.5	30.1	33.7	37.0	37.8	45.2	45.0	33.5	25.0	37.3
Bemistop	12.1	37.0	45.8	51.0	20.1	54.9	36.8	46.0	37.0	35.1	34.6	31.5	31.0	35.9

The insect growth regulator, Admiral showed lower effect against the leaf miner *L. trifolii* larvae, the mean reduction for it after 15 days from application was 60.5%, while, the reduction of *L. trifolii* larvae reached 50.6, 52.8, 54.8, 61.4, 71.0 and 72.7 %, respectively, after 1st application.

Botanical insecticide Bemistop was the least effective against the *L. trifolii* larvae, the mean reduction for it after 15 days from application was 54.9 % and it had a moderate effect after 2, 3, 5, 7, 10 and 15 days from treatment where the reduction percentages were 71.3, 62.0, 58.9, 49.8, 46.6 and 41.0 % respectively, after 1st application.

The same trend of results occurred in the 2nd application of these compounds. Trigard was significantly the most effective against *L. trifolii* larvae, the mean reduction percentages from 2 to 15 days after treatment of Trigard, Actellic, vertimec, Admiral and Bemistop were 81.0, 75.8, 75.3, 66.2 and 57.1 % respectively (Table, 1).

Toxicity of Tested Treatments Against the Larval Parasitoid, *Diglyphus isaea*: Harmful effects of tested compounds on ecto-larval parasitoid *Diglyphus isaea* were evaluated in the present study and the data were tabulated in Table (2). Data indicated that the insect growth regulator, Trigard was the severest to *D. isaea* larvae and pupae, the reduction percentages after 2, 3, 5, 7, 10 and 15 days after treatments were 87.5, 100, 100, 100, 100 and 78.1 %, respectively, the mean reduction of the insecticide residues from 2 to 15 days after Trigard treatment was 94.3 % in the 1st application. These results agree with Evleen *et al.* [18] who mentioned that the mean of residual effect (mean of % reduction at 5,7,10 and 15 days post treatment by Trigard) was 90.2 % reduction in *D. isaea* populations.

The organophosphorus compound Actellic was the second compound as harmful to D. isaea larvae and pupa. The reduction of *D. isaea* individuals after 2, 3, 5, 7, 10 and 15 days from treatment by Actellic were 67.0, 74.7, 89.5, 77.0, 71.5 and 70.8 %, respectively, the overall mean reduction of Actellic after 15 days from application was 75.1 % in the 1st application. This result corresponds to El-Haemeasy *et al.* [19] who showed that some insecticides such as Anthio, Nexion and sumithion were effective in reducing the number of mines and killing *L. Trifolii* larvae infesting broad bean plants. The third harmful compound was the microbial insecticide vertimec, which resulted an overall mean reduction of 60.4% after 15 days from 1st application and the reduction in *D. isaea* counts after 2, 3, 5, 7, 10 and 15 days from treatment by vertimec were 44.4, 76.7, 72.0, 68.2, 51.0 and 50.0 %, respectively, in the 1st application.

The least effective compounds harmful to *D. isaea* larvae and pupa were Admiral and Bemistop; the mean reduction after 15 days from application was 33.7 and 36.8 % respectively. The reduction percentages of *D. isaea* after 2, 3, 5, 7, 10 and 15 days from treatment by Admiral were 52.8, 45.6, 31.0, 24.3, 18.5 and 30.1 %, respectively, while those were 12.1, 37.0, 45.8, 51.0, 20.1 and 54.9 %, respectively, after the 1st application. Also, the same trend of results occurred after the 2nd application of the same treatments. Trigard was the most harmful to *D. isaea* larvae and pupa, followed by Actellic, vertimec, Admiral and Bemistop mean reductions of 91.4, 66.6, 50.6, 37.3 and 35.9 %, respectively (Table 2). The results on the effects of insecticides application that showed negative effects on survival and fitness of *Liriomyza* natural enemies agree with Kaspi and Parrella [20]; Tran *et al.* [21] and Hossain and Poehling [22].

Efficacy of the Ecto-Larval Parasitoid, *Diglyphus isaea* against *Liriomyza trifolii*: This piece of work was carried out under glasshouse conditions at (25±3°C and 65 ±5% R.H.) the parasitoid was released after one week of artificial infestation and repeated 10 days later. Data in Table (3) revealed that the number of alive *L.trifolii* larvae per plant was decreased after first release of the parasite as compared with initial number of alive larvae in pre-release. On contrary, the number of alive larvae/ plant

Table 3: Effect of different rates of release the parasitoid, *Diglyphus isae* releases on number of a live *Liriomyza trifolii* larvae in common bean leaves.

	Average number of alive larvae/ plants															
		First release			Second release											
Rate of release	Prerelease	3	5	7	10	Total	Mean	3	5	7	10	17	24	30	Total	Mean
4/30*	7.0	6.0±0.5	3.0±0.6	2.0±0.3	0.6±0.2	18.6±1.5	4.6±0.4	1.6±0.3	0.0±0.0	0.0±0.0	1.0±0.5	1.5±0.8	1.0±0.3	1.0±*0.4*	6.1±2.5	0.87±0.3
2/30*	8.0	7.0±0.5	5.0±1.1	1.0±1.1	1.0±0.4	22.9±3.1	5.5±o.8	2.0±0.7	0.1±0.2	0.0±0.0	1.6±0.6	1.7±0.6	2.0±0.8	1.0±0.4	8.4±3.2	1.2±0.5
1/30*	5.0	8.0±1.2	6.0±1.1	2.3±1.2	3.0±0.3	24.3±2.8	6.07±0.9	1.0±0.3	0.6±0.3	0.0±0.0	5.6±0.8	12.0±1.7	22.0±2.9	31.0±4.3	72.2±110.0	10.31±1.4
Control	6.0	9.6±1.2	9.3±0.7	8.3±0.4	5.0±0.4	38.2±2.7	9.55±0.7	3.5±0.6	2.3±0.4	1.0±0.3	1.7±1.9	58.0	85.5±3.5	86.0±4.0	285.7±14.0	40.81±2.0

*= Released parasitoids/ 30 larvae

Table 4: percentage of parasitism after first and second releases.

	Average number of alive larvae/ plants														
	Days after first release						Days after second release								
Rate of release	3	5	7	10	Total	Mean	3	5	7	10	17	24	30	Total	Mean
4/30*	3.0±0.3 (33.2)	5.0±0.4 (55.0)	8.0±0.7 (80.0)	5.3±0.3 (88.7)	21.3±1.8	5.3±0.4 (64.4)	2.0±o.3 (58.3)	2.0±0.3 (100.0)	2.0±0.4 (100.0)	6.0±0,5 (87.5)	8.0±0.6 (85.0)	13.0±0.7 (92.9)	11.0±1.0 (91.7)	42.0±4.2	6.0±0.6 (87.8)
2/30*	2.0±0.4 (27.3)	5.0±0.8 (50.0)	8.0±0.5 (80.0)	6.0±0.5 (85.6)	22.0±2.3	5.5±0.6 (60.7)	2.0±0.6 (50.0)	1.1±0.2 (87.21)	1.2±0.2 (100.0)	6.33±0.7 (81.4)	8.3±0.8 (83.3)	13.0±1.2 (86.7)	14.0±1.2 (93.3)	45.93±5.0	6.6±0.7 (83.0)
1/30*	2.0±0.5 (20.0)	4.0±0.6 (40.0)	6.6±0.6 (66.6)	5.0±0.4 (62.5)	17.6±2.0	4.4±0.4 (47.3)	2.0±0.3 (50.0)	1.0±0.3 (66.6)	2.0±0.3 (100.0)	6.0±0.5 (84.6)	7.0±1.1 (35.0)	10.0±0.6 (30.3)	10.1±0.6 (23.7)	38.1±3.5	5.5±0.6 (50.6)

*= Released parasitoids/ 30 larvae

was increased in control to reach 8.05±0.73 larvae/plant. The three rates of releases (4/30, 2/30 and 1/30 parasite/ larvae resulted a significantly, lower counts of alive larvae/plant (2.9±0.4, 3.5±0.8 and 4.8 ±0.9 larvae/plant) than in case of control plants (8.05 ± 0.73 larvae/plant). Results after the second release, indicated that the two parasite releasing levels (4/30 and 2/30 parasite/larvae) resulted fewer alive larvae of *Liriomyza*/plant (0.87±0.3 and 1.2±0.5 larvae/plant, respectively) as compared with control plants which manifested the greater number of alive larvae / plant (40.81±2.0 larvae). Also, it was noticed that number of alive larvae was increased after the 7th day from the second release to reach (1.0±0.4, 1.0±0.4 and (31.0±4.3 larvae/plant for the three rates of release, respectively). While, no. alive larvae were counted in leaves, 7 days after second release.

Data in Table (4) showed that the percentages of parasitism increased after the first release to reach their maxima on the 10th day after release (88.7, 85.6 and 62.5%, respectively). Hence, the mean percentage of parasitism after the first release reached (64.6, 60.7 and 47.3% for three levels of release, respectively). Also, the percentages of parasitism increased after the second release to reach (87.5, 81.4 and 48.6% for three release rates (4/30, 2/30 and one /30 larvae, respectively). Also, the percentages of parasitism increased after the second release to reach (87.5, 81.4 and 48.6% for the three releasing rates, respectively). However, the percentage of parasitism reached its maximum (100%) at all releasing rates at the 7th day from the second release then decreased and increased at the end of 2nd release especially, at the two parasite rates (4/30* and 2/30* parasite/ larvae) to reach 91.7 and 93.3%, respectively. While, the percentage of parasitism at the rate of 1/30* (parasite/ larvae) recorded the minimum percentage of parasitism at the end of 2nd release (23.8%; Table, 4).

It could be recommended that, the leaf miner *Liriomyza trifolii* need weekly releases of *D. isaea* for reaching considerable control of *L. trifolii*. Also, the rates of 4/30* and 2/30* (parasite/larvae) induced satisfactory control of this pest. The present results agree with Chuan *et al.* [23]; Tellez *et al.* [24] and Kim *et al.* [25] who mentioned that *D. isaea* proved as an effective biocontrol agent in greenhouse vegetables. In addition, Fadl and El-Khawas [26] found that the population density of the leaf-miner, *L. trifolii* attacking early summer plantations of tomato fields and percentages of parasitism on it.

CONCLUSION

It could be concluded that all treatments caused suppression in common bean pest leaf miner, *liriomyza trifolii* population, in addition to cause a reduction on the population of their ectoparasitoid, *D. isaea* during the two application. The insect growth regulator, Trigard, was the most active compound following by the organophosphorus insecticides, Actellic. While, microbial insecticides, Vertimec and insect growth regulator, Admiral were moderately toxic. On the other hand, a botanical insecticides Bemistop gave the lowest reduction in pest population and their ectoparasitoid. Also, the leaf miner, *L. trifolii* need a weekly release of *D. isaea* for controlling it. Thus, the rate of 4/30 and 2/30 (parasite/larvae induced as a satisfactory control of the pest.

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