"Evaluation of Exotic parasitoid, *Diglyphus isaea* against *Liriomyza trifolii* on Tomato in Protected Cultivation in India"

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Abstract

Greenhouses in India are suffering from serious pest infestations mainly sucking pests and soil borne pathogens. As growers in India are largely dependent on insecticides and biorationals, commercial availability of bioagents is virtually negligible, thereby hampering ecofriendly objectives. A trial on evaluation of exotic parasitoid, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) against leaf miner, *Liriomyza trifolii* (Burgess) (Diptera) on tomatoes was conducted in a study plot of 150 m^2 which was separated into two walk- in tunnels of 75 m^2 each. The parasitoid was supplied by the commercial unit of Biobee Sde Eliyahu Ltd Israel. The trial included there treatments i.e. releases of *D. isaea* @ 0.5 wasps/m^2 against leaf miner (T_1), no pesticide application (T_2) and management with chemical pesticides (T_3). *D. isaea* was found effective in reducing the population of leaf miner significantly in tomato crop grown under protected structures.

Key words: Leaf miner, Biological control, greenhouse, *Diglyphus isaea*, tomato

Introduction

Although the role of protected cultivation is increasing globally , greenhouse cultivation in India is still a new and emerging field for growing vegetables and flowers. To counter the sustained demand of fresh vegetables and flowers throughout the year this technology is fast picking up, but the greater threat is in the form of pest problems encountered in greenhouses and it is a challenge to control the menace of pests. Leaf miner, *Liriomyza trifolii* (Burgess) (Diptera:

Agromyzidae), is one of the most serious pests of various floricultural and vegetable crops. The farmers are almost totally dependent on chemical pesticides for containing the pest under protected cultivation which results in resistance development causing a more severe challenge to pest control (Herron and Rophail 1993). *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) have been used in several countries for the management of leaf miner (Ozawa *et al.* 1993; Ulubilir and Sekeroglu 1997). However commercial natural enemies are not being used by commercial growers in India in spite of rising number of green houses and incidence of the pest (Chakraborty 2011; Sharma and Chandel 2011; Chaudhuri and Senapati 2004).The present work is an attempt to evaluate the exotic parasitoids *D. isaea* to manage the leaf miner, *L. trifolii* in tomato crop under protected cultivation. The parasitoid was supplied by the commercial unit of Biobee Sde Eliyahu Ltd Israel through their Indian counterpart.

Materials and methods

The trial was carried out by the National Centre for Integrated Pest Management New Delhi in collaboration with Biobee Biological systems Pvt Ltd New Delhi in the greenhouse facility of Centre for Protected Cultivation Technology (CPCT), Indian Agricultural Research Institute, New Delhi. The experiment was conducted with tomato (cv. Avinash III) in two walk- in tunnels of 75 m^2 each with three treatments and seven replications in RBD. Tomato plants were transplanted on 1^{st} October 2011 at CPCT. The treatments included releases of *Diglyphus isaea* (twice - November 4^{th} and 16^{th} 2011) @ 0.5 wasps per m^2 in T_1, no treatment of any kind against leaf miner in T_2 and application of chemical pesticide against leaf miner in T_3. Each treatment had three rows of tomato with 8 plants per replicate.

Collection of field samples of Tomato leaves

Three leaves (having leaf miner tunneling symptoms) were randomly selected at weekly interval from each replication. A total of 21 leaves from each treatment were collected for laboratory examination under a stereo microscope. The observations were recorded on number of live leaf miner larvae, dead (host fed) leaf miner larvae, parasitized leaf miner larvae by *D. isaea*, and pupae of the same.

Data Analysis: The data was analyzed statistically using one way ANOVA. For all statistical comparisons in different tables $P< 0.5$ was taken.

Meteorological data: The mean maximum temperature, minimum temperature and relative humidity remained 28.4ºC, 11.8 ºC and 90%, respectively, during the trial period.

Results

Active larvae of leaf miner, *Liriomyza trifolii*

The initial observations made on the progressive population of active leaf miner larvae (Table 1) (Fig.1) indicated 2 larvae/leaf in both T_1 (biological) and T_2 (no treatment) treatments against 1 larva/leaf T_3 (recommended chemical). After the 1^{st} release of *D. isaea* the population of leaf miner in 2^{nd} observation, reached almost the same level (1.4-1.5 larvae/leaf) in all the treatments.

Second release of *D. isaea* resulted in significant reduction in the population of leaf miner during 3rd observation in T_1 as compared to other treatments. In the last observation (6th) a slight increase in the population of leaf miner was recorded in T_2 and T_3 whereas in T_1 the population remained zero. On an average, the lowest active population of larvae (0.6) was observed in T_1 as compared to T_2 and T_3 (0.9 larvae per leaf).

Emergence holes of leaf miner

Emergence holes of leaf miner larvae were found in all the treatments throughout the season ranging from 0.4 to 1.0 (av. 0.7) in T_1, 0.5-2.0 (av.1.0) in T_2 and 1.0 to 2.0 (av. 1.1) in T_3 (Table 1) (Fig. 2)

Dead host fed larvae of leafminer

Population of dead larvae was almost similar i.e. 0.7, 0.5 and 0.8 larva/leaf in T_1, T_2 and T_3 treatments, respectively, in the first observation (Table 2) (Fig. 3). After the 2nd release of *D. isaea* (16th Nov., 2011), the biological treatment T_1 showed an increase in the larval mortality in the second observation whereas treatment T_2 and T_3 showed a decline in the mortality. The differences among treatments were statistically significant. A decline in the mortality was recorded in third (0.5 larva/leaf) and fourth (0.3 larva/leaf) observations in T_1, whereas T_2 and T_3 indicated an increase in the mortality in third observation. On an average, highest mortality (0.6 larvae/ leaf) was recorded in T_1 against 0.3 larvae/ leaf each in T_2 and T_3

Parasitization of leaf miner larvae by *Diglyphus isaea*

Observations on progressive parasitization of leaf miner larvae indicated low parasitization in first observation of T_1 (0.2 larva/leaf) which increased to maximum level (1.1 larvae/leaf) just after the 2nd release of *D. isaea* (16th Nov., 2011) in second observation and thereafter declined in third observation (0.6 larva/leaf) as no active larva (Table 1) was available for parasitisation by *D. isaea* wasps. No parasitization was recorded in subsequent observations (ob.3rd and 4th) in T_1. In other treatments no parasitization was recorded (Table 3) (Fig. 4).

***D. isaea* pupae**

Observations on *D. isaea* pupae indicated presence of pupae in all the treatments during 2nd, 3rd and 4th observations. The highest number of pupae were recorded in T_1 (range 0.2-1.0; av.1.0) followed by T_2 (range 0.1-1.0; av. 0.4) and T_3 (range 0.2-0.6; av. 0.3) (Table 3) (Fig. 5 & 6)

Emergence holes of *D. isaea*

Emergence holes of *D. isaea* (Fig. 2) were found in treatment T_1 in 2nd observation. In other treatments they remained absent as the parasitoid was released in T_1 only. In subsequent observations (3rd to 5th) the emergence holes were recorded in all the treatments apparently due to the presence of escaped exotic species alongside. Emergence holes remained highest in treatment T_1 as compared to T_2 and T_3 throughout the season. On an average the number of emergence holes was 0.5, 0.2 and 0.2 in T_1, T_2 and T_3, respectively (Table 3). Emergence of healthy parasitoid adults of D. isaea were observed as one of the indicators of successful completion of their life cycle on leafminer (Fig. 7)

Table-1: Leaf Miner larvae that continued feeding and finally escaped through emergence holes for pupation

Date/No. of observation	4th Nov'11		11th Nov'11		17th Nov'11		25th Nov'11		1st Dec'11		8th Dec'11		Avg	
	1		2		3		4		5		6			
Treatments	Larvae/leaf	Emergence holes/leaf	Larvae/leaf	Emergence holes/leaf	Larvae/leaf	Emergence holes/leaf	Larvae/leaf	Emergence holes/leaf	Larvae/leaf	Emergence holes/leaf	Larvae/leaf	Emergence holes/leaf	Larvae/leaf	Emergence holes/leaf
T_1	2 (1.555) ^{a}	1 (1.075) ^{a}	1.5 (1.38) ^{a}	1 (1.014) ^{a}	0.3 (0.861) ^{a}	1 (1.08) ^{a}	0.15 (0.808) ^{a}	1 (1.144) ^{a}	0 (0.707) ^{a}	0.5 (0.97) ^{a}	0 (0.707) ^{a}	0.4 (0.928) ^{a}	0.6	0.7
T_2	2 (1.391) ^{a}	1 (0.996) ^{a}	1.4 (1.349) ^{a}	1.3 (1.3) ^{a}	1.5 (1.383) ^{a}	2 (1.405) ^{a}	0.7 (1.048) ^{a}	1.1 (1.254) ^{a}	0.1 (0.777) ^{a}	0.5 (0.96) ^{a}	0.35 (0.914) ^{a}	1 (1.128) ^{a}	0.9	1
T_3	1 (1.379) ^{a}	1 (1.063) ^{a}	1.4 (1.34) ^{a}	2 (1.526) ^{a}	1.5 (1.42) ^{b}	1 (1.102) ^{a}	0.5 (0.973) ^{a}	1 (1.072) ^{a}	0.15 (0.795) ^{a}	1.2 (1.27) ^{a}	0.3 (0.896) ^{a}	1 (1.101) ^{a}	0.9	1.1
CD	NS	NS	NS	NS	0.2820	NS	NS	NS	NS	NS	NS	NS		

Figures in parenthesis indicate sqrt transformed values and the values in the columns represented by same letters are not significant statistically

Table-2: Dead (host fed) leaf miner larvae in different treatments in tomato

Treatments	No. of dead larvae /leaf					Avg
	11^{th} Nov	17^{th} Nov	25^{th} Nov	1^{st} Dec	8^{th} Dec	
	1	2	3	4	5	
T_1	0.7 (1.037) ^{a}	1.3 (1.33) ^{b}	0.5 (0.988) ^{a}	0.3 (0.861) ^{a}	0.3 (0.89) ^{a}	0.6
T_2	0.5 (0.943) ^{a}	0 (0.707) ^{a}	0.4 (0.924) ^{a}	0.5 (0.969) ^{a}	0.1 (0.766) ^{a}	0.3
T_3	0.8 (1.09) ^{a}	0 (0.707) ^{a}	0.6 (0.994) ^{a}	0.3 (0.861) ^{a}	0.1 (0.766) ^{a}	0.3

CD	NS	0.2111	NS	NS	NS	

Figures in parenthesis indicate sqrt transformed values and the values in the columns represented by same letters are not significant statistically (P<0.05)

Table 3: Indicators of parasitization of leaf miner larvae by *D. isaea*

Date and no. of	11th Nov'11			17th Nov'11			25th Nov'11			1st Dec'11			8th Dec'11			Avg.		
	1			2			3			4			**5**					
Treatments	Parasitized *D. isaea* larva/leaf	emergence holes	No. of pupa/leaf	Parasitized *D. isaea* larva/leaf	emergence holes	No. of pupa/leaf	Parasitized *D. isaea* larva/leaf	emergence holes	No. of pupa/leaf	Parasitized *D. isaea* larva/leaf	emergence holes	No. of pupa/leaf	Parasitized *D. isaea* larva/leaf	emergence holes	No. of pupa/leaf	Parasitized *D. isaea* larva/leaf	emergence holes	No. of pupa/leaf
T_1	0.2 (0.795) ^{a}	0 (0.707) ^{a}	-	1.1 (1.264) ^{b}	0.3 (0.878) ^{b}	1 (1.035) ^{a}	0.6 (1.031) ^{b}	0.6 (1.012) ^{a}	1 (1.05) ^{a}	0 (0.707) ^{a}	1 (1.217) ^{a}	1 (1.157) ^{a}	0 (0.707) ^{a}	0.5 (0.946) ^{a}	0.2 (0.837) ^{a}	0.4	0.5	1
T_2	0 (0.707) ^{a}	0 (0.707) ^{a}	-	0 (0.707) ^{a}	0 (0.707) ^{a}	0 (0.707) ^{a}	0 (0.707) ^{a}	0.2 (0.79) ^{a}	1 (1.081) ^{a}	0 (0.707) ^{a}	0.6 (1.043) ^{a}	0.6 (0.993) ^{a}	0 (0.707) ^{a}	0.1 (0.778) ^{a}	0.1 (0.737) ^{a}	0.0	0.2	0.4
T_3	0 (0.707) ^{a}	0 (0.707) ^{a}	-	0 (0.707) ^{a}	0 (0.707) ^{a}	0 (0.707) ^{a}	0 (0.707) ^{a}	0.3 (0.852) ^{a}	0.5 (0.946) ^{a}	0 (0.707) ^{a}	0.4 (0.917) ^{a}	0.6 (1) ^{a}	0 (0.707) ^{a}	0.1 (0.737) ^{a}	0.2 (0.781) ^{a}	0.0	0.2	0.3
CD	NS	NS	-	0.1522	0.1292	NS	0.1926	NS	NS	NS	NS	NS	NS	NS	NS			

Figures in parenthesis indicate sqrt transformed values and the values in the columns represented by same letters are not significant statistically (P<0.05)

Discussion:

The study on the efficacy of *D. isaea* on *L. trifolii* showed that *D. isaea* was effective in reducing the population of leafminer (*L. trifolii*) significantly in tomato crop under protected cultivation under Indian conditions. The results were further confirmed by the mortality of leafminer larvae due to *D. isaea* active parasitization i.e presence of active *D. isaea* larvae and pupae feeding on *L. trifolii* larvae under field conditions. Agromyzid leafminers, in particular, the serpentine leafminer, *L. trifolii* is one of the most serious pests of various floricultural and vegetable crops (Kiel and Parella 1990).

Considerable work has been conducted using *D. isaea* as an effective biocontrol agent in greenhouse vegetable crops (Chuan, 2007; Tellez *et al.* 2006; Kim *et al.* 2007). Field studies have also been carried out to determine the population density of the leaf-miner, *L. trifolii* attacking early summer plantations of tomato fields and percentages of parasitism on it (Fadl and El-Khawas 2009).

The most promising non-chemical approach for controlling *Liriomyza* leaf miners in greenhouses is the augmentative releases of the oligophagous larval ectoparasitoid *D. isaea* (Boot *et al*. 1992; Minkenberg and Van Lenteren, 1987 and Ozawa *et al*. 2001). The parasitoid is being successfully utilized in many countries against leaf miner (Ozawa *et al.* 1993; Ulubilir and Sekeroglu 1997; Talebi *et al.* 2005; Shaaban Abd- Rabou 2006), however, the parasitoid has never been exploited in India.

It is evident from the present study that *D. isaea* has proved effective in reducing the population of leafminer *(L. trifolii*) significantly in tomato crop under protected cultivation under Indian conditions. The results were further confirmed by the mortality of leafminer larvae due to *D. isaea* parasitization and presence of active *D. isaea* larvae feeding on *L. trifolii* larvae under field conditions.

In conclusion our findings show that the parasitic wasp Diglyphus isaea has shown the potential to be exploited for the management of leafminers under greenhouse conditions
In the present study as the population of leaf miner remained low, no further releases were made but when the population is higher further releases may be required.

Fig. 1. Active larvae of leaf miner on tomato

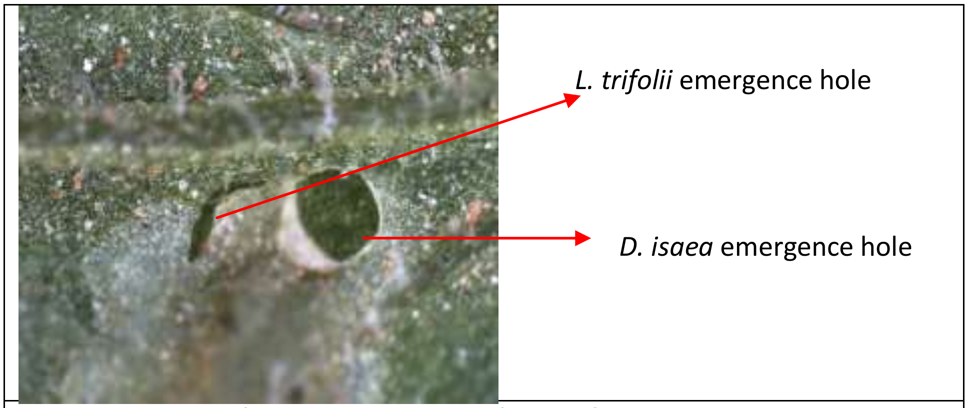

Fig. 2. Shape of emergence holes of *L. trifolii* and *D. isaea*

Fig. 3. Dead larvae of *L. trifolii*

Fig. 4. Parasitization of leaf miner larvae by *Diglyphus isaea*

Fig. 5. Larval, Prepupal and Pupal Stages of *D. isaea*

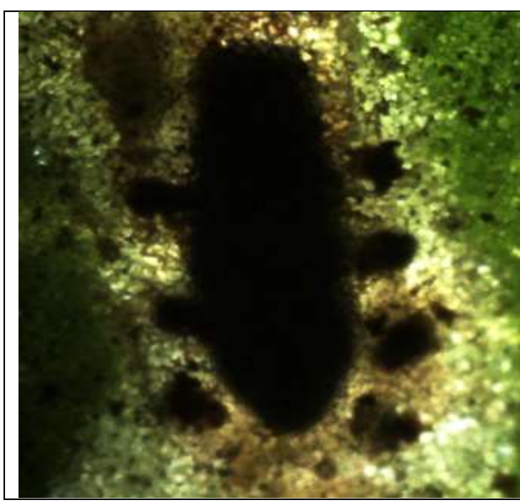

Fig. 6. *D. isaea* pupa with meconia	**Fig. 7.** Emerged *D. isaea* adult

References

Abd-Rabou S. (2006). Biological control of Leafminer *Liriomyza trifolii* by introduction, releasing, evaluation of parasitoids *Diglyphus isaea* and *Dacnusa sibirica* on vegetable crop in Egypt. *Archives Of Phytopathology and Plant Protection 39*,439- 443.

Boot, W.J., Minkenberg, O.P.J.M., Rabbige, R., & De Moed, G.H. (1992). Biological control of leafminer *Liriomyza bryoniae* by seasonal inoculative releases of *Diglyphus isaea*: Simulations of parasitoid host system. *Netherlands journal of plant pathology 98*,203-212

Chakraborty, K. (2011).Incidence and abundance of Tomato Leaf Miner, *Liriomyza trifolii* , Burgess in relation to climatic conditions of Alipurduar, Jalpaiguri, west Bengal, India. *Asian Journal Exp.Biol.sci. 2*, 467-473.

Chaudhuri, N., & Senapati, S.K. (2004) Incidence and Biology of Leaf Miner(*Liriomyza trifolii* Burgess) on Tomato as influenced by weather conditions. *Annals of plant protection sciences*. *12* 55-58.

Chuan, B.Y., Shu, G.H., & Hong, X.W. (2007). Parasitic wasps of leaf miners on vegetable crops and their protection and potential application. *China Vegetables 12*, 39-41.

Fadl, H.A.A.A., & El-Khawas, M.A.M. (2009). Incidence of parasitoids on the leaf-miner species, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), in tomato fields, at Qaluobia Governorate, Egypt. *Egyptian Journal of Biological Pest Control*, *19*, 93-97

Herron, G.A., & Rophail, J. (1993). Clofentezin and Hexythiazox resistance in Tetranychus urticae Koch in Australia. *Experimental and Applied Acarology 17*, 433-440.

Keil, C.B., & Parrella, M.P. (1990). Characterization of insecticide resistance in two colonies of *Liriomyza trifolii* (Diptera: Agromyzidae). *J. Econ. Entomol*, *83*, 18–26.

Kim, J.H., Byoun, Y.W., Lee G.S., & Kim H.Y. (2007). Evaluation of Biological control of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) using *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae in three seasonal culture types of tomato greenhouse. *Korean Journal of Applied Entomology 46*, 71-78

Minkenberg, O.P.J.M., &Van Lentern, J.C .(1987). Evaluation of parasitic wasps for the biological control of leafminers , Liriomyza spp, in greenhouse Tomatoes. *IOBC / WPRS Bulletin*. *10*,116-120.

Ozawa, A., Saito, T., & Ota, M. (2001). Biological control of American serpentine leafminer, *Liriomyza trifolii* (Burgess), on tomato in greenhouse by parasitoids. II. Evaluation of Biological control by *Diglyphus isaea* (walker) and *Dacnusa sibirica* Telenga in commercial greenhouses. *Japanese journal of Applied entomology and zoology 45*, 61-74

Ozawa,A., Kobayasi, H., Amano, T., Ikari, T., Saito,T. (1993). Evaluation of imported parasitic wasps as biological control agents of legume leafminer *Liriomyza trifolii* Burgess, in Japan.II. A field test on cherry tomatoes in plastic geenhouse in Shizuoka prefecture. *Proceedings of Kanto-Tosan Plant Protection Society*; 40, 339-241.

Sharma, P.L., & Chandel, A. (2011) Seasonal incidence dispersion and management of Serpentine Leaf Miner, *Liriomyza trifolii* (Burgess) on tomato under subtemprate conditions. *Journal of insect science 24*, 291-294.

Talebi, A.A., Asadi, R., Fathipour, Y., Kamali, K., Moharramipour, S., & Rakhshani, E. (2005). Eulophid parasitoids of Agromyzid leafminers genus Liriomyza (Dip: Agromyzidae) in Tehran, Iran. *Bulletin OILB/SROP 28* ,263-266

Tellez, M. M., Tapia, G.M., & Lara, L.Y. (2006). *Diglyphus isaea*, an efficient parasitoid for the control leafminers. *Horticultura Internacional*, *13*, 76-77

Ulubilir, A., & Sekeroglu, E. (1997) Biological control of *Liriomyza trifolii* by *Diglyphus isaea* on unheated greenhouse tomatoes in Adana Turkey. *Bulletin OILB/SORP*; *20*, 232- 235