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Rearing of Trilocha varians (Lepidoptera: Bombycidae) on Ficus benjamina and its Parasitization with Trichogramma chilonis

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Cover Page Footnote

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REARING OF *TRILOCHA VARIANS* (LEPIDOPTERA: BOMBYCIDAE) ON *FICUS BENJAMINA* AND ITS PARASITIZATION WITH *TRICHOGRAMMA CHILONIS*

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ABSTRACT

Ficus benjamina commonly known as weeping fig is an ornamental that planted alongside the road to increase the aesthetic value. Leaf eating caterpillar, *Trilocha varians* was observed on *Ficus benjamina* and many other ornamental plants in the surrounding area of Muhammad Nawaz Shareef University. Large larval population was recorded on fig plants with 100 % defoliation. The rearing of *Trilocha varians* on *F. benjamina* was carried out under controlled conditions and parasitism of *Trichogramma chilonis* on eggs were checked. Incubation period was 5-6 days while single female laid 150-210 eggs in her whole life period. With five larval instars, duration period of 1st, 2nd, 3rd, 4th and 5th was 2.0 days, 3-5 days, 2-4 days, 3-6 days and 3-7 days, respectively. Pupa was obtect in nature. Mated and unmated female were long lived as compared to male, 7.50 and 9.25 % egg parasitization was recorded at 16 and 24 hours of exposure, respectively. The coefficient variations were 89.50, 55.92, 42.21 and 29.59 at 12, 16, 20 and 24 h. To determine the larval damage, five different host plants belonging to moraceae family were used. The moderate damage of larvae was observed on *Ficus religiosa.* The severe feeding damage was observed on *F. benjamina* and *F. virens,* while *M. alba* and *F. elastica* were found not favorable hosts of *T. varians.* The study concluded that *Trichogramma chilonis* was recorded the most suitable parasitoid of *T. varians* and further need to record the natural enemies of the pest in the country.

Keywords: *Ficus benjamina*; figs; *Trilocha varians*; bombycid moth; Pakistan

INTRODUCTION

Genus *Ficus* belongs to Moraceae family with approximately 850 species of woody trees, vines, shrubs, epiphytes, and hemiepiphytes. The most important *Ficus* species are *Ficus benjamina, F. altissima, F. microcarpa, F. elastica, F. annulate, F. religiosa*, *F. benghalensis* and *F. infectoria* (Ramzan et al., 2019). These species have distributed throughout the tropics and semi-warm temperate zones of the world. The most important *Ficus* species is *F. benjamina* which commonly known as weeping fig. This species is medically very important and grown for landscaping purposes. This tree can be consumed as food for humans and wildlife. This evergreen species is planted alongside the road to increase the beauty (Ramzan et al., 2021b).

Several sucking and chewing insect pests and various diseases attack on this woody tree in the world especially Pakistan. The major insect pests of weeping figs are whitefly, mealybug and

thrips (Basri et al., 2019; Ramzan et al., 2020b). The most important and serious pest of weeping fig is *Trilocha varians* which belongs to order Lepidoptera and family Bombycidae. This is widely distributed in various countries such as Pakistan (Ramzan et al., 2023; Mansoor et al., 2022), China, India, Sumatra, Malysia, Taiwan, Java, and Nepal (Singh and Brar 2016). The aesthetic value of the country reduces due to its severe attack. 100 % defoliation of plant has reported by many researchers during favorable environmental conditions and high pest population (Ramzan *et al.* 2020a; Basri et al., 2019). Larval stage of this Bombycid moth is very dangerous for all parts (leaves, tender tips and twigs) of *Ficus* spp. in the various countries of the world like India, China and Malaysia. Some researchers have reported death of the plant caused due to severe attack of its larvae (Zolotuhin and Witt 2009; Daimon et al., 2012).

It is now becoming major insect pest of various other ornamental plants (Aziz et al., 2013; Ramzan et al., 2020b) because it caused 100 % defoliation of *F. benjamina* and now attacked on jackfruit and *F. virens* (Navasero et al., 2013). There is need to control this pest to overcome the losses of ornamental plants in the country. Before going to adopt management strategies, biological parameters of this species must be known. The current study was conducted to get information about biological and morphological parameters of pest under controlled conditions. This is the first study in Pakistan which conducted to check the parasitism of parasitoids on egg stage of pest.

MATERIAL AND METHODS

Stock Culture of Trilocha varians

An experimental study was carried out in Southern Punjab Pakistan (30.2° N and 71.4° E) at 123-meter-high above the sea level. Newly laid eggs of *Trilocha varians* were collected from different places of southern Punjab and brought to ecology laboratory for maintaining the stock culture. Eggs were placed into petri dishes and fresh leaves of *F. virens* and *F. benjamina* were used for larval feeding while eggs monitored on daily basis to check their hatchability. New hatched larvae were placed into plastic jars with *F. benjamina* and *F. virens* leaves for food and mouth of jars were covered with muslin cloth. These two hosts were best host for the current pest. The pre-pupae and pupal stages of pest were kept into separate plastic containers/jars for adult emergence. The emerged adults were placed into rearing cage for obtaining eggs. The same procedure was followed to maintain the laboratory colony.

Culture of Trilocha varians

Fresh eggs of *T. varians* were removed from cage with the help of forceps and placed in petri dishes for hatching. There were three replications with each containing ten *T. varians* eggs. Different developmental stages (Incubation period, instars durations, total larval period, pupal period and total developmental period from egg to adult) were noted on daily basis.

Culture of Trichogramma chilonis

T. chilonis were obtained from Biological Laboratory, Department of Entomology, University of Agriculture Faisalabad. *T. chilonis* was maintained in Ecology Laboratory at Institute of Plant Protection (IP^2) on the egg of *Sitotroga cereallela* by using rearing procedure of early researchers (Saljoqi et al., 2012).

Efficacy of Trichogramma chilonis

One day old 240 eggs of *T. varians* were collected and used in the study. These eggs were pasted on the paper strips

with the help of fine gum film. Then these cards were exposed to ultraviolet light for an hour for sterilization. The study was conducted by using CRD having six treatments including control and each treatment contained four replications. Each replication contained 10 counted number of larvae. *T. varians* eggs were exposed to adult *T. chilonis* parasitoids for 1, 4, 8, 12, 16, 20, and 24 hours on paper strips.

Treatments

T1: one female
T2: Two females
T3: Three females
T4: Four females
T5: Five females
T6: Control

Percentage Parasitism Data

After oviposition, the colour of parasitized eggs were changed into black and easily recognized (Saljoqi *et al.* 2012) while emerging parasitoids reached the pupal stage within 5-6 days. The emergence rate of insect was also determined by counting the number of dark eggs with holes showing hatchability. Percent (%) parasitization was also checked by using this formula.

$$\text{Percent parasitization} = \frac{\text{Total No.of black eggs}}{\text{Total number of eggs}} \times 100$$

This experiment was conducted at controlled conditions *i.e.* 26 ± 2 °C with a 60 ± 10% RH.

Life History

The incubation period was also checked. The larval and pupal duration were determined by using 50 neonate larvae that randomly selected from insect colony and each larva was reared individually on apical leaves of *F. virens* and *F. benjamin*a. The pupation and adult emergence was checked on daily basis. The oviposition and eggs hatching period was observed throughout the study period. The selection of pupation site and mating of adult was also recorded.

Extent of Damage and Host Suitability

The extent of damage due to larvae was observed for 24 hours. For this purpose, different hosts were selected and ten larvae were placed on each host to check their suitability and damage for 24 hours. The procedure of early researcher was followed to check the extent of damage (Navasero et al., 2013).

Statistical Analysis

The means were calculated statistically and compared by Tukey test using Statistic 8.1.

RESULTS

Eggs and Larvae

The 5-6 days were the incubation period that recorded in the study. A single female was laid 150-210 eggs in her life period (Table 1). The duration period of 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} was 2.0 days, 3-5 days, 2-4 days, 3-6 days and 3-7 days, respectively. The total larval periods of male and female were 16-18 days and 17-21 days, respectively (Table 1).

Pupa and Adult

The colour of pupa was whitish and change their colour before adult emergence. The larvae pupate in silk like cocoon on the walls of container or on the muslin cloth in laboratory (Table 1). The male adult was fast flayer while female short flayer. The male body size was short as compared to female. The female was short lived than male. It was observed that mated female was short lived than unmated female. The developmental stages with their ranges and means were described in detail (Table 1).

Table 1: Biological parameters of *T. varians* under laboratory conditions

Developmental stage	Range		Mean	
	Male	Female	Male	Female
Egg incubation	5-6	5-6	5.5	5.5
Larva				
1st instar	2.0	2.0	2.00	2.0
2nd instar	3-5	3-5	4.33 ± 0.82	3.68 ± 1.12
3rd instar	2-4	2-4	3.00 ± 0.63	3.89 ± 1.05
4th instar	3-5	4-6	3.50 ± 0.84	4.56 ± 1.33
5th instar	4-5	3-7	4.337 ± 0.52	5.00 ± 1.22
Total larval period	16-18	17-21	17.17 ± 0.98d	19.1 ± 1.36
Pupa	5-6	5-6	5.17 ± 0.41	5.56 ± 0.53d
Total developmental period (Egg-adult)	26-29	28-33	27.33 ± 1.21	30.89 ± 1.67d

Table 2: Post developmental stage of *T. varians*

Post developmental stage	Range	Mean ± SE
Pre-oviposition	2-3	2.34 ± 0.52
Oviposition	2-4	2.63 ± 0.74
Post-oviposition	1-3	1.88 ± 0.64

Table 3: Host plants belonging to family Moraceae tested as potential hosts and feeding damage of larvae after 24h of post treatment

Sr. No.	Host Plant		Extent of Damage*		
	Common Name	Scientific Name	Trial 1	Trial 1	Trial 1
1	Weeping fig	*Ficus benjamina*	+++	+++	+++
2	White fig	*Ficus virens*	+++	+++	+++
3	Peepal	*Ficus religiosa*	+	+	+
4	Mulberry	*Morus alba*	-	-	-
5	Rubber tree	*Ficus elastica*	-	-	-

**(+) = slight feeding damage, (++) = moderate feeding damage, (+++) = severe feeding damage and (-) = no feeding damage*

The post developmental periods like preoviposition, oviposition and post oviposition of female adults were recorded 2.34 ± 0.52, 2.63 ± 0.74 and 1.88 ± 0.64, respectively (Table 2). In the current study, five different host plants belonging to moraceae family were tested to check the larval damage on these hosts. The moderate damage of larvae was observed on *Ficus religiosa.* The severe feeding damage was observed on *F. benjamina* and *F. virens. M. alba* and *F. elastica* were found not favorable hosts of *T. varians* (Table 3).

The study resulted that parasitoid didn't give best control or parasitoized the eggs at 1-4 hours. The mean percent parasitization of 0.75 ± 0.75 was recorded at one exposure time (hour) in treatment 5thwhich found highly significant difference from all other treatment in the same time. No significant difference was observed at four hours. The mean percent parasitization of eggs at 1, 4, 6 and 8 h

were 0.75 ± 0.75, 0.25 ± 0.25, 1.25 ± 1.25 and 2.5 ± 2.50, respectively in treatment 5th (Table 4). The mean percent parasitization of eggs were 0.0 ± 0.00, 0.25 ± 0.25, 0.25 ± 0.25, 0.0 ± 0.00 and 2.5 ± 2.50 in treatment, 1st, 2nd, 3rd, 4th and 5th, respectively at 8 h.

Table 4: Percent parasitization of eggs of *T. varians* by *T. chilonis* at 1, 4, 6 and 8 hours of egg cards Mean ± S.E.

%age parasitization				
	Exposure time (Hours)			
Treatments	**1**	**4**	**6**	**8**
1	0.0 ± 0.00b	0.0 ± 0.00a	0.0 ± 0.00b	0.0 ± 0.00b
2	0.0 ± 0.00b	0.0 ± 0.00a	0.0 ± 0.00b	0.25 ± 0.25b
3	0.0 ± 0.00b	0.0 ± 0.00a	0.0 ± 0.00b	0.25 ± 0.25b
4	0.0 ± 0.00b	0.25 ± 0.25a	0.0 ± 0.00b	0.0 ± 0.00b
5	0.75 ± 0.75a	0.25 ± 0.25a	1.25 ± 1.25a	2.5 ± 2.50a
Control	0.0 ± 0.00b	0.0 ± 0.00a	0.0 ± 0.00b	0.0 ± 0.00b
Grand mean	0.1250	0.0833	0.2083	0.5000
CV	163.30	346.41	97.98	166.67

Table 5: Percent parasitization of eggs of *T. varians* by *T. chilonis* at 12, 16, 20 and 24 hours of egg cards Mean ± S.E.

%age parasitization				
	Exposure time (Hours)			
Treatments	**12**	**16**	**20**	**24**
1	0.25 ± 0.25b	0.5 ± 0.50c	1.25 ± 1.25cd	3.25 ± 3.25bc
2	0.0 ± 0.00b	0.75 ± 0.75bc	2.25 ± 2.25bcd	5.5 ± 5.50b
3	0.25 ± 0.25b	1.25 ± 1.25bc	5.0 ± 5.00bc	6.5 ± 6.50ab
4	1.25 ± 1.25b	3.5 ± 3.50b	6.0 ± 6.00ab	6.5 ± 6.50ab
5	5.0 ± 5.00a	7.5 ± .50a	9.25 ± 9.25a	9.25 ± 9.50a
Control	0.0 ± 0.00b	0.0 ± 0.00c	0 ± 0.00d	0 ± 0.00c
Grand mean	1.1250	2.2500	3.9583	5.2083
CV	89.50	55.92	42.21	29.59

The mean percent parasitization of eggs was recorded 7.5 and 9.25 at 16 and 24 hours of exposure. In control treatment, all eggs were developed into larvae and not a single egg showed the parasitoized symptoms during study period. The coefficient variations were 89.50, 55.92, 42.21 and 29.59 at 12, 16, 20 and 24 h (Table 5).

DISCUSSION

The insect pests of family bombycidae such as wild silkmoth (*B. mandarina*) and domesticated silkmoth, (*Bombyx mori*) have been studies many times by many researchers all over the world. Silkworm, *B. mori* belongs to the same family (Zolotuhin and Witt 2009) of current pest and an important domesticated insect. It is use for the production of silk thread that used for making various products such as cloth. It has narrow host range like mulberry and has no any alternative host plat while *T. varians* feed on many ornamental plants and belong to subfamily bombycinae (Lamaire and Minet 1998).

215 ± 32.87 eggs laid by female in 3 to 6 rows, on the walls of used

containers in controlled conditions, while on the upper as well as lower side of leaves in the field as reported by many researchers. Our findings are almost similar to the previous researcher findings (Ramzan et al., 2020a). The colour of egg, larva and pupa was light yellow, whitish and brown whitish, respectively, similar to the findings of other scientists (Navasero and Navasero 2014).

The current study was conducted to rear the pest and check the parasitism of parasitoid on eggs of *T. varians.* In the current study, *T. chilonis* was recorded the best parasitoid of pest, while larval and pupal parasitoids of *T. varians* had recorded or reported first time by Kedar et al. (2014) in India. They reported that *Goryphus* sp. ichneumonid wasp and *Encicospilus* sp.

The several factors (biotic and abiotic) are involved in the development and growth of silkworm like *T. varians*. The fertility, fecundity, hatching uniformity and rearing performance was affected by temperature (Basri *et al.* 2019; Ramzan et al., 2021a), humidity and host availability. The egg lying capacity and silk production of silkworm is low in Pakistan as compared to other countries (Hussain et al., 2011).

T. varians is only the pest of *Ficus* species and some other ornamental plants such as jack fruit (Udayagiri 1988). The larval duration of silkworm was recorded at the range of 21.04- 22.28 days. Our findings are in line with the previous study (Pakhale et al., 2014) have reported the larval duration 21.04 days to 22.28 days. The similar findings have been reported by Daimon et al. (2012). Adults are medium sized, dark brown to grayish brown. The colour of each instar was found little different from each other. A caudal horn was present on each larva and similar results have recorded by many scientists. During the study, larvae were found very active and can move very fast outside the containers.

In 2017, a study was planned to determine the behavior of domesticated and wild silkworm larvae on mulberry plant. The larvae of *B. mandarina* were recorded very moveable than *B. mori* (Kômoto 2017; Biram et al., 2009). They have reported the similar findings about larval movement. It was recorded that various biocontrol agents were studies against domesticated silkworm and among those, *T. chilonis* found best control. The various environmental factors such as temperature and food affect the performance of *T. chilonis* (Hoffmann et al., 2001) under both laboratory as well as field conditions. The parasitoid was found most effective against *T. varians* eggs at 24 hours followed by 20 and 16 hours. The maximum eggs were parasitoids at 24 hours and minimum at 1 hours of post treatment.

CONCLUSION

T. varians is the most serious pest of *Ficus* spp. especially *F. virens* and *F. benjamina* in the country. Larval stage is the most damaging stage of the pest. *T. chilonis* has potential to control this damaging pest. The current study findings are providing basic understanding about the natural enemies existing in nature, There is need to identify them and used against this pest which will be helpful in pest management and protect the plants from pest attack.

AUTHOR CONTRIBUTION

Each author has equal contribution in this study.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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