Plant Archives Vol. 24, No. 2, 2024 pp. 1601-1607 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.226

EVALUATION OF FOUR TRICHOGRAMMATIDS AS BIOLOGICAL CONTROL AGENTS FOR *PECTINOPHORA GOSSYPIELLA* (SAUNDERS) IN INDIA

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(Date of Receiving-02-03-2024; Date of acceptance-23-05-2024)

ABSTRACT

Egg parasitoids (Trichogramma) play a crucial role in the pink bollworm *Pectinophora gossypiella* management. In this study, we assessed and compared the efficacy of four different Trichogramma species (*Trichogrammatoidea bactrae*, *Trichogramma chilonis*, *T. pretiosum*, and *T. achaeae*) on the eggs (0-, 1-, 2- and 3-day-old) of the pink bollworm, *P. gossypiella*, examining their parasitizing efficacy, emergence rates, and development periods under laboratory conditions. All four Trichogramma species parasitized *P. gossypiella* eggs at all stages of development, but they displayed varying preferences, showing a stronger inclination towards younger eggs. *T. bactrae* exhibited parasitization rates of 69%, 63%, 56%, and 65% on 0-, 1-, 2-, and 3-day-old eggs respectively within a 24-hour period, indicating its superior suitability and effectiveness across all egg ages. In contrast, *T. chilonis* showed the lowest parasitization and poorer host acceptance. Both *T. chilonis* and *T. pretiosum* displayed similar developmental periods when parasitizing eggs of all ages of *P. gossypiella*. However, *T. achaeae* and *T. bactrae* showed significantly slower development across all egg ages of *P. gossypiella*, although no significant differences were observed in adult emergence rates. These findings underscore the biological efficacy of *T. bactrae*, positioning it as a promising biocontrol agent against *P. gossypiella*. Consequently, it suggests its potential integration into bio-intensive Integrated Pest Management (IPM) strategies for cotton to mitigate the threat posed by *P. gossypiella*.

Key words: Biological control, Host age IPM, Pink bollworm, parasitization, *Trichogramma*,

Introduction

Cotton, a crucial commercial crop in India, occupies an extensive cultivation area of 130.49 lakh hectares, contributing substantially to global cotton production with an approximate share of 21% (ICAR-AICRP Cotton Annual Report, 2022-23). Cotton is a plant that nature seems to have designed specifically to attract insects (Rainwater, 1952). The indeterminate growth characteristics of the cotton crop offer food and shelter to a broad range of insects both directly and indirectly. Nearly 130 insect pest species plague Indian cotton, necessitating the management of around a dozen of these arthropods to optimize cotton yields, while globally, over 1300 herbivorous insects are identified in cotton systems (Hargreaves, 1948), though only a fraction are prevalent, and fewer still hold economic significance.Bollworms and sap sucking insects are important pests of cotton crop from the time it is sown until harvest. Bollworms, particularly the pink bollworm, are notorious for causing significant damage to cotton crop and reduce the economical yield (Ghosh, 2001). Several studies have shed light on the role of egg parasitoids, *Trichogramma* spp. in managing wide range of insect pests that affect cotton crops in different regions around the globe (Peter, 2022). Egg parasitoids (*Trichogramma*) are significant bio-agents in bio-intensive IPM strategy and successfully used against many larval pests including cotton bollworms (Cock, 1985; Ahmed *et al*., 1998; Malik, 2000; Charles *et al*., 2000 and Nadeem *et al*., 2009). Egg Parasitoid, *Trichogramma* is cosmopolitan in distribution and capable of parasitizing pink bollworm eggs (Ahmad *et al*., 2011). Various factors, such as adaptability to local environments (Pizzol *et al*., 2010, 2012; Zhang *et al*., 2014), effectiveness in dispersal (Ayvaz *et al*., 2008; Bueno *et al*., 2012), and preferences for host species or age (Zhu *et al*., 2014; Du *et al*., 2018), can significantly influence the field performance of *Trichogramma* parasitoids. It is imperative to examine and widely disseminate indigenous *Trichogramma* species for successful biological control programs, as they may possess unique characteristics tailored to specific host species and environmental conditions (Smith, 1996). Several studies have indicated that the efficiency of parasitization is impacted by the age of the host (Pak, 1986). Egg age emerges as a critical consideration when selecting appropriate *Trichogramma* species for pest biological control, as it affects the ethological and physiological dynamics of parasitoid-host interactions (Jarjees and Merritt, 2004; Pizzol *et al*., 2012); certain parasitoid species exhibit a preference for younger eggs.

In order to develop effective biological control programs, it is crucial to investigate the parasitization and suitability of Trichogramma parasitoids on *P. gossypiella* eggs at different stages of development. In this study, we assessed four Trichogramma species-*Trichogrammatoidea bactrae*, *T. chilonis*, *T. pretiosum*, and *T. achaeae*on *P. gossypiella* eggs under controlled laboratory conditions. The aim was to determine the parasitic capacity and suitability of these Trichogramma species for *P. gossypiella* eggs at various developmental stages, with the ultimate goal of identifying the most effective Trichogramma species for controlling this pest.

Materials and Methods

The experiment was carried out in the ICAR-CICR Insect Biocontrol facilities in Nagpur, India. The following *Trichogramma* species were utilized: *Trichogramma achaeae* (Nagaraja), *Trichogramma pretiosum* (Riley), *Trichogramma chilonis* (Ishii), and *Trichogrammatoidea bactrae* (Nagaraja). The factitious host employed for maintaining the culture was Corcyra cephalonica. Host culture (*Pectinophora gossypiella*) was maintained on artificial diet recommended by Naik *et al*., 2017. Each species of *Trichogramma* was reared in glass vials as described by (Morison, 1970) at 27°C temperature and 65 per cent relative humidity was maintained.

Preliminary assays revealed that *P. gossypiella* eggs incubated for 4-5 days under laboratory conditions before hatching. Therefore, eggs aged 0, 1, 2, and 3 days were chosen for the study. Following the outlined procedure, mated females of each of the four *Trichogramma* species were initially introduced into separate replicated glass tubes, each containing a paper card with 100 pink bollworm eggs of a single age group (0-, 1-, 2-, or 3-day-old eggs). After allowing the female parasitoids to oviposit for 24 hours, they were removed. Eggs were then monitored for parasitization after being incubated for 8, 9, and 10 days, indicated by the darkening of the vitelline membrane. Since the eggs were not treated with UV, the number of host larvae hatching simultaneously was also recorded. Additionally, the eggs were inspected daily to observe the emergence of *Trichogramma* adults from the host eggs until no parasitoids emerged. Each *Trichogramma* species evaluated separately on various ages of pink bollworm eggs and was replicated four times. The parameters were calculated using the following equations.

$$\text{Per cent parasitization} = \frac{\text{Number of Parasitized eggs}}{\text{Total number of host eggs}} \times 100$$

$$\text{Per cent Adult emergence} = \frac{\text{Number of eggs with emergence holes}}{\text{Total number of parasitized eggs}} \times 100$$

Developmental time (d) = represents the number of days from parasitization to wasp emergence

Data analysis

The per cent parasitized eggs and adult emergence and the development time were analyzed by a two-way ANOVA with parasitoid species (five levels) and host egg age (four levels) as factors at P<0.05.

Results and Discussion

Parasitization of *Trichogramma* species on *P. gossypiella* eggs at various ages

Trichogramma species and host egg age both significantly impacted the parasitization. Also, the interaction of egg age and *Trichogramma* spp on the proportion of parasitized eggs found significant ($F_{3,12}$=6.004, P<0.001). *Trichogramma* species parasitization was significantly impacted by *P. gossypiella* egg age; as egg age grew, the % parasitization reduced.

T. chilonis had the highest parasitizationon 0-day-old eggs (72.25%) and parasitized fewer hosts with egg age increasing. *T. bactrae* had a similar parasitizationon 0, 1, 2 and 3-day-old eggs. *T. pretiosum* and *T. achaeae* significantly preferred 1-day-old eggs to older eggs. There were significant differences in *Trichogramma* parasitization on various eggs ages with an exception of

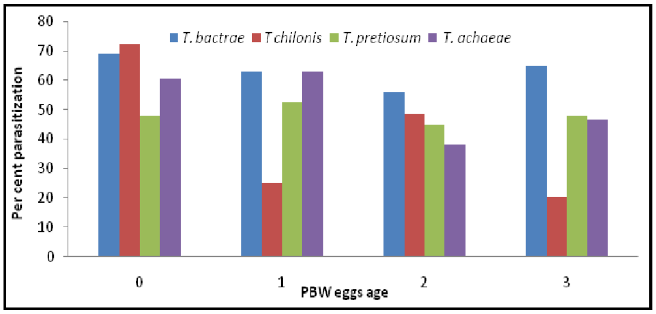

Fig. 1: Per cent of *P. gossypiella* eggs parasitized by the four *Trichogramma* species within 24 h at various ages.

0-day-old and 2-day-old eggs (0-day-old: $F_{3,12}$= 3.06, P=0.06; 1-day-old: $F_{3,12}$=15.42, P<0.001; 2-day-old: $F_{3,12}$ = 1.28, P= 0.32 and 3-day-old: $F_{3,12}$ = 8.37, P<0.01).

Values in parentheses are arc sin transformed

T. bactrae had the highest parasitization on all host egg ages. More specifically, there were 69.00, 63.00, 56.00, and 65.00 host eggs parasitized in 24 h on 0-, 1-, 2-, and 3-day-old eggs, respectively. In general, *T. chilonis* parasitized the least host eggs at all egg ages compared to the other *Trichogramma* species (Fig. 1).

Development of the *Trichogramma* species on *P. gossypiella* eggs at various ages

The PBW egg age, the *Trichogramma* species, and the interaction of these factors significantly affected the parasitoid developmental time (Table 2). The developmental time of all *Trichogramma* species except for *T. achaeae* increased with the host egg age. In addition, when parasitoid females parasitized older *P. gossypiella* eggs, the development time of offspring development was shorter. *T. chilonis* and *T. pretiosum* had a similar developmental time on all ages of host eggs. The developmental time of different *Trichogramma* species was significantly different on various host egg ages (0-day-old eggs: $F_{3,12}$=7.92, P<0.001; 1-day-old eggs: $F_{3,12}$=5.88, P<0.001; 2-day-old: $F_{3,12}$=8.54, P<0.001 and 3-day-oldeggs: $F_{3,12}$=1.32, P<0.001). At room temperature, the greatest developmental duration was recorded for *T. achaeae* (7.95 days), whereas *Trichogrammatoidea bactrae*, *T. chilonis*, and *T. pretiosum* took 7.25, 6.25, and 5.75 days, respectively. *T. achaeae* and *T. pretiosum* generally displayed the longest and shortest developmental times, respectively. There was highly significant difference found in the developmental stages of *Trichogramma* species on the pink bollworm eggs.

Adult Emergence of *Trichogramma* species on *P. gossypiella* eggs at various ages

PBW egg age and *Trichogramma* species had no significant effect on the Per cent of adult emergence.

Table 1: Results of the factorial ANOVA used to analyze (A) the numbers of *P. gossypiella* eggs parasitized (parasitization), (B) the Per cents of emergence of parasitoids from parasitized eggs, and (C) the developmental time of *Trichogramma* spp in *P. gossypiella* eggs. Main factors tested were the age of *P. gossypiella* eggs (‘‘Host egg age’’ factor) and best performing *Trichogramma* spp for control of *P. gossypiella*

Parameters	Source of variation	Df	F	P value
Per cent Parasitization	PS	3	6.99	<0.001
	HA	3	5.51	<0.01
	PS*HA	9	6.00	<0.001
	Error	48		
Per cent adult emergence	PS	3	1.26	0.29
	HA	3	0.53	0.66
	PS*HA	9	0.94	0.50
	Error	48		
Developmental period	PS	3	6.45	<0.001
	HA	3	1.95	<0.001
	PS*HA	9	8.05	<0.001
	Error	48		

*PS parasitoid species, HA host eggs

However, the interaction between egg age and *Trichogramma* species had no significant effect (Table 2).

The parasitoid emergence rate increased in younger *P. gossypiella* eggs that were 0–1-day-old. The pattern was not exactly the same when *P. gossypiella* eggs were 2–3-day-old, the emergence rate decreased with the increased PBW eggs ages except for *T. pretiosum* and *T. achaeae* where the emergence rate increased; the highest emergence rate (91.51 %) was observed for *Trichogrammatoidea bactrae*. Despite different patterns between the four host egg ages tested, the two factors ‘‘Parasitoid’’ and ‘‘Host egg age’’ did not interact significantly. No significant differences were found on the emergence Per cent of *T. bactrae*, *T. chilonis*, *T. pretiosum* and *T. achaeae* from 0- to 3-day-old host eggs (Table 2).

The per cent of parasitized eggs, the number of adult emergences, the development period of female progeny, and the suitability of *Trichogramma* species are typically used to evaluate parasitization and suitability (Pak 1986; Miura and; Kobayashi *et al.*, 1998; Monje *et al.*, 1999; Takada *et al.*, 2000; Zhang *et al.*, 2014; Song *et al.*, 2015). Furthermore, the availability and acceptance of parasitoids as hosts are known to be influenced by the host’s age (Vinson, 1976; Pak, 1986).

Four *Trichogramma* species were compared for

Table 2: Comparisons of development time and per cent of emergence of the four *Trichogramma* species on differently aged *P.gossypiella* eggs.

Parameters	**Species**	**PBW eggs age (day)**			
		0	**1**	**2**	**3**
Developmental period (day)	*T. bactrae*	8 ± 0^{b}	9 ± 0^{d}	6 ± 0^{b}	6 ± 0^{b}
	T. chilonis	6 ± 0^{a}	7 ± 0^{b}	6 ± 0^{b}	6 ± 0^{b}
	T. pretiosum	6 ± 0^{a}	6 ± 0^{a}	6 ± 0^{a}	5 ± 0^{a}
	T. achaeae	8 ± 0^{b}	7 ± 0^{c}	8.75 ± 0.25^{c}	7 ± 0^{c}
Adult Emergence (%)	*T. bactrae*	90.45±5.51(77.03)	95.90±1.36(79.85)	93.48±3.79(79.42)	86.19±13.80(77.99)
	T. chilonis	86.20±7.22(71.74)	83.34±13.37(71.45)	75.23±9.80(63.91)	64.83±7.48(53.85)
	T. pretiosum	84.38±6.54(67.77)	71.31±11.62(58.82)	91.88±2.89(75.64)	84.03±9.10(70.71)
	T. achaeae	91.51±3.14(75.35)	76.26±15.64(63.53)	80.94±11.40(70.97)	86.97±4.68(71.61)

Mean± SE are presented. Means in a column followed by the same lower case letter and means in a row followed by the same upper case letter do not differ significantly (P<0.05).

parasitization, adult emergence, and developing period on *P. gossypiella* eggs at varying ages. The results showed that *T. bactrae* had a higher rate of parasitization and a shorter developmental time than the other *Trichogramma* species. Moreover, *T. bactrae* had the greatest number of adults that emerged from parasitized *P. gossypiella* eggs at ages of 0-2 days. Out of all the examined *Trichogramma* species, *T. bactrae* had the most potential for suppressing *P. gossypiella*.

Three-day-old host eggs were found to be less parasitized than younger eggs, which is consistent with the theory that host egg age influences the parasitization rate of *Trichogramma* parasitoids (Pizzol, 2004). The outcomes of this study are consistent with those of other research (Brand *et al*., 1984; Calvin *et al*., 1997; Pak *et al*., 1986; Moreno *et al*., 2009). According to our research, all four of the native *Trichogramma* species are able to parasitize the youngest eggs, and they all accepted the *P. gossypiella* eggs at all ages. It appears that *T. bactrae*, *T. chilonis*, *T. pretiosum*, and *T. achaeae* were better suited to parasitize younger *P. gossypiella* eggs because they only demonstrated increased parasitization efficiency on 0 to 1-day-old eggs. Malik's (2001) findings, which indicated that *Trichogrammatoidea bactrae* could parasitize up to 95.71% of pink bollworm eggs which is in support to the parasitization rate (56-69%) in our study. The previous investigation revealed that *T. bactrae* had a strong parasitization potential and can parasitize 24 hours old eggs, or one day old eggs (87.66%), followed by 48 hours old eggs (73.50%), and the lowest Per cent was found in 72 hours old eggs (58.33%) (Asha *et al*., 2019). However, we found that *T. bactrae* apparently showed a relative low acceptance on 48 hours (56%) old eggs. *T. achaeae* exhibited good parasitic ability on all aged eggs of *P. gossypiella*, especial for 0 to 1-day-old eggs with a parasitization rate of 60.75 and 63 respectively suggesting that it was less adopted to old eggs. *T. chilonis* exhibited good parasitic ability on 0-day-old eggs with a parasitization rate of 72.25 per cent. Hou *et al*., 2018 also found that *T. chilonis* exhibited similar preference for 0-2-day old *M. separata* eggs to 3-day-old eggs, suggesting that it was less adapted to old eggs.

Trichogramma parasitoids inject venom at the time of stinging, which eventually inhibits the developing host embryo from reaching adulthood, although they do not oviposit on host eggs older than 4-5 days (Benoit and Voegele, 1979). If older *P. gossypiella* eggs do not allow the host embryo to successfully develop, this could account for the reduced parasitization success in those eggs, leading to the collapse of the host egg.

According to Godfray (1994) and Vinson (1998), parasitoid females are known to evaluate the hosts they come into contact with. Their behavior is determined by cues they detect, particularly during ovipositor probing, or at the moment of stinging, though other mechanisms might also be at play (Outreman *et al*., 2001; Desneux *et al*., 2009a). It's possible that older eggs were rejected by *Trichogramma* females due to possible competition with host embryos that were already developed. Even though a parasitoid egg was deposited as a result of the probing, Asgari and Rivers (2011) report that the venom injected during the stinging likely caused the death of all probed host eggs, as has previously been documented in *Trichogramma* parasitoid species (Benoit and Voegele, 1979; Klomp *et al*., 1980). Further studies would have to be carried out to clarify this point.

For some parasitoids, the combination of host species and age may affect host selection (Vinson, 1976; Pak, 1986; Zhang *et al*., 2014). According to Pizzol *et al*., (2012), the *Trichogramma* species in the earlier experiments show varying acceptability for host eggs at varying ages. As a case study, *T. chilonis* favored eggs that were intermediate in age (1-2 days old) (Pak, 1986; Miura and Kobayashi, 1998), while our findings showed that *T. chilonis* preferred *P. gossypiella* eggs that were 0 days old (Miura and Kobayashi, 1998). These variations imply that some parasitoids may pick their hosts based on the interaction between the host's age and species. Budhwant *et al*., (2008) also reported that fresh 24-hour-old eggs (60.3%) had the best efficiency of *Trichogramma chilonis* Ishii against lepidopteran pests, followed by 48-hour-old eggs (49.5%) and 72-hour-old eggs (37.3%). It is consistent with the current study's findings that *T. chilonis* had the highest Per cent of parasitization at 0-day-old eggs (72.25%).

In this study, however, all *Trichogramma* species preferred to parasitize younger *P. gossypiella* eggs. This preference may lead to increase their offspring's survival or easily parasitize hosts. Pak (1986); and Honda and Luck (2000) reported that the first 75% of embryological development of the host egg was suitable for *Trichogramma* larvae development, although the pattern of vulnerability with host age varies among species. In our study, the four *Trichogramma* species had a lower Per cent of egg parasitized on 3-day-old *P. gossypiella* eggs. The possible mechanism underlying these phenomena is the differences in host quality associated with host egg age and the increasing defense capacity with the development of host egg (Mattiacci and Dicke, 1995).

Our results showed that *Trichogramma* species had high parasitization and good suitability on 0-1-day-old *P. gossypiella* eggs, and equal or more than one *Trichogramma* adults emerged from a single *P. gossypiella* egg. Our laboratory study demonstrated the *P. gossypiella* egg ages could affect the parasitization efficiency of the four *Trichogramma* species, providing valuable information for selecting suitable species to control *P. gossypiella*. In general, *Trichogrammatoidea bactrae* exhibits the highest parasitization capacity and suitability on all egg ages, and suites the best in controlling *P. gossypiella*. Practically, suitability is based on the ability of parasitoid to search for host eggs, the parasitoid quality, and the adaptability to pesticides and environment. Further research will be carried out under field conditions to measure the concrete indexes ensuring effective and practical biological control strategy.

Acknowledgement

The authors extend their heartfelt appreciation to Dr.Panjabrao Deshmukh Krishi Vidyapeeth and ICAR-Central Institute for Cotton Research for the invaluable support provided throughout their work. Their assistance, guidance, and resources have been instrumental in the successful completion of this project.

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