

***Trichogrammatoidea bactrae* (Hymenoptera: Trichogrammatidae): A Potential Biocontrol Agent of Rice Leaf Folder**

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ABSTRACT. *Rice leaf folder, Cnaphalocrocis medinalis is a major pest, in many rice growing areas of Sri Lanka. Biological control of this pest is encouraged due to ill effects of insecticide application on rice ecosystem. Use of egg parasitoids is advantageous over larval and pupal parasitoids, as they could destroy pests at the egg stage. Trichogrammatoidea bactrae present in rice fields in Sri Lanka, is an egg parasitoid of rice leaf folder. This study was conducted to assess the potential of T. bactrae as a biocontrol agent for the management of rice leaf folder. T. bactrae was reared on Coccyra cephalonica and its fecundity, longevity and parasitism were assessed. Acceptance of stored host eggs and parasitoid emergence from stored parasitized eggs were examined. Dispersal ability of parasitoid was also examined. T. bactrae adults showed a mean longevity of 7.9 days (range 1-15 days) and laid 55 eggs /female during first 5 days; of which, 56 % of eggs was laid on the first day. There was a significant reduction ($F=5.26$; $df=14$; 89 ; $P < 0.001$) in parasitism, 66-35 %, with increasing number of generations on C. cephalonica. Parasitism steadily decreased as the number of generations increased. Acceptance of C. cephalonica eggs varied significantly with temperature ($F=187$ $df=1$; 126 $P<0.001$). Mean acceptance at 4 and 8 °C was 30 and 42 % respectively. Acceptance also significantly varied with the duration stored ($F=385$; $df=6$; 126 ; $P<0.001$). Parasitized eggs of T. bactrae could be successfully stored under low temperature (4 °C) up to 40 d. Over 80 % parasitism was achieved with 150 parasitoids released within 25 cm radius circle. Hence, T. bactrae is a promising candidate to be promoted as a biocontrol agent of C. medinalis.*

Keywords: Dispersal, fecundity, longevity, parasitization, Trichogrammatoidea bactrae

INTRODUCTION

Rice leaf folder (RLF), *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) is considered as one of the major rice leaf feeding insect in many parts of Sri Lanka (Nugaliyadde *et al.*, 2000). RLF females lay eggs singly or in small clusters on the leaf blade of rice plants. After four days, egg hatch and larvae emerge (Ranasinghe, 1992). Damage due to defoliation caused by RLF larvae demands the implementation of control measures, usually application of insecticides. This practice adversely affects the colonizing natural enemies in rice ecosystems (Roger *et al.*, 1991; Lawler, 2001). Natural enemy community in rice ecosystem has a greater impact on other pest species of rice as well (Barrion and Litsinger, 1994; Ooi and Shepard, 1994; Heong *et al.*, 2005). Hence, use of non-chemical

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strategies to manage RLF has been considered to enhance the colonization of natural enemy community in the rice ecosystem.

Of the non chemical strategies, biological control of RLF using egg parasitoids is the most promising. Use of larval and pupal parasitoids to manage rice leaf folder has practical limitations. RLF larvae have already damaged the rice foliage when larval parasitoids begin to destroy the larvae. In contrast, egg parasitoids destroy the RLF at the egg stage, prior to leaf damage by larvae. *Trichogrammatoidea bactrae* Nagaraja has been identified as a naturally occurring egg parasitoid that can be used against RLF (Hemachandra *et al.*, 2014). The potential of this egg parasitoid in the control of RLF has not been properly investigated.

Success of a biocontrol programme depends much on the biology of the biocontrol agent and the convenience of rearing of the parasitoids on surrogate host eggs to produce adequate numbers for field release. Therefore, the objective of this study was to assess *Trichogrammatoidea bactrae* as a potential biocontrol agent. Its fecundity, longevity, dispersal ability of adults in searching of host eggs, effects of number of generations of parasitoids on parasitization capacity, acceptance of cold stored eggs for parasitization were investigated.

METHODOLOGY

This study was conducted based in the laboratories of the Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya from November, 2011 to 2013. The egg parasitoid, *T. bactrae* was reared on *Cocyrta cephalonica* eggs to obtain parasitoids for experimentation adopting the rearing protocol of Nathan *et al.* (2006). *C. cephalonica* was reared on crushed corn as described by Sreekumar and Paul (2000).

Assessment of fecundity of *T. bactrae*

To determine the number eggs laid by a female parasitoid in its lifetime (fecundity), newly emerged female parasitoids were held singly in separate plastic vial (2.5 cm dia. and 5.5 cm height) containing 80, one day old *C. cephalonica* eggs, prepared as an egg card. A drop of undiluted bees honey was placed on the inner surface of the lid of each vial. Vials were held at room temperature. After every 24 h, parasitized egg card were replaced with fresh egg cards bearing 80 *C. cephalonica* eggs. This procedure was continued until no oviposition was observed. The parasitized egg cards were observed daily and number of parasitized eggs (those turned black) was counted. Twenty five females were used in this experiment.

Assessment of longevity of *T. bactrae*

To determine the longevity of *T. bactrae*, newly emerged 100 adult parasitoids were held singly in clear vials. Adults were fed with 50 % diluted bees honey on a filter paper. Vials were maintained at room temperature (28 °C) and examined daily for dead insects. Dead insects were removed and a count was made.

Effect of parasitization capacities of *T. batrae* with varying generations of *C. cephalonica* eggs

It has been reported that egg parasitoids lose their egg laying capacity when they are reared continuously on alternative host eggs. Relevance of this effect for *T. batrae* was investigated. Newly emerged (one day old) female parasitoids were placed singly in separate plastic vials (2.5 cm dia. and 5.5 cm height) containing a one day old egg card of *C. cephalonica*. Each egg card had 100 *C. cephalonica* eggs. Undiluted bees honey was provided as food. The experiment was replicated with nine female parasitoids. After five days, number of parasitized eggs was counted. One day old adult females that emerged from parasitized eggs were held with a one day old *C. cephalonica* egg card and the number of parasitized eggs was counted. The same procedure was followed for fifteen generations. Mean parasitism level was evaluated for each generation.

Acceptance of cold-stored *C. cephalonica* eggs for parasitization by *T. batrae*

In mass rearing, when there is an oversupply of eggs, it is useful to store excess eggs for later parasitization. Suitability of such eggs was tested by storing *C. cephalonica* eggs at 4 and 8 °C for different durations: 1, 3, 7, 10, 20, 30, and 40 days and exposing the eggs for parasitization by *T. batrae*. Acceptance of host eggs was evaluated in terms of level of parasitism by *T. batrae*. The methodology followed was adapted from Nadeem *et al.* (2010).

Effect of storage of parasitized eggs at low temperature on emergence of *T. batrae*

To examine the effect of storing parasitized eggs, 2,500 *C. cephalonica* eggs parasitized by *T. batrae* were held at 4 and 8 °C for different durations and examined the adult emergence. Egg-cards (1 x 2 cm), bearing 60 eggs per card, held for 1, 3, 7, 10, 30, 40, 50 and 60 days in storage (4 and 8 °C) were held for at room temperature for emergence of adults. Numbers of parasitoids emerged were counted and recorded.

Dispersal ability of *T. batrae*

To determine the dispersal ability of parasitoid, the method of Ayvaz *et al.* (2008) was adopted. In a plant house, potted rice plants were arranged to simulate the plant arrangements in a rice field. Egg cards having *C. cephalonica* eggs were placed in concentric circles at distances of 0, 5, 25, 50, 75, 100, 125, 150 cm. Only one circle was used at a time, starting from 0 and continued up to 150 cm.

The same experimental procedure was used to examine the relationship between dispersal and released parasitoid density. A similar plant set up was used and the egg cards were placed in a circle of 150 cm radius, and the level of parasitism in relation to release rates was assessed.

The data from above experiments were analyzed using Chi square tests when data were non-parametric and ANOVA was used for parametric data with SYSTAT Ver. 11.

RESULTS AND DISCUSSION

Assessment of the fecundity of *T. bactrae*

Trichogrammatoidea bactrae oviposited over a period of five days, at a decreasing rate. The rate of oviposition significantly varied with the age of the parasitoids. One day old parasitoids had the highest oviposition rate (31.25 eggs per female per day), and it was 56.05 % of total fecundity. The mean number of eggs from second to fifth day was 13.5 ± 0.5 , 7.9 ± 0.6 , 2.9 ± 0.3 and 0.4 ± 0.2 respectively. These represent 24, 14, 5 and 0.7 % of the total fecundity. These findings agree with Naranjo (1993) who reported that *T. bactrae* has higher rate of oviposition very early in its adult life. Presence of relatively higher rate of oviposition among newly emerged females on release is a significant positive feature of a successful biological control agent. In addition, this feature is of convenience in mass rearing. Mean fecundity of *T. bactrae* was 55.75 eggs per female. Our findings on mean fecundity of *T. bactrae* were similar (55 eggs per female) to those of Naranjo (1993). Fecundity of *T. bactrae* could change with the type of host eggs. Fecundity of *T. bactrae* was 48.9 eggs/female when *T. absoluta* was used and 39 when *S. cerealella* was used as the host (Virgala and Botto, 2010). *T. bactrae* shows lower fecundity compared with other trichogrammatid egg parasitoid species. *Trichogramma chilonis* and *T. ostriniae* show a fecundity 135.6 and 117.5 eggs per female at 28°C (Hirashima *et al.*, 1990). *Trichogramma minutum* lays 236.8 eggs per female at 25 °C when *Cydia pomonella*, the natural host is used (Yu *et al.*, 1984).

Assessment of the longevity of *T. bactrae*

The longevity of *T. bactrae* varied from 1-15 days with mean longevity of 7.9 ± 0.37 days, when fed on 50 % bees honey. Longevity was 6.2 on *T. absoluta* and 6.1 d on *S. cerealella* (Virgala and Botto, 2010). Longevity of *T. bactrae* is comparatively shorter than in other egg parasitoid species. *Trichogramma minutum* adults have longevity of 28.5 ± 5.4 days when fed on 40 % bees honey (Leatemia *et al.*, 1995). This difference may be associated with the species used or environmental conditions under which experiments were conducted. However, effect of longevity appears to be less in *T. bactrae* because it lays 56 % of its egg load within the first day when hosts eggs are provided. On the other hand, high longevity provides an opportunity to the adult female to select the most suitable eggs for laying eggs which has an effect on the biological fitness of the offspring.

Effect of parasitization capacities of *T. bactrae* with varying generations of *C. cephalonica* eggs

There was a significant variation ($F=5.26$ $df=14$; $89 P < 0.001$) in the level of parasitism with increasing generations, on the progeny numbers. The ability for parasitization in *T. bactrae* was reduced when the progeny continued on alternative hosts (Bueno *et al.*, 2006). For some unexplainable reason, level of parasitism recorded in generation one was low (35.5 %). Some parasitoids did not oviposit at all. On exclusion of those, a significant variation in parasitism was recorded with increasing generations (Figure 1). Parasitism levels steadily decreased from 66 to 35 %, 2nd to 15th generations. Similar results on parasitism have been reported by Hopper *et al.* (1993) and it was thought to be associated with genetic changes in the colony or adaptation to lab conditions.

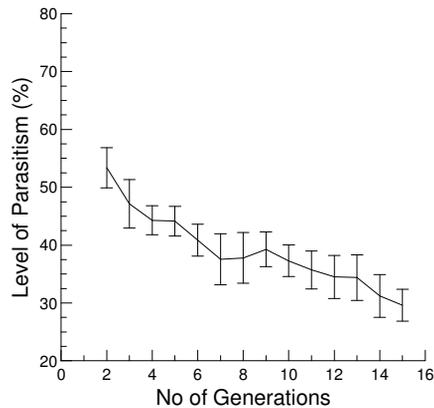


Fig. 1. Variation in level of parasitism in *T. batrae* with number of generations when reared on *C. cephalonica* eggs, under laboratory conditions.

Certain other egg parasitoids have shown a much higher potential for rearing on alternate host over many generations without losing the quality: level of parasitization, sex ratio, and longevity. Bueno *et al.* (2006) reported that *Trichogramma pretiosum* when reared on *Anagasta kuehniella* eggs over 100 generations were not affected until the 20th generation. The quality of *T. pretiosum* can be reestablished using the natural host even after 70th generations. In this study, *T. batrae* regained the former quality when it was reared on the natural host, *C. medinalis*. Quality of the egg parasitoids is very important as it affects its efficacy of suppressing the pest population. On the other hand, ability to mass rear while keeping the quality is important, especially, when it is intended to be used in augmentation and release programmes.

Acceptance of cold-stored *C. cephalonica* eggs for parasitization by *T. batrae*

Level of acceptance of host eggs significantly varied with temperature ($F=187$ $df=1$; 126 $P<0.001$) and duration of storage ($F=385$; $df=6$; 126; $P<0.001$). In addition, the interaction of temperature x duration ($F=2.3$; $df=6$; 126; $P=0.035$) was not significant at 1%. Overall, acceptance of *C. cephalonica* eggs stored durations significantly varied with temperature ($F=10.5$; $df=1$; 138; $P<0.001$). At 8 °C, mean acceptance of host eggs was 41.5 ± 2.4 and 30.1 ± 2.4 at 4 °C. Overall acceptance of *C. cephalonica* eggs stored at 4 and 8 °C, significantly varied with the storage duration ($F=156.3$; $df=6$; 133; $P<0.001$). Level of acceptance steadily decreased with the increasing storage duration (Figure 2).

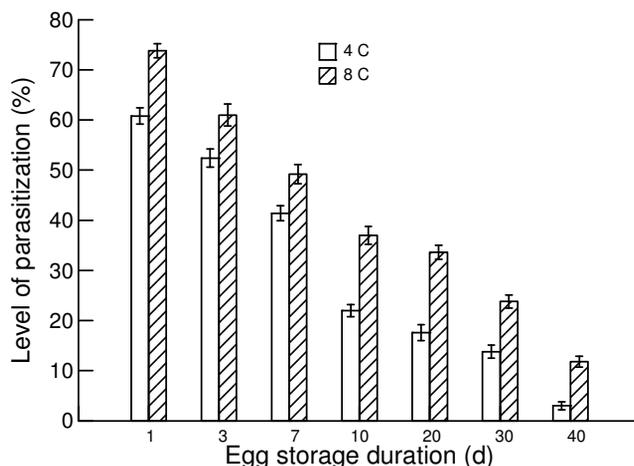


Fig. 2. Parasitization of *Cocyra cephalonica* eggs by *T. batrae* following storage at 4°C and 8°C for different durations.

Clain *et al.* (2008) reported that *T. chilonis* accepted stored eggs for 9 weeks for parasitization and they recommend storing eggs up to 7th week during mass rearing of *T. chilonis*. Acceptance of cold storage eggs is important to meet the fluctuating demand for adult parasitoids in biocontrol programmes.

Effect of storage durations of parasitized eggs at low temperature on emergence of *T. batrae*

Parasitized eggs were stored at low temperatures in order to extend the shelf-life of the parasitized eggs. This is particularly important when the demand for parasitoids is variable. The level of parasitoid emergence significantly varied with storage temperature ($F=24.5$; $df=1$; 56 ; $P<0.001$) and duration ($F=140.7$; $df=6$; 56 ; $P<0.001$). The interaction of temperature and duration ($F=0.859$; $df=6$; 56 ; $P=0.531$) was not significantly different.

Parasitoid emergence of across all egg storage durations at 4 °C was $37.6\pm 2.3\%$ while that of at 8 °C was 41.9 ± 2.2 . Parasitoid emergence significantly varied with different egg storage durations and across the two storage temperatures ($F=103.5$; $df=6$; 63 ; $P<0.001$). The highest adult emergence was recorded from one day of storage ($53.8\pm 1.9\%$) at 8 °C and the lowest at 40 d storage (9.8 ± 0.9 %) at 4 °C. When adult emergence was considered separately for temperatures 4 and 8 °C, it was steadily decrease with increasing stored durations (Figure 3).

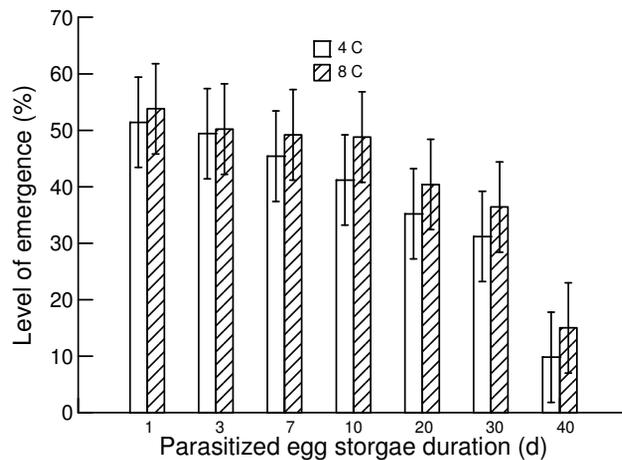


Fig. 3. Emergence of *T. bactrae* adults from parasitized *Cocyza* eggs stored at 4 and 8°C, for different durations.

Kumar *et al.* (2005) have conducted a similar study and found that adult emergence of trichogrammatids was very high (95.6%) after one day of storage. Similar results have been obtained for other egg parasitoid species. Eggs parasitized by *T. chilonis* can be stored up to 30 days without a loss in emergence rate (72.4%) (Pathak *et al.*, 2010). Similarly, eggs parasitized by *Anaphes iole* Girault (Hymenoptera: Mymaridae) can be stored up to 20 days at 10 °C when parasitoids are at the pupal stage within the host eggs (Abdullah *et al.*, 2009; Ramos and Cate, 1992).

Dispersal ability of *T. bactrae*

C. cephalonica eggs placed at different distance were parasitized and the level of parasitism varied significantly with the distance ($F=1623$; $df=7$; 792 ; $p<0.001$). *T. Bactrae* successfully reached 25 cm distance but the number reaching beyond 25 cm was significantly dropped. However, even the egg cards kept at 150 cm from point of release had been parasitized, indicating that few insects were capable of reaching 150 cm distance (Figure 4).

When a higher number of parasitoids were released, increasing numbers of insects dispersed over long distance. When egg cards were kept at a distance of 150 cm, over 80% of host eggs were parasitized upon the release of 140 egg parasitoids. Findings of these two experiments indicated that parasitoids dispersal is dose dependent (Figure 4 and 5) phenomenon. This idea is in agreement with Ayvaz *et al.* (2008), who reported that, when 1,000 *Trichogramma evanescens* were released, parasitism of host eggs was 11.41 %, which increased to 29.75 % and 62.06 % when 2000 and 3000 parasitoids were released respectively.

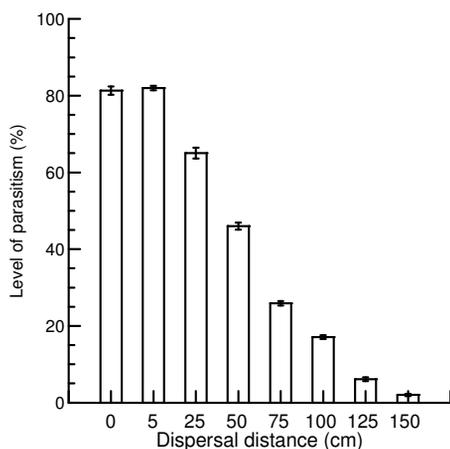


Fig. 4. Dispersal distance of *T. bactrae* in experimental arena and level of parasitism of host eggs

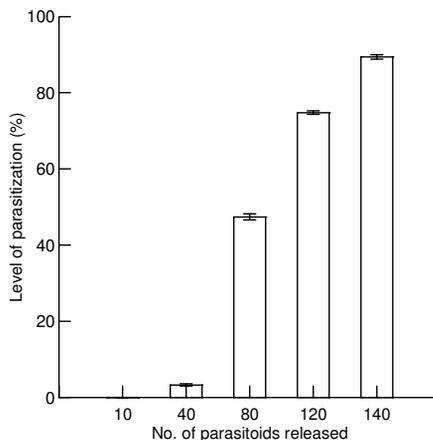


Fig. 5. Release rate of parasitoids and level of parasitism of host eggs cards at 150 cm radius circle in a simulated rice field.

CONCLUSIONS

On the average *Trichogrammatoidea bactrae* lives 7.9 ± 0.37 days and lays 55 eggs/female over 5 days, depositing 56 % of egg load within the first day. *T. bactrae* parasitized *C. cephalonica* eggs stored at low temperature (4 and 8 °C) and parasitized eggs also can be stored at low temperature resulting in successful parasitoid emergence. These characters of *T. bactrae* are conducive for mass rearing. Hence, it can be concluded that *T. bactrae* can be easily mass cultured which is an essential feature of a biocontrol agent. *T. bactrae* is also capable of dispersing over 150 cm. In order to parasitize host eggs within 150 cm radius releasing, 150 parasitoids are required to achieve >80 % parasitism. Based on these findings, it can be concluded that *T. bactrae* is a promising candidate to be used as an effective biocontrol agent in augmentation releases in the management of rice leaf folder.

ACKNOWLEDGEMENT

National Research Council (NRC) of Sri Lanka is acknowledged for financial assistance (Grant No: 11-116).

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