

Trichogramma japonicum is periodically colonised in summer low-land rice (*Oryza sativa*) to biocontrol the yellow stem borer (*Scirpophaga incertulas*).

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ABSTRACT

A significant pest of low-land rice (*Oryza sativa* L.), yellow stem borer [*Scirpophaga incertulas* (Walker)] reduces yield by 30–40%. Inundative discharges of the egg parasitoid *Trichogramma japonicum* Ashmead are one of the biological control agents used to reduce yellow stem borer (YSB) populations and damage across Asia. To suppress YSB in summer low-land rice, however, information on the timing and quantity of parasitoid releases is missing. In order to standardise the timing and quantity of *T. japonicum* releases for efficient biocontrol of YSB, an experiment was carried out in the summer of 2018 and 2019 at ICAR-National Institute of Biotic Stress Management, Raipur, Chhattisgarh. Three distinct treatment schedules were used in the experiment: T1 (four releases of *T. japonicum* at weekly intervals on the 7th, 14th, 21st, and 28th days after planting (DAT); T2 (four releases of *T. japonicum* at weekly intervals on the 14th, 21st, 28th, and 35th DAT); T3 (four releases of *T. japonicum* at weekly intervals on the 21st, 28th, 35th, and 42nd DAT); and T4, Control (no release of wasps). In comparison to untreated control plots (T4), which had 16.8% dead heart and 3.4% white ear in the summer of 2018, the treatment schedule (T2), which included four releases of *T. japonicum* (@50,000 wasps/ha/release) at weekly intervals on the 14th, 21st, 28th, and 35th DAT of rice, significantly reduced the symptoms of YSB-induced damage. Similarly, in the summer of 2019, the therapy (T2) reduced white ear and dead heart by 46.15% and 43.35%, respectively, compared to the control. The higher grain output in (T2) from 2018–19, or 12.74–20.18% over the seasons, correlated with the notable decrease in YSB-induced damages. The findings imply that throughout the summer months in low-land rice, the (T2) augmentative releases provide ecologically feasible bio-control of YSB.

Keywords: *Trichogramma japonicum*, *Scirpophaga incertulas*, summer rice, periodic colonisation

Introduction

The main staple food crop in China, India, and other southeast Asian nations is rice (*Oryza sativa* L.). An estimated 104.3 million tonnes (2404 kg/ha) of rice are produced in India on 43.39 million hectares of farmed land (Annamalai et al. 2018). Yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) (Lepidoptera: Crambidae), is a significant lepidopteran borer pest that reduces low-land rice output by 30–40% a year. "Dead heart and white ear" are the signs of feeding damage to rice plants (Gangwar 2015). YSB control in rice is made more difficult by chemical pesticides' incapacity to reach the cryptic feeding places of YSB larvae (Kumar and Khan 2005) and the development of insecticide resistance as a result of applying the same chemical treatments year after year (Senthil-Nathan 2013).

IICAR-National Institute of Biotic Stress Management, Chhattisgarh, Raipur. *Corresponding author email: rcrkm2013@gmail.com *Trichogramma* spp. parasitoids. (Family: Trichogrammatidae) are known to target the eggs of over 200 insect pest species belonging to the orders Lepidoptera, Neuroptera, Diptera, Hemiptera, Coleoptera, and Hymenoptera, with a predilection for the Lepidoptera (Tanwar et al. 2006). According to Singh et al. (2013) and Mohapatra and Nayak (2015), sequential releases of 5–7 rounds of *Trichogramma* spp. parasitoids at varying densities ranging from 50,000–200,000 wasps/ha have been shown to increase grain yields while reducing rice plant damage (68.2%–78.8%) caused by Lepidoptera pests of low land rice in India.

The pest biology and ecology within the particular climatic circumstances influence the effectiveness of *Trichogramma* spp. (Cicero et al. 2017). As a result, the quantity and timing of *Trichogramma* spp. releases to control rice YSB have varied by region and nation. For each area to effectively reduce insect pests of rice, such

as the YSB, information on the optimal time for releases and the quantity of parasitoid releases must be determined (Kaur and Brar 2008).

The ideal period after planting and the quantity of *T. japonicum* parasitoid releases for successful YSB biocontrol are reported in this research.

METHODS AND MATERIALS

Rice moth (*Corcyra cephalonica* Stainton) mass culture: In the Trichogramma mass-production plant of ICAR-National Institute of Biotic Stress Management, Raipur, Chhattisgarh (21°22'56.9"N 81°49'29.6"E), the *C. cephalonica* was raised on broken pearl millet [*Pennisetum glaucum* (L.) R. Br.] grain-based medium. The freshly developed adult *C. cephalonica* were collected 35 days after inoculation and placed in oviposition cages of 27 cm in height and 28 cm in diameter. The cages had wire mesh on the bottom and lateral sides for ventilation. Adult meal consisted of a 50% honey solution. Every day, eggs were collected from the bottom of a blotting paper in a plastic tray and sieved to get rid of insect appendages and wing scales. Cleaned eggs were added to broken pearl millet grains in a plastic container (45 cm × 30 cm × 10 cm) at a rate of 18,000 to 20,000 eggs per 2.5 kg of grains. The mixture was then fortified with 10 g of yeast and covered with a tightly woven cotton fabric. The culture was carefully kept free of illnesses, red flour beetles, and storage mites by combining it with 5 g of wettable sulphur 80 wp and 0.5% *C. cephalonica* streptomycin sulphate.

Culture of egg parasitoid *Trichogramma japonicum*: The *T. japonicum* colony was allowed to parasitise irradiated 1-day-old eggs of *C. cephalonica* at the ICAR-National Institute of Biotic Stress Management's Trichogramma production plant in Raipur, Chhattisgarh. After being sterilised for 20 minutes under a UV light, the 1-day-old *C. cephalonica* eggs were adhered to white cardboard (4000 eggs on an 8 cm by 2 cm card) using 10% acacia gum. After being exposed to *T. japonicum* individually at 1:6 ratios, each egg card, which contained 18,000 to 20,000 eggs, was placed in a polythene bag (35 cm × 26 cm) and kept in a plastic tray (45 cm × 30 cm × 10 cm) until emergence (7–8 days after exposure). Trichogramma cultures were maintained at 70–80% relative humidity and $27 \pm 1^\circ\text{C}$. A 10% honey solution was given to the adults. On the eighth day after exposure to the card, adult parasitoids appeared.

Field assessment of several *T. japonicum* delivery schedules against rice yellow stem borer: At the ICAR-National Institute of Biotic Stress Management research farm in Raipur, Chhattisgarh, three release schedules of parasitoid wasps, each consisting of four releases of wasps at a weekly interval for four weeks, were assessed under field conditions in rice (cv. MTU 1010) against YSB during the summer of 2018 and 2019. Three schedules were used to release the parasitoid wasps: T1 (four releases of *T. japonicum* at weekly intervals on the 7th, 14th, 21st, and 28th days after planting (DAT); T2 (four releases of *T. japonicum* at weekly intervals on the 14th, 21st, 28th, and 35th DAT); T3 (four releases of *T. japonicum* at weekly intervals on the 21st, 28th, 35th, and 42nd DAT); and T4, Control (no release of wasps).

The transfer of the egg parasitoid *T. japonicum* from one replication to another inside the treatment cannot be controlled since it is continuously moving through the environment. As a result, a 100 m² plot was designated as the test plot area for every treatment. Five plots, each measuring 20 m², were chosen at random within the treatment. Each plot and sub-plot's percentage of dead heart and white ear was recorded (randomly in each sub-plot's five hills, each measuring 20 m²) and was regarded as a single replication. A discharge of 500 wasps per 100 m² was given to each treatment. An untreated check (control) was a treatment (100 m²) in which no wasps were discharged. Tricho cards with 500 7-day-old parasitised *Corcyra* eggs were fastened to the tip of each release station and raised to the height of the plant canopy. These cards are expected to generate adult parasitoids the next day. Throughout the study, no pesticide was used. Wasps were prevented from moving from one treatment plot to another by using an isolation gap of 15 m between two treatments. White ear was noted on the 90th DAT, but the percentage of dead heart was noted on the 21st, 28th, 35th, 42nd, 49th, and 56th DAT. The white ear measurement was taken either at the physiological maturity stage or close to harvesting. The yield was measured and reported in tonnes of grains per hectare. In a similar vein, the experiment was conducted again in the summer of 2019 using the same technique.

Statistical analysis: The percentages of dead heart and white ear from field tests carried out in the summers of 2018 and 2019 were examined independently. ANOVA was performed on the dead heart and white ear data following the arcsine transformation. Prior to analysis, the yield values were converted using the square root. Tukey (HSD) was used to separate the means for multiple comparison.

OUTCOMES AND TALK

Evaluation of *T. japonicum* in the field against rice yellow stem borer: The release timetable (T2) for the four

releases of

In low land rice (cv. MTU 1010), *T. japonicum* at a density of 50,000 wasps/ha/release at weekly intervals, starting on the 14th DAT and continuing sequentially by the 21st, 28th, and 35th DAT, reduced dead heart symptoms by 45.83% (9.1% dead heart) compared to the control plots (6.8% dead heart) during summer 2018 (Table 1). The effectiveness of *T. japonicum* released in accordance with the T2 schedule (14th DAT) differed markedly from the other release dates, which began on either the 7th DAT (T1) or the 21st DAT (T3), with 11.3% and 12.3% dead heart symptoms, respectively (Table 1).

In contrast to all other treatments ($F_{3,19} = 90.91$; $P < 0.001$) (T1, 6.7% dead heart) and (T3, 7.7% dead heart) scheduled releases, which had 11.2% dead heart in the control (T4), the (T2) second schedule of releases had the lowest percentage of dead heart symptoms (5.4%) (Table 1). On the 28th, 35th, 42nd, 49th, and 56th DAT, the treatment (T2) continued to be significantly at the lowest for dead heart, with results of 9.1% ($F_{3,19} = 365.48$; $P < 0.001$), 12.3% ($F_{3,19} = 184.72$; $P < 0.001$), 8.0% ($F_{3,19} = 97.24$; $P < 0.001$), 9.9% ($F_{3,19} = 82.83$; $P < 0.001$), and 9.8%

Table 1 Effect of split release of *Trichogramma japonicum* on per cent dead heart by YSB in low-land rice during summer 2018

Treatment	Per cent dead heart						Mean	Per reduction cent over control
	21 DAT	28 DAT	35 DAT	42 DAT	49 DAT	56 DAT		
T1	6.7b	11.2b	17.3b	9.6b	11.3b	12.6b	11.3	32.74
T2	5.4a	9.1a	12.3a	8.0a	9.9a	9.8a	9.1	45.83
T3	7.7c	14.6c	16.8b	9.2b	11.9b	13.4c	12.3	26.79
T4	11.2d	20.4d	23.3c	13.2c	15.2c	17.4d	16.8	-
SE(d)	0.387	0.205	0.355	0.299	0.302	0.281	-	-
CD ($P < 0.01\%$)	1.129	0.599	1.084	0.914	0.923	0.859	-	-

Details of the treatment are provided under Materials and Methods. DAT stands for days after planting. *Mean of three replications; ANOVA was performed on the means after an arcsine transformation; Tukey's test revealed no significant difference ($P < 0.01\%$) between the means followed by the same letter or letters.

($F_{3,19} = 38.54$; $P < 0.001$), in contrast to the control group's 20.4%, 23.3%, 13.2%, 15.2%, and 17.4% (Table 1).

In comparison to all other treatment schedules T1 (2.3% white ear; 32.35% reduction over control), T3 (1.5% white ear; 55.88% reduction over control), or the untreated control (3.4%), treatment schedule (T2) beginning on the 14th DAT significantly suppressed YSB damage, resulting in the lowest white ear symptom of 1.8% (47.05% reduction over control) at 90th DAT (Table 2). The treatment schedule (T2) reduced the symptoms of YSB-induced damage, resulting in the maximum yield of 6.9 t/ha ($F_{3,19} = 17.89$; $P < 0.001$), compared to 5.4 t/ha in the untreated control (Table 2).

In contrast to the untreated control (16.1% dead heart), the treatment schedule T2 significantly reduced the YSB-induced dead heart symptom during the summer of 2019 with a reduction of 43.35% over control (9.1% dead heart) (Table 3). The release schedules T1 (11.2% dead heart; 30.43% reduction over control) and T3 (11.9% dead heart; 26.09% reduction over control).

Table 2 Effect of split release of *Trichogramma japonicum* on per cent white ear by YSB and yield in low-land rice during summer 2018

Treatment	Per cent white ear 90 DAT	Per cent reduction over control	Yield (t/ha)	Per cent increase over control
T1	2.3b	32.35	6.1b	11.48
T2	1.8a	47.05	6.9a	21.74
T3	1.5a	55.88	6.5ab	16.92
T4	3.4c	-	5.4c	-

SE(d)	0.407	-	0.043	-
CD	1.243	-	0.132	-
(P<0.01%)				

Details of the treatment are provided under Materials and Methods. DAT stands for days following transplantation. *Mean of three replications; ANOVA was performed on the white ear means following arcsine transformation; ANOVA was performed on the yield means following square root transformation; Tukey's test revealed no significant difference (P<0.01%) between the means followed by the same letter or letters.

In comparison to either the commencement of release on the 7th DAT (7.4% dead heart) or the 21st DAT (8.7% dead heart) throughout summer 2019, the T2 schedule of releases, commencing on the 14th DAT, considerably reduced the YSB-induced symptom, resulting in the lowest dead heart of 6.2%. The results for the next period of measurements followed the same pattern, with T2 showing a significant decrease in dead heart, with the lowest percentages being 6.2% (F3,19 = 2945.56; P<0.001), 5.8% (F3,19 = 1151.08; P<0.001), 8.1% (F3,19 = 3685.11; P<0.001), 11.1% (F3,19 = 7733.64; P<0.001), 11.7% (F3,19 = 38.54; P<0.001), and 11.8% (F3,19 = 4339.04; P<0.001).

In comparison to the other treatment schedules of T1 (2.7% white ear; 30.77% reduction over control), T3 (2.9% white ear; 25.64% reduction over control), and untreated control (3.9% white ear), the treatment schedule T2 had the lowest YSB-induced white ear on the 90th DAT (2.1% white ear; 46.15% reduction over control) (F3,19 = 409.47; P<0.001). In comparison to the control (5.26 t/ha), T2 enhanced rice grain production (F3,19 = 16.26; P<0.001), yielding the greatest yield of 6.59 t/ha (20.18% increase over control) (Table 4).

In comparison to the summer of 2018, the incidence of YSB in terms of the development of dead heart symptoms was somewhat lower in 2019, and the opposite was true for white ear. The split releases of parasitoids, which started on the 14th DAT (T2) and were followed sequentially by the 21st, 28th, and 35th DAT at weekly intervals, were the best times for releases and the total number of releases to significantly reduce YSB feeding damage compared to control plots, according to results regarding dead heart, white ear, and yield that were concurrent across both seasons. According to Deshpande et al. (2023), the decrease of YSB feeding damage symptoms under the periodic releases of *T. japonicum* maximum enhanced grain output in T2 compared to other release schedules (T1 and T2) and untreated control in both seasons (Summer 2018 and 2019).

The total density requirement of *Trichogramma japonicum*/ha was determined using a biological approach.

Table 3 Effect of split release of *Trichogramma japonicum* on per cent dead heart by YSB in low land rice during summer 2019

Treatment	Per cent dead Heart						Mean	Per cent reduction over control
	21 DAT	28 DAT	35 DAT	42 DAT	49 DAT	56 DAT		
T1	7.4b	7.4b	11.6b	12.9b	12.6ab	15.0b	11.2	30.43
T2	6.2a	5.8a	8.1a	11.1a	11.7a	11.8a	9.1	43.35
T3	8.7c	9.6c	11.2c	12.4c	13.2b	16.1c	11.9	26.09
T4	12.6d	13.4d	15.4d	17.7d	16.8c	20.6d	16.1	-
SE(d)	0.071	0.134	0.062	0.038	0.413	0.062	-	-
CD (P<0.01%)	0.216	0.411	0.189	0.115	1.261	0.188	-	-

Details of the treatment are provided under Materials and Methods. DAT stands for days following transplantation.

*Mean of three replications; ANOVA was performed on the means after arcsine transformation; Tukey's test revealed no significant difference (P<0.01%) between the means followed by the same letter or letters.

Table 4 Effect of split release of *Trichogramma japonicum* on per cent white ear by YSB and yield in low land rice during summer 2019

Treatment	Per cent white ear 90 DAT	Per cent reduction over control	Yield (t/ha)	Per cent increase over control
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T1	2.7b	30.77	6.08b	13.49
T2	2.1a	46.15	6.59a	20.18
T3	2.9c	25.64	6.32ab	16.77
T4	3.9d	-	5.26c	-
SE(d)	0.093	-	0.042	-
CD	0.285	-	0.128	-
(P<0.01%)				

Details of the treatment are provided under Materials and Methods. DAT stands for days following transplantation. *Mean of three replications; ANOVA was performed on white ear means after arcsine transformation; ANOVA was performed on yield means after square root transformation; Tukey's test revealed no significant difference ($P<0.01\%$) between means followed by the same letter or letters. In general, 2,00,000 wasps (Tang et al. 2017) were divided into four equal portions at a rate of 50,000/ha/release and tested against yellow stem borer in low-land rice fields throughout the summer of 2018 and 2019. The damages produced by YSB were considerably minimised by four weekly releases of *T. japonicum* at a rate of 50,000 wasps/release/ha on the 14th, 21st, 28th, and 35th DAT. These were the best times and numbers of wasp releases for the effective control of YSB in low land rice throughout the summer. Similarly, Kaur et al. (2008) showed that rice leaf folder and stem borer could be effectively controlled with seven weekly releases of *T. chilonis* and *T. japonicum* at a rate of 100,000 each beginning at 30 DAT. According to reports, the introduction of *T. japonicum* at a rate of 100,000/ha was better in lowering the damage caused by YSB to rice tillers (50.1 to 63.5%) (Mishra and Kumar 2009). However, it was noted that 40% of the eggs in the bottom layer of the yellow stem borer egg mass could not be parasitised, suggesting that *T. japonicum* had a dubious effect on the control of rice yellow stem borer (Manjunath 2023). This can be refuted by Yunus's (2018) earlier claim that *T. japonicum* has been suggested as an effective species for controlling Lepidoptera pests that produce egg masses because it has a relatively long ovipositor to parasitise eggs that are in deeper layers of egg mass.

Periodic releases of *T. japonicum* parasitoid wasps were more beneficial than inundative releases of wasps, according to the current field trials and findings. This might be because the parasitoid releases are more suited to the climate, pest biology, and ecology of the rice habitat. In an attempt to quickly reduce pest populations, some new research proposes the use of inundative releases of the parasitoids *T. japonicum* and *T. chilonis* at various wasp densities. They do not, however, take into account the long-term effects (Perera et al. 2015, Tang et al. 2017, Visalakshi and Bhavani 2020). Though they consistently fail to offer effective biological control of Lepidopteran pests, such inundative discharges of *Trichogramma* parasitoids are an effort to outnumber the number of eggs deposited by the target insect. This is due to the fact that the research did not take into account factors such as seasonal abundance, number of broods, peak egg mass load availability, pest biology, ecology, and climate conditions (Edwin et al. 2016). Similar to chemical pesticides, adopting an inundative technique of parasitoid release to quickly decrease the pest population does not eliminate the broods of future hosts, nor does it create an established parasitoid population for long-term pest suppression. Therefore, when a more long-term pest management is needed, inundative biological control methods are not environmentally feasible (Murali-Baskaran et al. 2021).

The IPM of rice YSB has a clear suggestion for the inundative release of *Trichogramma*. For a periodic colonisation approach, the release dates would need to be adjusted or re-examined to accommodate various geographical areas with varying climates and pest biology. Even the two less successful release schedules (T1 and T3) produced the second and third-best schedules to effectively manage YSB and lessen dead heart and white ear symptoms, according to the results of the three schedules of periodic colonisation of parasitoids.

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