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Periodic colonization of *Trichogramma japonicum* for bio-control of yellow stem borer (*Scirpophaga incertulas*) in summer low-land rice (*Oryza sativa*)

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ABSTRACT

Yellow stem borer [*Scirpophaga incertulas* (Walker)], is a major pest of low-land rice (*Oryza sativa* L.), causing 30–40% yield losses. The use of biological control agents to suppress yellow stem borer (YSB) population and damage across Asia includes inundative releases of the egg parasitoid, *Trichogramma japonicum* Ashmead. However, information on the time and number of parasitoids' releases are lacking to control YSB in summer low-land rice. Therefore, an experiment was conducted during summer seasons of 2018 and 2019 at ICAR-National Institute of Biotic Stress Management, Raipur, Chhattisgarh to standardize the time and number of *T. japonicum* releases for effective biocontrol of YSB. Experiment consisted of 3 different treatment schedules, viz. T_1, Four releases of *T. japonicum* at weekly intervals on the 7th, 14th, 21st, 28th days after planting (DAT); T_2, Four releases of *T. japonicum* at weekly intervals on the 14th, 21st, 28th, 35th DAT; T_3, Four releases of *T. japonicum* at weekly intervals on the 21st, 28th, 35th, 42nd DAT; and T_4, Control (no release of wasps). Significant reduction of YSB-induced damage symptoms was observed in the treatment schedule (T_2) consisting of four releases of *T. japonicum* (@50,000 wasps/ha/release) at weekly intervals on the 14th, 21st, 28th, 35th DAT of rice which significantly reduced the dead heart (9.1%, 45.83% reduction over control) and the white ear (1.8%, 47.05% reduction over control), as compared to untreated control plots (T_4) (16.8% dead heart, 3.4% white ear) in summer 2018. Similarly, in summer 2019, the reduction in dead heart and white ear by the treatment (T_2) was 43.35% and 46.15% over control, respectively. The significant reduction of YSB-induced damages corresponded with the increased grain yield in (T_2) from 2018–19 i.e. 12.74–20.18% across the seasons. The results suggest that the (T_2) augmentative releases provided ecologically viable bio-control of YSB during the summer seasons in low-land rice.

Keywords: Periodic colonization, Summer rice, *Scirpophaga incertulas*, *Trichogramma japonicum*

Rice (*Oryza sativa* L.) is the primary staple food crop in India, China and other countries of south-east Asia. In India, cultivated rice covers an area of 43.39 million ha with an estimated production of 104.3 million tonnes (2404 kg/ha) (Annamalai *et al*. 2018). Among lepidopteran borer pests, yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) (Lepidoptera: Crambidae) is a major pest, causing 30–40% yield loss annually in low-land rice. The symptoms of feeding damage to rice plants are called 'dead heart and white ear' (Gangwar 2015). The inability of chemical insecticides to reach the cryptic feeding sites of YSB larvae (Kumar and Khan 2005) and development of insecticide resistance due to repeated use of the same chemical treatments each year, compounds the problem of YSB management in rice (Senthil-Nathan 2013).

Parasitoids of the *Trichogramma* spp. (Family: Trichogrammatidae) are known to attack eggs of more than 200 species of insect pests in the Orders: Lepidoptera, Neuroptera, Diptera, Hemiptera, Coleoptera and Hymenoptera with a special preference to the Lepidoptera (Tanwar *et al*. 2006). Sequential releases of 5–7 rounds of *Trichogramma* spp. parasitoids at different densities ranging from 50,000–200,000 wasps/ha have been reported to reduce rice plant damage (68.2% –78.8%), from Lepidoptera pests of low land rice in India, while also providing an increase in grain yields (Singh *et al*. 2013, Mohapatra and Nayak 2015).

The efficacy of *Trichogramma* spp. depends in part on the pest biology and ecology within the specific climatic conditions (Cicero *et al*. 2017). Thus, the number and timing of releases of *Trichogramma* spp. in managing rice YSB have been varying with geographic locations and from country to country. Hence the information on the best timing for releases and the number of parasitoid releases needs to determine for each location to be effective in reducing insect pests of rice, like the YSB (Kaur and Brar 2008).

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In this study, we report on the optimal time after planting, and the number of releases of *T. japonicum* parasitoids for effective biocontrol of YSB.

MATERIALS AND METHODS

Mass-culture of rice moth (Corcyra cephalonica *Stainton)*: The *C. cephalonica* was reared on broken pearl millet [*Pennisetum glaucum* (L.) R. Br.] grain-based medium in the *Trichogramma* mass-production unit of ICAR-National Institute of Biotic Stress Management, Raipur, Chhattisgarh (21^{o}22’56.9”N 81^{o}49’29.6”E). The newly emerged *C. cephalonica* adults collected 35 days after inoculation, were contained in oviposition cages of size (27 cm height and 28 cm diameter), with a wire mesh at the bottom and lateral sides for ventilation. Adults were provided with a 50% honey solution as adult food. Daily, eggs were gathered from the bottom of a blotting paper placed in a plastic tray, and sieved to remove wing scales and insect appendages. In a plastic container (45 cm × 30 cm × 10 cm), cleaned eggs were sprinkled over broken pearl millet grains at a rate of 18,000 to 20,000 eggs/2.5 kg of grains, fortified with 10 g of yeast, and covered with a tightly woven cotton cloth. Care was taken to maintain the culture free of storage mite, red flour beetle, and diseases by mixing it with 5 g of wettable sulfur 80 WP and streptomycin sulphate 0.5% *C. cephalonica.*

Culture of egg parasitoid, Trichogramma japonicum: In the *Trichogramma* production facility of ICAR-National Institute of Biotic Stress Management, Raipur, Chhattisgarh, the *T. japonicum* colony were permitted to parasitize irradiated 1-day-old eggs of *C. cephalonica*. The 1-day-old *C. cephalonica* eggs were sterilized under a UV lamp for 20 min and pasted on white cardboard (4000 eggs in 8 cm × 2 cm card), using 10% acacia gum. Each egg card, containing 18,000 to 20,000 eggs was contained in a polythene bag (35 cm × 26 cm) after exposing to *T. japonicum* separately at 1:6 ratios and maintained in a plastic tray (45 cm × 30 cm × 10 cm) till emergence (7–8 days after exposure). *Trichogramma* cultures were maintained at 27 ± 1°C, 70 to 80% RH. The adults were provided with a 10% honey solution. Adult parasitoids emerged on 8th day after exposure from the card.

Field evaluation of various schedules of releases of T. japonicum *against rice yellow stem borer*: Three release schedules of parasitoid wasps, each consisting of 4 releases of wasps at a weekly interval for 4 week were evaluated under field conditions in rice (cv. MTU 1010) against YSB during the summer season of 2018 and the 2019 at the research farm of ICAR-National Institute of Biotic Stress Management, Raipur, Chhattisgarh. The parasitoid wasps were released according to three schedules, viz. T_1, Four releases of *T. japonicum* at weekly intervals on the 7th, 14th, 21st, 28th days after planting (DAT); T_2, Four releases of *T. japonicum* at weekly intervals on the 14th, 21st, 28th, 35th DAT; T_3, Four releases of *T. japonicum* at weekly intervals on the 21st, 28th, 35th, 42nd DAT; and T_4, Control (no release of wasps).

Since the egg parasitoid, *T. japonicum* is constantly moving through the environment, its movement from one replication to another within the treatment cannot be restricted. Thus, a plot measuring 100 m^2 was established as the test plot area for each treatment. Within the treatment, 5 plots were randomly selected, each one measuring 20 m^2. The per cent dead heart and white ear were recorded in each plot and sub-plot (randomly in the 5 hills of each sub-plot measuring 20 m^2) and was considered as one replication. Each treatment received a release with 500 wasps/100 m^2/release. A treatment (100 m^2) without released wasps was considered as an untreated check (control). Tricho cards (likely to produce adult parasitoids next day) with 500 7-days old parasitized *Corcyra* eggs, were tied at the tip of each release station, being erected to the height of plant canopy. No insecticide was applied during the trial. An isolation distance of 15 m between two treatments was used to restrict the movement of wasps from one treatment plot to another. The per cent dead heart was recorded on 21st, 28th, 35th, 42nd, 49th, 56th DAT, while white ear was recorded on the 90th DAT. The white ear measurement was taken near harvesting or at the physiological maturity stage. The yield was recorded and expressed as tonne of grains/ha. Similarly, the trial was repeated with same methodology during summer 2019.

Statistical analysis: The per cent dead heart and white ear measured from field experiments conducted during both summer seasons of 2018 and 2019 were analyzed separately. Data on dead heart and white ear were subject to ANOVA after using arcsine transformation. The yield data were transformed with square root before analysis. Means were separated using Tukey (HSD) for multiple comparison.

RESULTS AND DISCUSSION

Field evaluation of T. Japonicum *against rice yellow stem borer*: The release schedule (T_2) for the 4 releases of *T. japonicum* at the density of 50,000 wasps/ha/release in low land rice (cv. MTU 1010) at weekly intervals, begun on the 14th DAT, followed sequentially by 21st, 28th and 35th DAT reduced dead heart symptoms by 45.83% (9.1% dead heart) as compared to the control plots (6.8% dead heart) during summer 2018 (Table 1). The efficacy of *T. japonicum* released as per the T_2 schedule (14th DAT), was significantly different to the other release dates started either on 7th DAT (T_1) or 21st DAT (T_3), resulting in 11.3% and 12.3% dead heart symptoms, respectively (Table 1).

The (T_2) second schedule of releases had the lowest percentage of dead heart symptom (5.4%), being significantly different from all other treatments ($F_{3,19}$ = 90.91; P<0.001) (T_1, 6.7% dead heart) and (T_3, 7.7% dead heart) scheduled releases, as compared to 11.2% dead heart in the control (T_4) (Table 1). Same trend was noticed with reference to dead heart that the treatment (T_2) did continue to be significantly at the lowest in all the period of measurements on 28th, 35th, 42nd, 49th and 56th DAT, resulting 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 cent reduction over control
	21 DAT	28 DAT	35 DAT	42 DAT	49 DAT	56 DAT		
T_1	6.7**b**	11.2**b**	17.3**b**	9.6**b**	11.3**b**	12.6**b**	11.3	32.74
T_2	5.4**a**	9.1**a**	12.3**a**	8.0**a**	9.9**a**	9.8**a**	9.1	45.83
T_3	7.7**c**	14.6**c**	16.8**b**	9.2**b**	11.9**b**	13.4**c**	12.3	26.79
T_4	11.2**d**	20.4**d**	23.3**c**	13.2**c**	15.2**c**	17.4**d**	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	-	-

Treatment details are given under Materials and Methods. DAT, Days after planting.

*Mean of three replications; Means were subject to ANOVA after making arcsine transformation; Mean followed by the same letter(s) are not significantly different by Tukey's test (P<0.01%).

($F_{3,19}$ =38.54; P<0.001), respectively while it was 20.4%, 23.3%,13.2%, 15.2% and 17.4% in control (Table 1).

Treatment schedule (T_2) starting from 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 which was significantly different compared to all other treatment schedules T_1 (2.3% white ear; 32.35% reduction over control), or the T_3 (1.5% white ear; 55.88% reduction over control), or with the untreated control (3.4%) (Table 2**)**. Reduction in the YSB-induced damage symptoms due to the treatment schedule (T_2) recorded the highest yield of 6.9 t/ha ($F_{3,19}$ = 17.89; P<0.001) while it was 5.4 t/ha in the untreated control (Table 2).

During summer 2019, the treatment schedule, T_2 reduced the YSB-induced dead heart symptom, significantly with a reduction of 43.35% over control (9.1% dead heart) which was significantly different from the release schedules: T_1 (11.2% dead heart; 30.43% reduction over control) and T_3 (11.9% dead heart; 26.09% reduction over control) (Table 3) in contrast to untreated control (16.1% dead heart).

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
T_1	2.3**b**	32.35	6.1**b**	11.48
T_2	1.8**a**	47.05	6.9**a**	21.74
T_3	1.5**a**	55.88	6.5**ab**	16.92
T_4	3.4**c**	-	5.4**c**	-
SE(d)	0.407	-	0.043	-
CD (P<0.01%)	1.243	-	0.132	-

Treatment details are given under Materials and Methods. DAT, Days after transplanting.

*Mean of three replications; Means of white ear were subject to ANOVA after making arcsine transformation; Means of yield were subject to ANOVA after making square root transformation; Means followed by the same letter(s) are not significantly different by Tukey's test (P<0.01%).

T_2 schedule of releases, starting from 14th DAT significantly suppressed the YSB-induced symptom, leading to the lowest dead heart of 6.2% when compared to either beginning of release on 7th DAT (7.4% dead heart) or 21st DAT (8.7% dead heart) during summer 2019. Results in subsequent period of measurements was in the same trend in which T_2 was distinct in reduction of dead heart, resulting the lowest of 6.2% ($F_{3,19}$ = 2945.56; P<0.001), 5.8% ($F_{3,19}$ = 1151.08; P<0.001), 8.1% ($F_{3,19}$ = 3685.11; P<0.001), 11.1% ($F_{3,19}$ = 7733.64; P<0.001), 11.7% ($F_{3,19}$ = 38.54; P<0.001) and 11.8% ($F_{3,19}$ = 4339.04; P<0.001), respectively during the period of observations on 21st, 28th, 35th, 42nd, 49th and 56th DAT while it was 12.6%, 13.4%, 15.4%, 17.7%, 16.8% and 20.6% in untreated control (Table 3).

On 90th DAT, the YSB-induced white ear was the lowest in the treatment schedule, T_2 which was 2.1% white ear (46.15% reduction over control) ($F_{3,19}$ = 409.47; P<0.001) and significantly different from other treatment schedules of T_1 (2.7% white ear; 30.77% reduction over control) and T_3 (2.9% white ear; 25.64% reduction over control) and untreated control (3.9% white ear) (Table 4). T_2 increased the yield of rice grain ($F_{3,19}$ = 16.26; P<0.001), resulting the highest yield of 6.59 t/ha (20.18% increase over control) in contrast to the control (5.26 t/ha) (Table 4).

The incidence of YSB in terms of symptoms development of dead heart was comparatively lower in the summer season 2019, than the summer season 2018, and *vice-versa* in case of white ear. However, the results with reference to dead heart, white ear and yield were concurrent across both the seasons, showing that the split releases of parasitoid begun on 14th DAT (T_2), followed sequentially by 21st, 28th and 35th DAT at weekly intervals were optimum times for releases and the total number of releases to significantly reduce YSB feeding damage compared to control plots. The reduction in YSB feeding damage symptoms under the periodic releases of *T. japonicum* increased grain yield in T_2 maximally than other schedule of releases (T_1 and T_2) and untreated control in both the seasons (Summer 2018 and 2019) as also reported by Deshpande *et al.* (2023).

A biological technique was employed in which the total density requirement of *Trichogramma japonicum*/ha

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		
T_1	7.4^{b}	7.4^{b}	11.6^{b}	12.9^{b}	12.6^{ab}	15.0^{b}	11.2	30.43
T_2	6.2^{a}	5.8^{a}	8.1^{a}	11.1^{a}	11.7^{a}	11.8^{a}	9.1	43.35
T_3	8.7^{c}	9.6^{c}	11.2^{c}	12.4^{c}	13.2^{b}	16.1^{c}	11.9	26.09
T_4	12.6^{d}	13.4^{d}	15.4^{d}	17.7^{d}	16.8^{c}	20.6^{d}	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	-	-

Treatment details are given under Materials and Methods. DAT, Days after transplanting.

*Mean of three replications; Means were subject to ANOVA after making arcsine transformation; Means followed by the same letter(s) are not significantly different by Tukey's test (P<0.01%).

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
T_1	2.7^{b}	30.77	6.08^{b}	13.49
T_2	2.1^{a}	46.15	6.59^{a}	20.18
T_3	2.9^{c}	25.64	6.32^{ab}	16.77
T_4	3.9^{d}	-	5.26^{c}	-
SE(d)	0.093	-	0.042	-
CD (P<0.01%)	0.285	-	0.128	-

Treatment details are given under Materials and Methods. DAT, Days after transplanting.

*Mean of three replications; Means of white ear were subject to ANOVA after making arcsine transformation; Means of yield were subject to ANOVA after making square root transformation; Means followed by the same letter(s) are not significantly different by Tukey's test (P<0.01%).

@2,00,000 wasps in general (Tang *et al*. 2017) was split into four equal parts @50,000/ha/release and evaluated under low land rice fields during the summer seasons of 2018 and 2019 against yellow stem borer. Four releases of *T. japonicum* @50,000 wasps/release/ha at a weekly interval on the 14th, 21st, 28th, 35th DAT significantly reduced the damages caused by YSB and were the optimum times and number of releases of wasps for the efficient management of YSB in low land rice during the summer seasons. Similarly, Kaur *et al*. (2008) demonstrated that 7 releases of *T. chilonis* and *T. japonicum* @100,000 each at weekly intervals starting at 30 DAT were effective in the management of rice leaf folder and stem borer in rice. The release of *T. japonicum* @100,000/ha was reported to be superior in reducing the rice tiller damage (50.1 to 63.5%), caused by YSB (Mishra and Kumar 2009). However, it was exclaimed that the *T. japonicum* seemed to have doubtful impact on the regulation of rice yellow stem borer as 40% of the eggs present in the lower layer of the egg mass of yellow stem borer could not be parasitized (Manjunath 2023). This can be overruled based on the earlier statement by Yunus (2018) who reported that *T. japonicum* has been recommended as an effective species for control of Lepidoptera pests, that produce egg masses, as this species has comparatively a long ovipositor to parasitize eggs which are in deeper layers of egg mass

The present field experiments and results show that periodic releases of *T. japonicum* parasitoid wasps were more advantageous than inundative releases of wasps. This may be due to the better adaptation of the parasitoid releases to fit into the climatic conditions, pest biology and ecology within the rice ecosystem. Some recent studies suggest using inundative releases of the parasitoids *T. japonicum* and *T. chilonis* at different wasp densities with the expectation of achieving rapid reduction in the pest populations. However, they do not consider the continuing impacts (Perera *et al*. 2015, Tang *et al*. 2017, Visalakshi and Bhavani 2020). Such inundative releases of *Trichogramma* parasitoid are attempting to overwhelm the number of eggs deposited by the target pest, but show consistent failure to provide effective biological control of Lepidopteran pests. This is because the studies did not consider the pest biology, ecology and climatic conditions, seasonal abundance, number of broods, or the time of availability of the peak egg mass load etc. (Edwin *et al*. 2016). While using an inundative strategy of parasitoid release to produce a rapid suppression of the pest population, similar to chemical insecticides, there is no elimination of future hosts' broods and this does not produce an established parasitoid population for sustained pest suppression. Thus, inundative biological control strategies are not ecologically viable when a more permanent pest control is desired (Murali-Baskaran *et al*. 2021).

The recommendation on inundative release of *Trichogramma,* is readily available in the IPM of rice YSB. However, the release dates may need to be revised or reanalyzed to fit into different geographic locations with different climatic conditions and pest biology for periodic colonization strategy. Results from the three schedules of periodic colonization of parasitoids show that even the two less effective release schedules (T_1 and T_3) still provided the second and third-best schedule to produce effective control of YSB and reduce dead heart and white ear symptoms.

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