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Research Article
Effectiveness of *Trichogramma japonicum* Utilization for Biological Control Agents on *Scirpophaga incertulas* in Indonesia

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Abstract

Background and Objective: One of the *Trichogramma* species commonly used to control rice stem borer is *Trichogramma japonicum* (*T. japonicum*). The following research aimed to (1) Investigate the effect of the age of the parasitoid and the age of the host egg on *T. japonicum's* parasitization ability and (2) Know the pattern of dispersal and the effect of the release of *T. japonicum* against the intensity of parasitization. **Materials and Methods:** The testing method used was a factorial experiment with a completely randomized design. The first factor consisted of five different life stages of parasitoids (UP), delineated from 0 up to 4 days (UP-0, UP-1, UP-2, UP-3 and UP-4). The second factor in this experiment consisted of the 5 life stages of the host egg (UI), which were the ages of the host eggs from 0-4 days (UI-0, UI-1, UI-2, UI-3 and UI-4). Twenty-five units were treated at a time and the treatment process itself was repeated 5 times so that the whole experiment used 125 units in total. The data were analyzed using one-way (ANOVA) and significant effect continued with Tukey HSD test ($p<0.05$). **Results:** One day old *T. japonicum* parasitoid was more effective biocontrol agent compared with older parasitoids. Levels of parasitization were highest upon available host eggs less than 24 h old. *Trichogramma japonicum* in the field can be scattered in all directions and can reach a distance of up to 6 m (36 m^2) within 7 h. The existence of *T. japonicum* in the field proved to successfully control the population of *Scirpophaga incertulas*, if the population of *S. incertulas* eggs in field was low, the population of the parasitoid was similarly reduced. A dosage of 250,000 released parasitoids ha^{-1} was more effective than a 125,000 dosage of parasitoids ha^{-1}. **Conclusion:** An increased dosage could increase the intensity of parasitization by 236% when compared to controls. This research also encountered *Telenomus rowani* and *Tetrastichus schoenobii* in the field and they also attacked *S. incertulas* eggs.

Key words: Rice stem borer, age of parasitoid, age of host egg, *S. incertulas*, *T. japonicum*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rice stem borer (RSB) are pests that can attack rice plants throughout the plant's growth phase[1,2]. There are six RSB species found in Indonesia, of which five species belong to the family Crambidae and one species belongs to the family Noctuidae. All are included in the order Lepidoptera. Species from the Crambidae family are: 1) White RSB, *Scirpophaga innotata* (Walker), 2) Yellow RSB, *Scirpophaga incertulas* (Walker), 3) Striped RSB, *Chilo suppressalis* (Walker), 4) Black heads RSB, *Chilo polychrysus* (Meyrick), 5) Shiny RSB, *Chilo auricilius* (Dudgeon). The one species belonging to the family of Noctuidae is the Pink RSB, *Sesamia inferens* (Walker)[3]. All six RSB have a common point of attack, namely the rice stem from the beginning to the end of plant growth[4].

Since the early 1900s, the rice stem borer (RSB) has been a major pest in Indonesia. From the beginning of the modern era, the populations of White RSB *Scirpophaga innotata* have experienced rapid periods of growth and decline. During a period of 1900-1940, the population of *S. innotata* exploded several times, namely in the years 1903, 1907, 1912, 1919, 1926, 1932, 1936 and 1937[5]. An often-cited trigger cause of such explosions is a change in the composition of natural enemies[6].

Various control techniques have been employed to try and curb the impact of RSB attacks, namely: (1) Flooding the field after harvest for up to 15 days, (2) The burning of straw, (3) Catching moths with light trap, (4) Collecting egg groups, (5) The release of parasitoid eggs and (6) The application of insecticides[7-9].

The Trichogrammatidae egg parasitoid is widely used as a biological control agent for the populations of Lepidoptera pests in some countries. In Indonesia, Trichogrammatidae parasitoids have been employed to attack some pest species, such as: (a) Those known to eat cabbage leaves[10], (b) The soybean pod borer[11], (c) The rice stem borer[12], (d) The boll borer[13] and (e) Some corn cob borers[14].

Some species of *Trichogramma* most commonly associated with RSB are *T. japonicum*, *T. minutum*, *T. australicum* and *T. dendrolimi*. All four parasitoid species have a fairly broad distribution area that includes the United States, Central America, China, the Philippines, India, Indonesia, Japan, Madagascar, Malaysia and Taiwan[9]. Nagarkatti and Nagaraja[15] reported that the parasitoid of *T. japonicum* has spread across several countries and has developed in different host species according to its location. In India, *T. japonicum* parasitized *S. incertulas* eggs, in Malaysia, *S. incertulas* and *C. polychrysus* eggs, in the Philippines, *S. incertulas* and *C. suppressalis* eggs, in Thailand, *C. polychrysus* eggs, finally in Indonesia *S. incertulas* and *S. innotata* eggs[5,16,17].

Having a good understanding of the way in which *S. incertulas* population control can be enacted through use of *T. japonicum* relies on an understanding of some issues of traditional predatory or parasitic behavior in the field. The success of the *S. incertulas* population depends heavily on the parasitoid population density, as well as the alignment between the host and its parasitoid phenology. The egg parasitoid in both larval and pupal stage can only live on the host eggs, so the continued life of the parasitoid was determined by the availability of appropriate host eggs.

The appearance of *T. japonicum* in the field often occurs at an inopportune time (in terms of its ability to effectively neutralize pest populations) because of the unavailability of host eggs in the surroundings. The parasitoid then has to fly away and will take several days to find other suitable host eggs. In such conditions, the age of parasitoid increases and this in turn affects the parasitoid's parasitization ability. The opposite phenomenon occurs when the host eggs are abundant in the field and *T. japonicum* was absent. By the time the parasitoid finds the eggs, the age of the host eggs will have increased and the nutritional biochemical composition of the eggs will have changed. This again affects *T. japonicum's* parasitization ability.

Other things that affect the parasitoid's rate of parasitization are the population density, the dose of parasitoid released into a particular field and the effects of the changing seasons. Ulrichs and Mewis[18] reported that the release of *Trichogramma evanescens* in a 4 time dosage of 150,000 parasitoids per hectare was effective in controlling *Maruca vitrata* (Lepidoptera: Pyralidae) on legume crops. The intensity of parasitization increased to 53% in the dry season and dropped to 43% in the rainy season. Suneel and Khan[19] also reported that the release of parasitoid *Trichogramma spp.* (In Pantnagar, India) succeeded in controlling an attack of *S. incertulas* in rice plants. Their indicator was that the parasitoid release decreased the amount of seedlings damaged. The usage of 50,000 parasitoids per hectare per application decreased the seedlings damaged by 50.1-61.3% of the total undamaged and 100,000 doses parasitoids per hectare per application decreased the amount of seedlings damaged 78.1-8.6% undamaged.

This research aimed to know ability of parasitization, the pattern of dispersal and the effect of the release of *T. japonicum* against the intensity of parasitization.

MATERIALS AND METHODS

Time and place of research: The research into the age implications of both the parasitoid *T. japonicum* and the *Corcyra cephalonica* host egg was performed in the Biological Control Laboratory of the Agriculture Faculty at the University of Gadjah Mada in 2012. The temperature was 29-32°C and air humidity was at 70-85%. Research into parasitoid dispersal pattern and the effect of parasitoid release against RSBs was done in a rice production center area in which rice stem borers attack endemically. This research was conducted in Karangdowo Sub-regency in Klaten Regency. The precise geographical position is 110°30'-110°45' East longitude and 7°30'-7°45' South latitude.

Preparation

Multiplication of *Corcyra cephalonica* eggs: The *T. japonicum* were bred using alternative hosts (factitious hosts), in this case the eggs of *C. cephalonica*. This method was used in consideration of the ease of maintaining the factitious hosts, maintaining the eggs of *S. incertulas* was much more difficult. The "Multiplication method" of *Corcyra cephalonica* eggs refers to the same method as used by Yunus *et al.*[20].

The source of the *C. cephalonica* eggs was the collection of the Laboratory of Pests and Plant Diseases of the Faculty of Agriculture at the University Tadulako. Male and female adult moths were captured using glass tubes, sized center-line (Ø) at 1.5 cm and with a length of 9 cm. Moths were then inserted into the nesting tubes made of PVC plastic measuring 8 cm and 20 cm high. The top and bottom of the tubes were covered with gauze as the laying of *C. cephalonica* eggs. After 1 day, the eggs attached to the gauze were harvested using a fine brush, before being placed on a petri dish sized 10 cm. A total of 300 egg crops were then sown into a container for the maintenance of *C. cephalonica* pre-adults. The container measured 40 cm long by 30 cm wide and was 6 cm high. One kilogram of corn flour was then fed into the container. The maintenance of the *C. cephalonica* was done sustainably throughout the course of the research.

Multiplication of *Trichogramma japonicum*: The parasitoid *T. japonicum* used for later release were obtained from a collection of *S. incertulas* eggs that had developed at the research location. The egg group was brought to the Biological Control Laboratory of the Faculty of Agriculture to be incubated until adult parasitoids appeared. Observations were made every day, parasitoids that appeared were then cultivated using a host egg substitute (*C. cephalonica*) glued onto a card.

"Multiplication" of the parasitoid refers to the method used by Yunus *et al.*[20]. The *C. cephalonica* eggs were multiplied to be used as a nest medium for *T. japonicum*. A card contained 500 eggs of *C. cephalonica* were put in a glass tube with a midline of 1.5 and 9 cm long to be preyed on. Parasitoid offspring of the multiplied result were then maintained in a tube and the adult parasitoids were fed a 10% solution of honey. Each parasitoid that emerged from a card would then be used to develop three cards and so on until the desired population was achieved.

Testing

Combined effect of parasitoid age and host egg age: The testing method used was a factorial experiment with a completely randomized design. The first factor consisted of five different life stages of parasitoids (UP), delineated from 0 up to 4 days (UP-0, UP-1, UP-2, UP-3 and UP-4). If a host egg was always available for the *T. japonicum* parasitoid, its age would only ever be short: In this experiment, it reached a maximum of 1.9 days. Further preliminary observations indicated that *T. japonicum* could live much longer if it didn't have access to host eggs for 4 days. The second factor in this experiment consisted of the five life stages of the host egg (UI), which were the ages of the host eggs from 0-4 days (UI-0, UI-1, UI-2, UI-3 and UI-4). When the adult parasitoids emerged, the age of the host eggs available to them was quite diverse. As a reference we ensured that we knew the length of time for which the host eggs would be fit for parasitization. Twenty five units were treated at a time and the treatment process itself was repeated 5 times, so that the whole experiment used 125 units in total.

The preparation of experimental materials was done gradually. Preparation of UP subjects occurred daily, with each day divided up according to the age of parasitoid being used. The first day was designated for 4 days old parasitoids (UP-4), the second day for the treatment of UP-3, the third day for the treatment of UP-2, the fourth day for treatment of UP-1 and the fifth day for treatment of UP-0. On the fifth day, each card contained 60 eggs of *C. cephalonica* to be incorporated simultaneously into a glass tube to be preyed on by a *T. japonicum* female according to the treatment as outlined above. The preparation of the host egg was done by much the same process. Fresh *C. cephalonica* eggs were harvested each day between 07.00-08.00 am. The first day was given over to the treatment of UI-4 eggs, the 2nd day to prepare for the treatment of UI-3, the 3rd day to prepare for the treatment of UI-2, the 4th day to prepare for the treatment of UI-1 and the 5th day to prepare for the treatment of UI-0. Each treatment consisted of 25 cards of *C. cephalonica* eggs inserted into a petri dish of 10 cm. Each petri dish was then stored in a refrigerator in low temperature conditions (4-7°C). The storage time used was in accordance with the treatment length required (0-4 days). On the 5th day all the petri dishes were taken from the refrigerator with the contents put together into a glass tube to be preyed upon in accordance with the parasitization protocols outlined above.

Observations were made every day. The variable under observation was the parasitization ability of *T. japonicum* in each separate treatment, by counting the number of host eggs preyed upon by *T. japonicum* per each card.

Parasitoid dispersal patterns: To determine the parasitoid dispersal pattern the eggs on a card trap were attached to the surface of rice leaves (according to the conditions often found in nature). Sixteen cards of eggs were divided in the four cardinal directions. Each direction had four mounting points at a distance of 1, 2, 4 and 6 m from the point of release. Each card trap contained 1 day old 100 *C. cephalonica* eggs. The observation area formed a square with the size of each side at 14 m. Exposure was performed for 7 h, from 08.00 am until 15.00 pm. After exposure, the egg trap was collected back and each was subsequently incubated in the laboratory until the imago appeared. The observation variable was parasitoid dispersal, determined by counting the number of occurrences of parasitoid per each card trap without averaging.

Effect of dose release of *Trichogramma japonicum* in the field: The release of the parasitoid was done in the middle of the observation plots. A release station was made from coconut shell parts fitted at a discharge point above the plant canopy with the aid of poles. A card contained trap eggs glued to the inside of the coconut shell. Each card carton contained parasitoid pupae (pupa living inside a host egg). The coconut shell was mounted face down, which protected a card from direct sunlight, rainfall and allowed it to maintain a stable temperature. The parasitoid was released in the morning (07.00-08.00 am). The release plot formed a square with each side comprising 14 m (the total area used was 196 m^2). The plot distance between each treatment was no less than 100 m. The release of the parasitoid began when the population group of *S. incertulas* eggs in the field was sufficient enough to be used as a sample: This was at least 16 eggs/group/observation plot.

The testing method used a randomized block design consisting of 5 treatments and 3 replications. Each treatments were: 1) Control, without the release of parasitoid with group traps of *S. incertulas* eggs (Si-0), (2) Release of parasitoid in a dose of 2,500 units, using group traps of *S. incertulas* eggs (Si-2500), (3) Release of parasitoid with a dose of 5,000 units using group traps of *S. incertulas* eggs (Si-5000), (4) Release of parasitoid in a dose of 2,500 units using group traps of *C. cephalonica* eggs (Cc-2500) and (5) Release of parasitoid in a dose of 5000 units using group traps of *C. cephalonica* eggs (Cc-5000).

Sixteen traps were installed per observation plot in the form of groups of *S. incertulas* eggs or in the form of a card of *C. cephalonica* eggs. The installation of trap a card was conducted concurrently with the release of *T. japonicum*. The placement of traps was done systematically according to the four cardinal directions and with a distance of 1, 2, 4 and 6 m from the point of release. A card traps were exposed for seven hours, from 08.00 am until 15.00 pm before a card were collected back to be incubated in the laboratory.

Observation was done every day. The observation variable was the intensity of parasitization at each treatment by calculating the average number of preyed eggs from each egg group after an incubation period. The number of *T. schoenobii* and *T. rowani* that appeared from the host eggs was counted.

Statistical analysis: The data were analyzed using one-way ANOVA followed by honesty significant difference (HSD) test ($p<0.05$) to compare the mean.

RESULTS

Age of parasitoid and age of host eggs: The variance of parasitoid age treatment (UP) and host age (UI) showed that the treatment in combination had a significant effect ($p<0.05$) on *T. japonicum's* parasitization ability with a value of $p = 0.00$. The highest parasitization levels occurred in the combination treatment of UP-1 and UI-0 (36.2±6.8 egg parasitized/egg-mass card). Some combination treatments were not significantly different, such as the UP-0 and UI-0, UP-0 and UI-1, UP-1 and UI-1, UP-2 and UI-0, UP-3 and UI-0, UP-3 and UI-1. Other combination treatments were significantly different, the lowest parasitization levels occurred in the combination of UP-0 and UI-4 (5.4±3.3 egg parasitized/egg-mass card) and UP-4 and UI-4 (5.6±3.9 egg parasitized/egg-mass card).

Dispersal pattern of *Trichogramma japonicum* in the field: Based on the parasitization intensity data taken from the traps set at various distances (1 until 6 m) from the point of release, it was concluded that *T. japonicum* could disperse and reach

Table 1: Dose effect of *Trichogramma japonicum* release on parasitization intensity in trap eggs*

	Average number of individual appear each trap**			
Treatments	*Trichogramma japonicum* (imago)	*Telenomus rowani* (imago)	*Tetrastichus schoenobii* (imago)	*Scirpophaga incertulas* (larva)
Cc-2500	2.04[a]	-	-	-
Si-0	3.85[a]	2.23	17.46	73.5
Cc-5000	4.19[a]	-	-	-
Si-2500	7.85[b]	2.58	18.48	56.75
Si-5000	9.10[b]	2.15	16.67	48.67

*Trap eggs in the form of a card containing 100 eggs of *Corcyra cephalonica*, or as group of *Scirpophaga incertulas* eggs. **Numbers followed by the same letter are not significantly different from test of Tukey test α = 0.05

all eggs traps set by the exposure of eggs for 7 h, but not parasitize upon all the eggs at each distance. On replication 1 and 2 seven a card/plot were parasitized upon (7/16 = 43.75%), while in replication 3 only 4 a card-plot were parasitized (4/16 = 25%). The parasitization intensity per trap averaged at a low rate and varied quite widely over different replications: replication 1 ranged between 2-14 parasitoids/a card, replication 2 ranged from 3-17 parasitoids/a card and replication 3 ranged from 12-15 parasitoids/a card.

Dose effect of parasitoid release on parasitization intensity of *Trichogramma japonicum*: Based on variance, the difference in dose release of the parasitoid *T. japonicum* in Karangdowo sub-regency of the Klaten regency had significant effect on the parasitization intensity of *S. incertulas* and *C. cephalonica* eggs in the traps, with value of p = 0.00. Based on the Tukey test α = 0.05, the parasitization intensities of *T. japonicum* treatments Si-2500 and Si-5000 were significantly different from the control, whereas treatment Cc-2500 and Cc-5000 were not significantly different than the control. The intensity at the treatments involving the same egg types (Si-2500 and Si-5000 and Cc-2500 and Cc-5000) were not significantly different (Table 1).

The release of the parasitoid was conducted 3 times on rice aged 24, 28 and 32 days after planting (DAP). *T. japonicum*'s parasitization intensity across the *S. incertulas* egg groups was naturally low, at an average of 3.85 eggs parasitized upon over each egg-mass. Increasing the dosage of *T. japonicum* released (0, 2,500 and 5,000 parasitoids/plot, respectively) increased parasitization intensity on *S. incertulas* eggs. The Si-2500 treatment reached an average of 7.85 eggs (an increase of 7.85/3.85 = 204%) and the Si-5000 treatment reached an average of 9.10 points (an increase of 9.10/3.85 = 236%). A higher dose of parasitoids tended to result in a higher parasitization intensity, although the Tukey test α = 0.05 between Si-2500 and Si-5000 and therefore the two do not show a significant difference ($p<0.05$). Occurrences of *T. schoenobii* and *T. rowani* were found only in *S. incertulas* egg traps. Occurrences of *T. schoenobii* tended to be higher than the other parasitoid species (Table 1).

DISCUSSION

The strongest parasitization ability occurred in the combination of parasitoids aged 1 day old and host eggs aged less than 24 h (UP-1 and UI-0). This might be explained by the fact that emerging female parasitoids produce eggs in their ovaries until they are 1 day old. Female parasitoids aged 1 day old have the maximum ability to produce eggs, such that if the parasitoid encounters the host eggs at this ideal age (less than 24 h), the parasitoid's parasitization ability is at its highest.

The other best combination treatment which were not significantly different from the combination of UP-1 and UI-0 were the combinations parasitoids aged 0, 1, 2, or 3 days old with a host egg aged at 0 or 1 day old. The parasitization abilities of a 4 days old *T. japonicum* greatly decreased, most likely because the eggs in her ovaries had already been reabsorbed to maintain energy for survival. In the combination of a 4 days old *T. japonicum* and 4 days old host eggs, the parasitoid's parasitization ability decreased sharply (5.6 ± 3.9 egg parasitized/egg-mass card).

It appears that the *T. japonicum* were relatively capable of maintaining parasitization ability until their 3rd day, after this point, their ability decreased. For the short-lived parasitoid, ensuring reproductive ability on initial appearance and contact with the host egg is very important, since in the wild mortality factors cannot be predicted[9]. The absence of host eggs on the 1st day did not affect on the parasitization ability (p = 0.137), but the absence did take effect on subsequent days[17].

Zang and Liu[21] reported that the parasitoid *Eretmocerus melanoscutus* (Hymenoptera:Aphelinidae) were most effective in killing *Bemisia tabaci* (Homoptera: Aleyrodidae) at the age of 6 h when compared to a younger age (0-3 h) or an older age (10-24 h). Silva-Torres *et al.*[22], reported that

Oomyzus sokolowskii (Hymenoptera:Eulophidae) parasitization ability on *Plutella xylostella* was optimal when the parasitoid was aged between 48 and 72 h. After this point it decreased up to a limit of 96 h. A parasitoid aged 2 or 3 days old failing to make contact with a host egg would reabsorb some eggs to ensure adequate energy levels for its continued survival[17,23], hence its parasitization ability also decreased. According to Fleury and Bouletreau[23], *Trichogramma brassicae*'s parasitization ability aged 4 days old decreased from an average of 81.2 when younger to 34 eggs/parent. Tarla[24] reported that the absence of a host from 2-10 days could reduce the number of *Trissolcus grandis* offspring (Hymenoptera:Scelionidae) from 132±4.4 to 97.4±4.9 eggs/parent.

The results of the above experiment showed that the lowest parasitization ability occurred in parasitoids aged 4 days combined with a host egg age of 4 days. The parasitization ability would likely continue to decrease until the parasitoid died. In accordance with the observations of Rohmani *et al.*[17], *T. japonicum* parasitoids which did not make contact with a host egg and died had no eggs in their ovaries because their eggs had been absorbed back.

According Hougardy and Mills[25], the absence of a host has an effect on the dispersal power of parasitoids. *Mastrus ridibundus* parasitoids without a host from between 1 and 2 days had an average scatter power of only 2.1 $m^2\ h^{-1}$, if no host could be found for more than 4 days, the scatter power of the parasitoid would be increased, moving up to 81.5 $m^2\ h^{-1}$.

This study experiment proved that the age of *T. japonicum* had significantly effect on its parasitization ability. *T. japonicum* aged between 0 and 3 days showed a high ability for parasitization, while any older age greatly reduced its abilities. Parasitoids aged 1 day or more could reabsorb the bladder eggs to ensure adequate energy levels for its survival, but this process has consequences for its parasitization ability, which slowly decreases until the parasitoid dies.

The ideal age of *C. cephalonica* host eggs for *T. japonicum* parasitization was from between 0 and 24 h old. Older eggs were less receptive as hosts and the rate of parasitization was consequently reduced. Herlinda *et al.*[26] also reported that *C. cephalonica* egg age affected *Trichogramma* spp parasitization ability. A host egg aged between 0 and 24 h were more receptive to parasitization (89-90.1% parasitized) than a host egg aged between 36 and 48 h old (77.8% until 79.3% parasitized).

According to Paul *et al.*[27], *Chelonus blackburni* parasitization ability on host eggs of a varying age followed a normal curve, reaching a peak at an egg age of 66 h, before decreasing until the age of 78 h. They theorized that this is because parasitization is influenced by the embryo maturation process. This finding was supported by Kapila and Agarwal[28], who found that the highest parasitization ability in *Uscana mukerjii* (Hymenoptera:Trichogrammatidae) on *Callosobruchus maculatus* eggs (Coleoptera:Bruchidae) occurred within the first 24 h and then decreased. Miura and Kobayashi[29] also reported that *T. chilonis* laid eggs at all tested host egg ages (0 until 3 days), the highest percentage of oviposition, however, was found to be at the host egg age of 1 day, with a decreased rate at a younger or older age.

Trichogramma sp. parasitization ability is influenced by changes in the biochemical composition of the host egg according to the egg age. According to Bhatt and Krishna[30], eggs of *C. cephalonica* aged less than 24 h contain 2 types of carbohydrates (glycogen and trehalose) and some free amino acids (aspartic acid, cystine, glutamic acid, serine and tyrosine). As the egg increases in age beyond 24 h, new amino acids form (arginine, leucine, lysine and phenylalanine). These changes in biochemical composition affect the host's receptivity to the parasitoid. Parasitization ability decreases, then, because the host eggs are too old and the content of nutrients in the egg rapidly change[31]. Reznik *et al.*[32] found that *Trichogramma* females given older host eggs require a higher motivation to lay their eggs, so their ability to parasitize decreases.

In real conditions in the field, *T. japonicum*'s parasitization ability in *S. incertulas* eggs aged between 0 until 2 days was higher (68.4-69.8% parasitized) than in eggs aged 4 days old (28.8% parasitized)[33]. According to Carpenter *et al.*[34], the effect of the host age difference between 1 until 3 days on the parasitization ability of *Trichogrammatoidea cryptophlebiae* (Hymenoptera:Trichogrammatidae) was not significantly different under conditions in which it could not choose the host egg age (in this case, the eggs of *Cydia pomonella*, (Lepidoptera: Tortricidae). The effect was significantly different ($p<0.05$), however, when the parasitoids were free to choose the host egg age.

From their release point, *T. japonicum* will disperse in all directions. Following an exposure of 7 h, *T. japonicum* could scatter and parasitize host eggs in a radius of 1-6 m. Some parasitoids chose to lay eggs further out in the 6 m radius rather than closer in. Buchori *et al.*[35], for instance, found that the parasitoid dispersal pattern was clumped and that they spread to a radius of 9 m from the point of release. They also reported that the parasitoids were able to survive for 7 days following release.

In an ecosystem, the dynamics of a pest population was affected by biotic and abiotic components. Under the concept of tritrophic interactions, population growth was strongly affected by bottom-up factors (living facilities that encourage the growth of pest population) and top-down factors (the study of natural enemies to reduce the pest population). The widespread availability of rice plants in the appropriate condition and weather type for the development of *S. incertulas* will result in a rapid population growth during each rice-cropping period. Frequently, natural enemies are unable to enact a biological control function because of interference from the application of pesticides. Overcoming these problems requires a variety of control efforts. One environmentally friendly control effort is the release of *T. japonicum*. *T. japonicum* has been long recognized as a potential biological agent to control infestations of *S. incertulas* in rice crops. The effectiveness of *T. japonicum* in the field is affected by the harmony between the host eggs availability at the time of the parasitoids' appearance and the desired function of parasitoid eggs when other species work as competitors[31,33].

The release of *T. japonicum* in the field had a significant effect on parasitization intensity (p = 0.00). The parasitization intensity of *T. japonicum* with *C. cephalonica* traps was good at doses of 2,500 parasitoids/plot (125,000 parasitoids ha^{-1}). 5,000 parasitoids/plot (250,000 parasitoids ha^{-1}) did not differ significantly from controls. The usage of an *S. incertulas* trap, both at a dose of 125,000 parasitoids ha^{-1} and 250,000 parasitoids ha^{-1} differed significantly from controls. The usage of a dose of 250,000 parasitoids ha^{-1} was more effective than a dose of 125,000 parasitoids ha^{-1}. With an exposure to the host egg for 7 h, the average parasitization ability reached 9.10 eggs parasitized/egg-mass and 7.85 eggs parasitized/egg-mass, whereas on the control only 3.85 eggs were parasitized/egg-mass.

The release of *T. japonicum* had no effect on the parasitization intensity of *T. schoenobii*. The average *T. schoenobii* parasitization intensity was higher than the parasitization intensity of *T. japonicum* and *T. rowani*, both in the conditions in which *T. japonicum* was released or whether there was no release at all. This proves that *T. schoenobii* have a good dispersal rate and parasitization ability in the rice habitats of this particular regency. Conservation efforts should thus be considered: The usage of pesticides should be reduced in order not to hamper the role of parasitoids in controlling pest populations.

T. japonicum's parasitization intensity in nature was relatively low (3.85 eggs parasitized/egg-mass). The release of as many as 3 times the naturally-occurring population showed a rise from between 7.85-9.10 eggs parasitized/egg-mass. *T. japonicum*'s parasitization intensity could be increased by periodic mass release efforts. This would ensure that it can take a useful, larger role in the rice field ecosystem. A continuous periodic release would increase the intensity of parasitization significantly.

CONCLUSION

This research found that the highest *T. japonicum* parasitization ability occurred in the combinational treatment of one day old parasitoids and a host egg 24 h old or less. After being released in the field, *T. japonicum* spread out in all directions and could reach a distance of between 1 and 6 m radius after an exposure of 7 h. Using *T. japonicum* in the field successfully controlled the population of *S. incertulas*. The *T. japonicum* parasitization intensity over each *S. incertulas* egg-mass on the rice cultivation area used was naturally low, at average of 3.85 eggs parasitized/egg-mass. The parasitoid release in a dosage of 250,000 parasitoids ha^{-1} was more effective in reducing the population of *S. incertulas* in the field and successfully increased the intensity of parasitization by an average of 9.10 eggs parasitized/egg-mass (9.10/3.85 = 236% compared to control). The spontaneous appearance of the parasitoids *T. schoenobii* and *T. rowani* only occurred on the *S. incertulas* egg traps, we found that the population of *T. schoenobii* tended to be higher than those of the other parasitoid species.

SIGNIFICANCE STATEMENT

Various control techniques can be used to overcome rice stem borer (RSB) attacks. This research found that a 1 day old *T. japonicum* parasitoid was a more effective biocontrol agent compared with older parasitoids. Levels of parasitization were highest upon available host eggs less than 24 h old. Other than the *T. japonicum* parasitoid, also encountered *Telenomus schoenobii* and *Tetrastichus rowani* in the field. These parasitoids also attacked *S. incertulas* eggs. The *T. schoenobii* population tended to be higher than those of the other parasitoid species.

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