

Quality Control of Mass Rearing of Egg Parasitoids of Yellow Rice Stem Borer *Scirpophaga incertulas* Walker

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Abstract— The study was conducted to find out: suitability of eggs of *Corecya cephalonica* Stainton as a factitious host of *S. incertulas* egg parasitoids and techniques of parasitoid stock provision, serially conducted from February 2011 to December 2011. The researches were carried out in four laboratory experiments, consisting of the study of ultraviolet (UV) irradiation on *C. cephalonica* eggs; suitability of *C. cephalonica* eggs as a factitious host of *Telenomus rowani* Gahan, *Trichogramma japonicum* Ashmead and *Tetrastichus schoenobii* Ferriere; storage method of *C. cephalonica* eggs; and storage method of parasitoids. The research design was suited to the research needs. The results showed that; The *C. cephalonica* eggs irradiated by the minimum of 28.48 minute 15 watt ultraviolet (UV) at a distance +15 cm between the lamp and the eggs could suppress the hatching eggs to zero (no emerged larvae). Among the three species of egg parasitoids of *S. incertulas* found in Jambi Province, only *T. japonicum* could be rearing on *C. cephalonica* eggs. The relationship between storage duration of *C. cephalonica* eggs irradiated by the 30 minutes 15 watts UV at 5°C and the emerged *T. japonicum* wasps was expressed by the equation of $y = 31.04 - 1.151x$, $R^2 = 0.865$, $P = 0.000$. The storage of *T. japonicum* pupae for six weeks at 5°C did not reduce the number of emerged *T. japonicum* wasps. The emerged wasps reduced significantly if the storage duration of *T. japonicum* pupae was increased to seven weeks or more.

Keywords— Quality Control; Egg Parasitoid; *S. incertulas*; Factitious Host; *C. Cephalonica*.

I. INTRODUCTION

Rice stem borer (RSB) is the most important pests in rice [1]. *Scirpophaga incertulas* Walker is the most important RSB spesies throughout the world [2], [3]. There are three species of RSB eggs parasitoid that important in RSB controlling. These species were *Tetrastichus schoenobii* Ferriere, *Telenomus rowani* Gahan and *Trichogramma japonicum* Ashmead [4], [5], [6], [7], [8], [9], [10], [11]. The ability of parasitoids to control RSB was very depend on its species [4].

Trichogramma has been researched extensively and used successfully in biological control programs and could be reproduced on factitious host [12]. A large number of parasitoid is needed in order to apply inundative control. Therefore, suitable factitious host is needed and should be available for rearing the parasitoids. Reference [13], [14] shows that *Ephistia kuhniella* Zell, *Sitotroga cerealella* and *Corecya cephalonica* Stainton could be factitious host of *Trichogramma* spp.

Otherwise, until now there is no information about the insect that can be used as a factitious hosts of *T. rowani* and

T. schoenobii. Reference [15] shows that *T. schoenobii* could only be reared in eggs of *Scirpophaga*. The effort of in vitro rearing of parasitoid as in [16] leads the use of *T. schoenobii* in biological control. Mass production of *Trichogramma* spp. by using *C. cephalonica* eggs as factitious host has been carried out successfully [17], [18], [19], [20]. Reference [18] shows that *C. cephalonica* could be propagated easily and its eggs were very suitable as a factitious host for growing and development of *Trichogramma* spp. Mass production of *C. cephalonica* was conducted successfully [21], [22].

Stock supplying of *C. cephalonica* eggs as a factitious hosts encounter frequently obstacles due to hatch quickly and damaged easily. Reference [23] shows that *C. cephalonica* eggs irradiated by 15 watts ultraviolet lamp for 30 minutes could be sterile (unhatchable). Before being used as a factitious host, *C. cephalonica* eggs need to be irradiated by 15 watts UV for 60 minute [24], but reference [25] shows that *C. cephalonica* eggs need to be irradiated by 15 watts ultraviolet lamp for 15 minute.

It should be understood that the effectiveness of UV irradiation to sterile *C. cephalonica* eggs depend on the

irradiation boxes specification (cabinets) used, and the irradiation duration. After parasitoids can be produced massively, the important thing should be considered is how to package and store parasitoids in a long time so the stock always available when it is needed.

The supporting information about mass production and supplying of parasitoid stock is very limited. Therefore the research was carried out. The purpose of the research were to know the suitability of *C. cephalonica* eggs as a factitious host of *S. incertulas* egg parasitoids and to know the techniques for supplying parasitoid stock sustainability.

II. MATERIALS AND METHODS

The experiment was conducted at the Plant Protection Laboratory of Agriculture Faculty of Jambi University from February 2011 to December 2011.

A. Study of Ultraviolet Irradiation on *Corcyra cephalonica* eggs

Provision of *C. Cephalonica* eggs was carried out based on the technique performed as in [18], [19], [23], [26], [22]. *C. Cephalonica* Eggs were glued by arabic gum glue on pias paper. The number of eggs glued were 100 eggs per pias, and then put into 15 watt UV treatment box. The distance between UV lamp and eggs is ± 15 cm.

The treatments were the UV irradiation durations on *C. Cephalonica* Eggs consisting of 15, 30, 45, 60, 75, 90 minutes and control (without UV irradiation). Each treatment was repeated five times. After treatment, each replicate was inserted into a test tube and covered with tulle fabric and observed for 10 days. During the observations the number of hatched eggs (number emerged larvae), the number of unhatched eggs were recorded, and then the percentage of both hatched eggs and unhatched eggs were calculated. Data were analyzed using simple linear regression.

B. The Study of Suitability of *Corcyra cephalonica* Eggs for Rearing of *Telenomus rowani*, *Trichogramma japonicum* and *Tetrastichus schoenobii*

Provision of *T. rowani*, *T. japonicum* and *T. schoenobii* was done through parasitoid feeding by using *S. incertulas* eggs from rice agroecosystem in Muaro Jambi District of Jambi Province. Moths of *S. incertulas* were captured from the field (vegetative phase paddy) and maintained individually by using plastic bottles with diameter of 3.5 cm and height of 5 cm. Pieces of fresh rice leaves inserted into the bottle for moths laying eggs. The resulted eggs were taken every day by cutting the leaf where the eggs attached along ± 4 cm by using scissors.

The one day old *S. incertulas* egg groups was glued on the leaves of paddy crops by a stapler. Clumps of the paddy were marked by plastic rope. After two days the eggs were taken back (harvested) by using scissor and inserted separately into test tubes and labeled. *S. incertulas* eggs collected were maintained at ± 25 °C in Plant Protection Laboratory. During maintenance the egg groups was observed every day for 12 days. Emerged parasitoids were identified and separated according to species.

Provision of *C. Cephalonica* eggs was carried out based on the technique performed as in [18], [19], [23], [21], [22].

50 grains of *C. cephalonica* eggs were glued on 2 x 8 cm pias paper. The pias paper was inserted into a test tube with diameter 2.5 cm and height 20 cm. A pair of one day old *T. rowani* wasps was invested into the test tube. The Test tube was covered by tulle fabric and incubated for 12 days. The same treatments were also carried out for *T. japonicum* and *T. schoenobii*. Each treatment was repeated five times.

During the incubation period, all experimental units were observed daily and the number of parasitized *C. cephalonica* eggs, the number of both male and female of emerged parasitoid wasps were recorded, and the sex ratio of male and female was calculated. The data obtained was presented in tabular form and analyzed descriptively.

C. Study of Storage of *Corcyra cephalonica* Eggs

The treatments were the storage durations of *Corcyra cephalonica* Eggs, consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 weeks *C. cephalonica* Eggs storage duration at in the refrigerator at ± 5 °C and control (no-storage treatment). Each treatment including control was repeated five times. and each replicate consisting of 50 *Corcyra cephalonica* Eggs.

The *C. cephalonica* eggs and *T. japonicum* wasps were needed for this study. Provision of *C. cephalonica* eggs and *T. japonicum* wasps were based on the technique performed as in [19]. Before being treated, the one day old *C. cephalonica* eggs were irradiated by 15 watts UV for 30 minutes. Storage duration treatments were done gradually every week, starting at 9 week storage duration treatment. Following that, the treatments of 8, 7, 6, 5, 4, 3, 2, 1 week storage duration treatment and control (without storage) were done consecutively.

50 *C. cephalonica* eggs of each treatment were glued on 2 x 8 cm paper pias and put into a test tube that its diameter 2.5 cm, height 20 cm. Into the test tube was invested a pair of one day old *T. japonicum* wasps. The *T. japonicum* wasps used were the results of multiplication in the laboratory. The test tubes were covered with tulle fabric and incubated for 12 days. Each treatment was repeated five times.

Each experimental unit was observed each day for 12 days. During observations the number of parasitized *C. cephalonica* eggs and emerged *T. japonicum* wasps were noted. The data obtained were analyzed using simple linear regression.

D. Study of Storage of Parasitoid

Completely randomized design was used in this study. The treatments were the storage durations of *T. japonicum* pupae, consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 weeks *T. japonicum* pupae storage duration in the refrigerator at a temperature of 5 °C and control (no-storage treatment). Each treatment including control was repeated five times and each replicate consisting of ± 100 *T. japonicum* pupae.

T. japonicum pupae was obtained with the modified parasitoids propagation method as in [19], [20]. On pias paper was made 50 circular form template that its diameter 6 mm by paper puncher. One day old *C. cephalonica* eggs irradiated by 15 watts UV for 30 minutes were glued on those 50 template of pias paper by arabic gum glue.

The pias paper that had 50 egg groups of *C. cephalonica* was inserted into a test tube that its diameter of 2.5 cm and height of 20 cm which already contains a pias of one day old *T. japonicum* wasps (about 2,000 *T. japonicum* wasps). Color of *C. cephalonica* eggs parasitized by *T. japonicum* changed to be black in 3-4 days after being invested by *T. japonicum* wasps, and become pupae at 6 day after invested.

The *T. japonicum* pupae (6 days old parasitoid pias) were excluded from the test tube and treated by storage duration, consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9 weeks pupae storage duration in the refrigerator at $\pm 5^\circ\text{C}$ and control (no-treatment of storage pupae). Each treatment including control was repeated five times and each replicate consisting of 1 group of *T. japonicum* pupae (± 100 *T. japonicum* pupae).

After storage treatment, parasitoid pias was removed from the freezer and placed in a room at $\pm 25^\circ\text{C}$. Observation were made to see the emergence of *T. japonicum* wasps. Observation was made after all appeared parasitoid wasps were dead. In observation the number of pupae treated, the number both male and female emerged parasitoid wasps were recorded. Then the proportion of emerged parasitoid wasps and the sex ratio of male and female were calculated. Data were analyzed by analysis of variance and Duncan multiple rang test at the level of $\alpha = 0.05$.

III. RESULTS AND DISCUSSION

A. Ultraviolet Irradiation on *Corcyra cephalonica* Eggs

The relationship between the 15 watt ultraviolet radiation at a distance of light and eggs + 15 cm and hatching of *C. cephalonica* eggs was expressed by the equation of $y = 81.26 - 2.853x$, $R = 0.963$, $R^2 = 0.927$, $P = 0.000$, where y = the number of hatched *C. cephalonica* eggs (%) and x = time duration of 15 watt UV irradiation (Fig. 1).

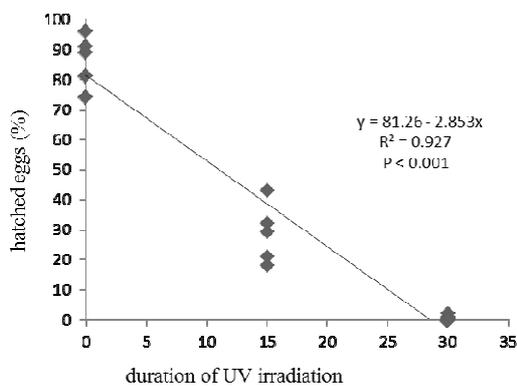


Fig.1. The relationship between the 15 watt ultraviolet radiation and hatching of *C. cephalonica* eggs

The model shows that hatched eggs proportion decrease linearly if duration of UV irradiation increase. In order to produce no hatched *C. cephalonica*, minimum 28.48 minutes of the 15 watt UV irradiation was needed. This is contrast with [24] shows that *C. cephalonica* eggs irradiated by 15 watts UV for 90 minutes still be able to hatch as much as 0.75%. This difference is likely due to differences in equipment specifications which were used. Reference [14] did not explain how the distance between the UV lamp and

C. cephalonica eggs. In this study, the distance between the UV lamp and *C. cephalonica* eggs was + 15 cm.

The model also indicates that *C. cephalonica* eggs could still hatch if being irradiated by 15 watt UV less than 28.48 minutes. The emerge larvae from *C. cephalonica* eggs used as factitious host for rearing parasitoid is very disturbing and will determine the success of parasitoid rearing. Emerge *C. cephalonica* larvae in parasitoid could eat and glue *C. cephalonica* eggs and parasitoid in it, finally parasitoid died. Therefore, the supply of *C. cephalonica* eggs as factitious hosts must be treated by 15 watt UV at least 28.48 minutes at the distance between lamp and eggs is ± 15 cm. For practical purpose, it is advised to use the 30 minutes duration of UV irradiation. This is in line with the [23] that shows there is not any hatching *C. cephalonica* eggs if it is irradiated by 15 UV watts for 30 minutes. Therefore it is 15 UV irradiation duration that shorter than 30 minutes is not good because most of the *C. cephalonica* eggs could still develop into larvae.

B. Suitability of *Corcyra cephalonica* Eggs for Breeding *Telenomus rowani*, *Trichogramma japonicum* and *Tetrastichus schoenobii*

The research shows that *T. rowani*, *T. schoenobii* could not be reared in *C. cephalonica* eggs. There was no *C. cephalonica* egg invested by *T. rowani* and *T. schoenobii* wasps that invested to *C. cephalonica* eggs in test tube. Appeared *T. rowani* and *T. schoenobii* wasps was not interested to lay their eggs on *C. cephalonica* eggs, so no parasitized *C. cephalonica* eggs, and failed continue their descendants. This in contrast to *T. japonicum* that could be reared on *C. cephalonica* eggs (Table 1).

TABLE I
CORCYRA CEPHALONICA EGGS SUITABILITY FOR BREEDING
TELENOMUS ROWANI, TRICHOGRAMMA JAPONICUM AND
TETRASTICHUS SCHOENOBII

Parasitoid Species	Sum of <i>Corcyra cephalonica</i> eggs treated(n)	Mean of parasitized eggs (grains)	Mean of off spring/betina		Specification
			male	famele	
<i>Tr</i>	50	0	-	-	Not compatible
<i>Tj</i>	50	23.4	4	16.4	Compatible
<i>Ts</i>	50	0	-	-	Not compatible

Ts = *Tetrastichus schoenobii*, *Tr* = *Telenomus rowani*, *Tj* = *Trichogramma japonicum*

The result of the study reinforce reference [12] shows that the *T. japonicum* could be reared massely with *factitious host*. Reference [4] shows that *T. schoenobii* could be cultured only in Scirpophaga eggs. The effort of in vitro rearing of *T. schoenobii* as in [16] leads the use of *T. schoenobii* in RSB biological control. Reference [4] shows that given the socio-economic conditions of rice farmers in Indonesia in general, presumably habitat management efforts to support the development of *T. schoenobii* much more appropriate than application of parasitoid release. For this purpose, the *T. schoenobii* bioecology is absolutely necessary to be understood.

Development of utilization of the three species of RSB parasitoid needs to be done continuously in various ways. Although *T. rowani* and *T. schoenobii* could not be reared on factitious hosts, increasing of the utilization of *T. rowani* and *T. schoenobii* might be done through conservation and captive breeding of RSB egg parasitoids to be released back to rice agro-ecosystem.

Conservation could be done by applying culture techniques of rice that might create a favorable environment for the development of the parasitoid. To maintain wild plants presence around the paddy crop as an alternative habitat and the parasitoid feed providers and to limit using pesticides minimally as few as possible are concrete actions that could be done to conserve the parasitoids.

Captive breeding of parasitoids might be done by collecting RSB eggs from the field. The RSB eggs collected is put in test tube and incubated in the laboratory until it develop to be RSB larvae or its parasitoids appear. The appearing RSB eggs parasitoid is released back out to the paddy agroecosystem, and the appearing RSB larvae are prevented to move from test tube to paddy crops by glue or gasolin or water, and than the larva died.

Another way for RSB eggs parasitoid captive breeding is by feeding (baiting) RSB egg parasitoids with *S. incertulas* eggs in the field and maintained in the laboratory. *S. incertulas* moths are caught from field (paddy crops). The *S. incertulas* moths collected are maintained individually in transparent plastic bottles. Pieces of fresh rice leaf put into the bottles for moths laying eggs. The eggs resulted are harvested every day by cutting the 4 cm long leaf where the eggs attached. The egg groups are used as RSB egg parasitoids bait. The RSB egg groups are attached on paddy leaf by using a stapler. Clumps of the paddy placed by the RSB egg are marked by plastic rope. After two days the eggs are taken back (harvested) by using scissors and put in a test tube and covered by a transparent colored tulle fabric. A test tube may contain one or several groups of eggs. Then the egg groups are incubated at $\pm 25^{\circ}\text{C}$ in plant protection laboratory until they develop to be RSB larvae or its parasitoids appear. The emerge RSB egg parasitoids wasps are released back to the paddy agroecosystem, and the emerge RSB larvae are prevented to move from test tube to paddy crops by glue or gasoline or water, and than the larva died.

The use of *T. japonicum*, beside it can be done through the conservation and captitive breeding, it can also be done through inundation. Inundation should be done at high levels *S. incertulas* population and low level *S. incertulas* eggs parasitization. Inundation must be supported by the parasitoid rearing ability massely. *T. japonicum* mass rearing can be done by using *C. cephalonica* eggs as factitious host.

C. Relationship between storage duration of *C.cephalonica* Eggs and emerged *T. japonicum* wasps.

The relationship between storage duration of *C. cephalonica* egg irradiated by 15 watt UV for 30 minutes at 5°C and emerged *T. japonicum* could be expressed by the equation of $y = 31.04 - 1.151x$, $R = 0.930$, $R^2 = 0.865$, $P = 0.000$, where y = the number of emerged *T. japonicum* wasps, and x = dutation (days) (Fig. 2).

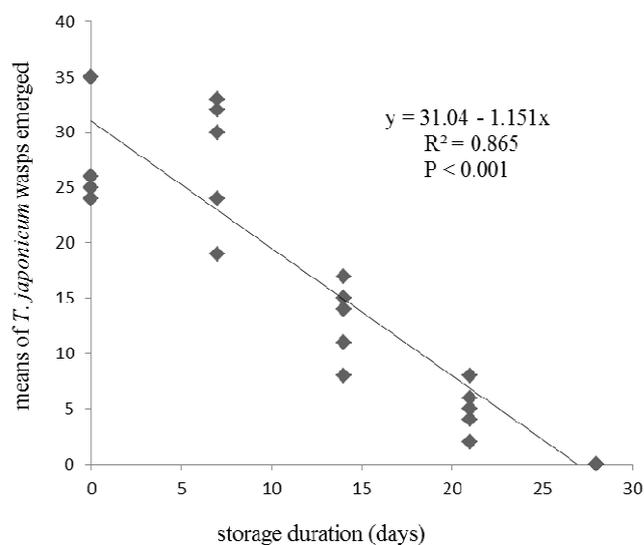


Fig.2. The relationship between storage duration of *C. cephalonica* egg irradiated by 15 watt UV for 30 minut at 5°C and emerged *T. japonicum*

From the formula is known that the *C. cephalonica* eggs store before being used as factitious host decreased their suitability for rearing *T. japonicum*. The model shows that the *C. cephalonica* eggs storage before being used as factitious host for rearing *T. japonicum* must decrease the number of *T. japonicum* wasps resulted. The number of emerged *T. japonicum* wasps decrease significantly if storage duration of *C. cephalonica* eggs used as factitious host is increased sharply. If *C. cephalonica* eggs used as factitious host for rearing *T. japonicum* was which had been stored at $+5^{\circ}\text{C}$ for 26.97 days or more, there was not *T. japonicum* wasps resulted. Reference [25] shows that *C. cephalonica* eggs before being used for breeding parasitoid could be stored in freezer but did not explain how storage duration could be done.

To determine how long duration *C. cephalonica* eggs storage must be done. It depends on how much maximum risk of failure may be incurred and the urgency of parasitoid rearing. The model indicates that if the acceptable maximum risk of *C. cephalonica* eggs storage duration is 50%, the maximum *C. cephalonica* eggs storage duration able be done is 13.48 days.

D. Parasitoid Storage

After being stored in a refrigerator at a temperature of 5°C for 6 weeks, *T. japonicum* pupae was still very good and did not interfere parasitoid development. The emerged *T. japonicum* wasps proportion from *T. japonicum* pupae stored for 1, 2, 3, 4, 5 and 6 weeks were not different significantly than controls. The emerged *T. japonicum* wasps proportion from *T. japonicum* pupae stored for 7 weeks or more was decreased significantly than controls, event though the emerged *T. japonicum* wasps proportion was still reached 0.586 (Table 2).

TABLE II

THE INFLUENCE OF TRICHOGRAMMA JAPONICUM PUPAE STORAGE DURATION AT 5 °C TO EMERGED TRICHOGRAMMA JAPONICUM WASPS

storage duration at 5 °C (week)	Total of pupae treated (n)	Means of emerged <i>Trichogramma japonicum</i> wasps proportion	Means of sex ratio of male and female
Tidak disimpan	501	0.820a	0.244a
1	579	0.818a	0.288a
2	566	0.814a	0.276a
3	564	0.798ab	0.304a
4	573	0.770ab	0.310a
5	570	0.764ab	0.292a
6	574	0.738abc	0.260a
7	600	0.694bc	0.256a
8	583	0.648cd	0.310a
9	592	0.586d	0.324a

Means within a column followed by the same letter are not significantly different according to the Duncan's Multiple Rang Test. at $p \leq 0.05$ level.

In all treatments the *T. japonicum* wasps was emerged 1-3 days after parasitoid was removed from the refrigerator. Therefore parasitoid storage need the ensure of electricity supply to refrigerator life continuously. The *T. japonicum* pupae may develop into *T. japonicum* wasps in refrigerator if storage temperature increase significantly that caused by electricity supply disconnected. Therefore the results of the research could be an input for designing packaging equipment to take parasitoid to field so it would not develop into wasps on the way.

IV. CONCLUSION AND RECOMMENDATIONS

A. Conclusion

Among the three species of egg parasitoids of *S. incertulas* found, only *T. japonicum* could be mass reared on eggs of *C. cephalonica*. *C. cephalonica* eggs irradiated by 15 watts ultraviolet at least 28.48 minutes at a distance between lamp and eggs ± 15 cm could suppress *C. cephalonica* eggs hatching to be zero (no larvae emerged) Irradiated *C. cephalonica* eggs storage used as factitious host for *T. japonicum* mass rearing could suppress emerged *T. japonicum* wasps. *C. cephalonica* eggs irradiated storage at ± 5 °C for 26.97 days or more may not be used as factitious host for *T. japonicum* mass rearing. *T. japonicum* pupae stored for maximum 6 weeks at 5 °C did not reduce the number of emerged *T. japonicum* wasps, but number of emerged *T. japonicum* wasps reduced significantly if *T. japonicum* pupae storage duration was done for seven weeks or more. *T. japonicum* pupae storage did not influence significantly to sex ratio of emerged *T. japonicum* wasps.

B. Recommendations

The utilization of egg parasitoids for control of RSB are suggested to do the three techniques compatibly, consisting of conservation, captive breeding and inundation. In *T. japonicum* mass production, it is suggested to use *C. cephalonica* eggs irradiated by 15 watt ultraviolet for 30 minutes at a distance between lamp and *C. cephalonica* eggs ± 15 cm as factitious host. *C. cephalonica* eggs used must be fresh, and do not use *C. cephalonica* eggs stored in the refrigerator for some days. For the supply of *T. japonicum* stockes are advised to deposit *T. japonicum*

pupae (parasitoid wasps aged 6 days after the investment) in a sealed container in the refrigerator at a temperature of 5 °C. In inundation, it is recommended to use parasitoid stockes stored no more than 6 weeks.

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