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SUITABILITY OF *CORCYRA CEPHALONICA* EGGS PARASITIZED WITH *TRICHOGRAMMA JAPONICUM* AS INTERMEDIATE HOST AGAINST SUGARCANE BORER *CHILO AURICILIUS*

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Abstract

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Chillo auricilius is pest of sugarcane causing decrease of production. Biology control utilizing eggs parasite *Trichogramma japonicum* was studied. This study aims to determine the suitability of *Corcyra cephalonica* eggs which was parasitized with *Trichogramma japonicum* on stem borer (*Chilo auricilius*) and to determine number of eggs of the rice moth (*Corcyra cephalonica*) which was most effective as alternative host. The experiment was conducted at laboratory of Agronomy Research Center of Sugarcane Plantation of Jatitujuh, West Java. Indonesia. The experiment was arranged in a completely randomized design with a factor consisting of six levels (number of parasitized *C. cephalonica* egg: 150, 300, 450, 600, 750, 900 eggs. Each level was added with 100 more eggs of *C. auricilius*) and they were replicated thrice. Parameters were: Correlation between meteorological and larva emergence; parasitized eggs; larva emerged; and period of larva emergence. Correlation analysis and Anova were applied. The results showed that humidity were correlated with larva emergence ($R^2 = 0.7147$). The treatment P6 resulted the highest parasitized (95.33%) and the lowest (3%). *T. japonicum* was effective parasitoid to control sugarcane borer, and *C. cephalonica* was suitable to be the alternative or the intermediate host to guaranty the availability and sustainability of host at all the time.

Key words: chemically; host; larva; parasitized; sustainability

Introduction

It is said in these decades, that the negative side effects of insecticides application have increased usage of natural enemies appears to be very helpful in biological control programs Nikbin et al., (2014). Biological control of pest will avoid ecology contamination of chemical pesticides, be more efficient, be sustainable, do not initiate destruction to ecology, and be compatible to other methods of pest control (Laba and Kartohardjono, 1998; Maneerat and Suasa-ard (2015). Chen et al. (2010) reported efficacy of *Trichogramma japonicum* (*Hymenoptera: Trichogrammatidae*) against paddy pests, and found that *T. japonicum* could control the paddy plant borers effectively.

Trichogramma spp. is an important egg parasitoid of lepidopterous pests with effective to control the sugarcane stem borers. Ahmad et al. (2012) stated that the interest in these parasitoids (*Trichogramma* spp.) as biocontrol agent is evident because they kill the pest at the most critical stage (the egg) before the damage occurs (Goebel et al., 2010). It was reported that release of *Trichogramma spp.* reduced the infestation level of sugar cane stem borer (Sattar et al., 2016).

It was reported that parasitism efficiency of *Trichogramma* spp. on the eggs *C. Cephalonica* was 93.86% (Begum et al., 2013). Rearing *Trichogrammaspp.*using *C. Cephalonica* eggs as alternative host is agro-technically feasible. Potential for using cool-stored eggs for parasitization was evident.

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This technique make possible the storing egg for long period of time (Singhamuni et al., 2015)

Rice moth *Corcyra cephalonica* is available in all seasons that it can be best as the intermediate host in time the stem borer is abstain in the field of sugarcane plantation. Famale of *Trichogramma spp* will oviposit their eggs in *C. Cephalonica* eggs as the alternative host.

Reported there is significant influence of rice moth *C.cephalonica* on development on survival and reproduction of predatory phytoseiid mites (Buchori et al., 2010; Nasr et al., 2015). Fictitious host insect *C.cephalonica* eggs are used for production of several natural enemies like *Trichogramma* spp. (Jalali et al., 2007).

Intermediate or fictitious host for *Trichogramma* spp. must be available at all the time because as stated by Buchori et al. (2010) that larva phase of parasitoid will only well develop on host of a certain phase (egg phase). If female parasitoid is to oviposit its eggs but at that time there is no host of the right phase, parasitoid will not be effective.

The intermediate host must be reared in well manner to keep it available at all the time it is needed. Generally, small animal as insect and moth are susceptible to the prevailing environmental condition as the meteorological elements (temperature and humidity). Sugarcane planters have to keep in mind and take into account the atmospheric condition to assure the *T.japonicum* is active and the sugarcane plantation free of the stem borer out-break (Parveen and Sultan, 2012) found that Trichogramma spp respects to rearing temperature and host egg age of the angoumo is grain moth, maximum parasitism was observed 84.3% at 28°C, while minimum parasitism was 39.6% at 32°C on *C.cephalonica* egg.

Outside the laboratory or in the field of sugar plantation application of *C.cephalonica* is susceptible to predation. Ant is the predator of *C.cephalonica* making the fictitious host unable to function properly. To encounter this occurrence, Geetha (2011) recommended some methods to escape the application of *C. cephalonica* from predation of ant by placing at least 100 eggs which were pasted in individual cards of size 8 x 3 cm.This technique will be the consideration for formulating the experimentation in laboratory. Mehendale and Shinde (2014) quoted other sources that the present investigation was manipulation for getting good quality eggs through enhanced nourishment of *C.cephalonica* larvae. Thus, good quality egg parasitoid, *Trichogramma* spp could be utilized through inundative release for the management of many lepidopterous insect pest.

It is very important to find another host to keep the parasitoid *T.japonicum* available at all the time, but the hint of difficult life and culture of *T. japonicum* and the intermediate host will be the consideration in formulation of treatment in the experiment to be carried out. Application of parasitoid *T.japonicum*species with intermediate or alternative or fictitious host of rice moth (*Corcyra cephalonica)* will make the control of stem borer *(C. auricilius)* more intensive and sustainable.

Materials and Methods

The study was conducted at the Laboratory of Natural Pest Control of the Research Centre for Agronomy Unit II of Jatitujuh Sugarcane Plantation, Majalengka subistrict. Its geographical coordinates are 6° 39› 0» South, 108° 13› 0» East, West Java Province, Indonesia, on April 2015 until June 2015.

Considering that placing eggs of *C. cephalonica*inthe field (plantation) is susceptible to predators as the experiment done by (Geetha, 2011) and (Perveen and Sultan, 2012) setting an experiment with treatment fresh eggs of 2 hours old of *C. Cephalonica* eggs at density more than 100.

In this laboratory experiment were tested the treatment levels with more than 100 eggs of *C. cephalonica*and the multiple of it.

Host and pest materials and treatments

Materials were parasitoid *Trichogramma japonicum* and fictitious/intermediate host used were eggs of *Corcyra cephalonica,* pest (eggs of *Chilo auricilius*) a day after the stem borers laid their eggs.Cards of *C. cephalonica* eggs which was parasited with *Trichogramma japonicum* (eggs of aday-old after being parasitized); sugarcane plants of 5 month-6 month-old. By referring to experiments conducted by Geetha (2011) and Parveen and Sultan (2012) the number of eggs as treatment were more than 100 eggs. Experimental design used was Completely Randomized Design with a factor of six levels and they were replicated thrice. The treatments were eggs of *C. cephalonica* parasitized with *Trichogramma japonicum,*and eggs of the stem borer (*Chilo auricilius)* were:

P_1 = Card1 (150 eggs of *Cc*parasitized with *Tj* with 100 eggs of *C. auricilius*)

P_2 = Card 2 (300 eggs of *Cc* parasitized with *Tj* with 100 eggs of *C. auricilius*)

P_3 = Card 3 (450 eggs of *Cc*parasitized with *Tj* with 100 eggs of *C. auricilius*)

P_4 = Card 4(600 eggs of *Cc*parasitized with *Tj* with 100 eggs of *C. auricilius*)

P_5 = Card5 (750 eggs of *Cc*parasitized with *Tj* with 100 eggs of *C. auricilius*)

P_6 = Card 6 (900 eggs of *Cc*parasitized with *Tj* with 100 eggs of *C. auricilius*).

Notes: Cc = *Corcyra cephalonica;Tj = Trichogramma-japonicum*

Observed and recorded data, and measurements

➢ Common and scientific senses say meteorological elements affect the life of living thing as plant pests. To reveal this assumption and prediction, room temperature and humidity were recorded twice a day at 06.00 AM and 15.00 PM. Data of temperature and humidity were analyzed with correlation analysis. They were correlated with the emergence of larva.

➢ Percentage of Parasitized *Chilo auriciliusegg* (%)

The percentage was measured with counting the parasitized eggs at 6 days after application. The counting formula as follow:

$$P = \frac{A}{B} \times 100\%$$ Pabbage and Tandiabang, (2011)

Notes: P = Percentage of parasitized eggs; A = Number of parasitized eggs; B = Total number of eggs.

➢ Period of stem borer larva emergence

Period of stem borer larva emergence was recorded at the sixth day, because at the average the eggs of the borer will hatch at the fifth days after being laid/oviposited.

➢ Number of larva of stem borer

Number of larva of stem borer per Ca rd were counted after all *Trichogramma japonicum* died. Counting was done under the objective of microscope.

Execution of Experiment

The experiment began with preparation of tested insects. Reproduction of population of borers (*Chilo auricilius)*, reproduction the intermediate host (*Corcyra cephalonica)*, replication population of parasitoid *Trichogramma japonicum*, and inoculation of parasitoid *Trichogramma japonicum* to *Chilo auricilius* by mean of intermediate *Corcyra cephalonica* eggs.

Preparing the Tested Insects, Reproduction of the Sugarcane Borers

Chilo auricilius was caught and collected from the sugarcane plantation at the larval stage. To avoid heterogeneity the stem borer were reared and reproduced in glass jars. Cutting of cane leaves were put and arranged inside the glass jars. The cane leaves were of the sugarcane plant of 5-7 months-old. Young borers were kept in the glass jars to ensure the sufficient number of eggs are always available.

Reproduction of Corcyra cephalonica

Reproduction of *Corcyra cephalonica* as intermediates host was conducted 6 weeks before the mass reproduction of *T.japonicum*. Life cycle of *C. cephalonica* around 5-7 weeks and the cycle was affected by environment temperature and humidity.

The reproduction and rearing of *C. cephalonicais* as follow: Rearing media of *Corcyra cephalonica* consists of broken rice and grounded corn. This mix of rice and corn were sterilized by mean of being roasted or dried in oven at 120°C for 20 minutes. The medium was put into a container forms as the medium layer of around 3 cm-thick, and the volume was about 5 kg container^{-1}.

Placing the Eggs or Larva of Corcyra cephalonica

Placing the eggs or larva of *C.cephalonica* in rearing container prepared with its feed, and population density is 2-3 eggs or larva cm^{-1}. The larva will grow and develop and become young wasp within six weeks. The young *C.cephalonica* was reared in the rearing container (glass jars). It is predicted within 2 months of culturing, there will be young C.*cephalonica.* The young rice moths reared in the container were translocated to other fresh media. The eggs of *C. Cephalonica* must be collected every day to keep the eggs in fresh and healthy. Translocation and handling reproduction of eggs especially for the reproduction of *T. japonicum* was performed by using brush. Parasitoid eggs must be sterilized by using ultra violet light as long as 20 minutes.

Preparing Cards

Cards of 8.5 cm x 4.5 cm. Put the eggs on the card pasted the eggs on the card by making a thin layer of glue, so as the eggs steadily stick on the card. The cards with the eggs of *C. cephalonica* are ready for the media or place of *T.japonicum* reproduction (Figure 1).

Reproduction of Trichogramma japonicum

Trichogramma japonicum reproduction was prepared in several ways. One way of them is: After cards are ready, take the parasitizing container and put the cards in it. Put into the card the starter parasitoid *Trichogramma* and the cards of eggs of *Corcyra cephalonica* with a proportion of 4 cards of starter parasitoid inoculated with 24 cards of sterile *C. cephalonica* eggs.

➢ Parasitizing container is covered with clothes black or dark in colour. This curtain (dark in colour) is to initiate the parasitoid to direct to lay eggs. The parasitoid is found of light then tie up with rubber string and put it on shelves file.

➢ Parasitizing process take place for 24 hours, and within 3-4 days the parasitized eggs of *Corcyra cephalonica* colour will turn to blackish. When the colour changed, it means the parasitizing is success.

Infestation of Parasitoid Trichogramma japonicum to Chilo auricilius.

Parasitoid *Trichogramma japonicum* was infested to eggs of *Chilo auricilius* as host. Number of eggs of the bor-

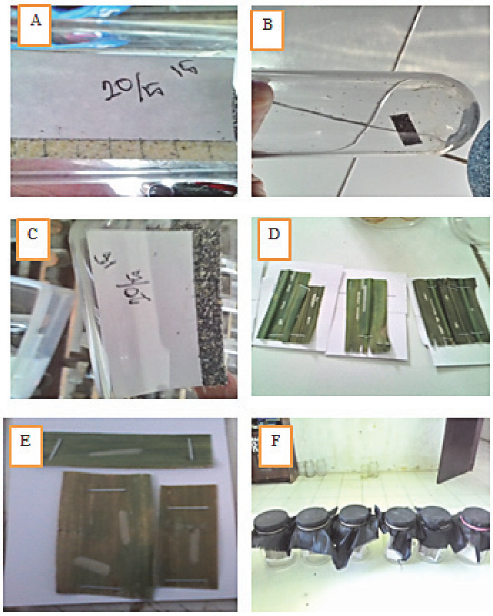

**Fig. 1. (A) *Corcyra cephalonica* eggs;
(B) *Trichogramma* sp.starter
(C) *Corcyra cephalonica* eggs parasitized with *Trichogramma*sp.
(D) and (E) *Chilo auricilius* eggs;
(F) Infestation *Trichogramma* sp. on cane stem borer**

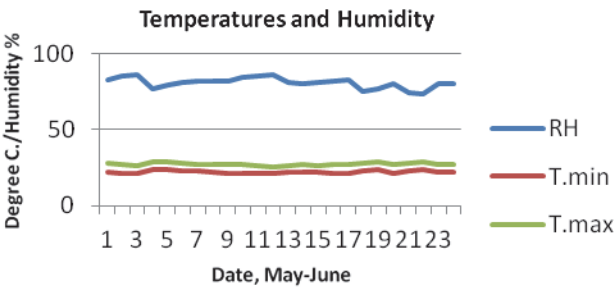

Fig. 2. Meteorological during study, days without preparing period

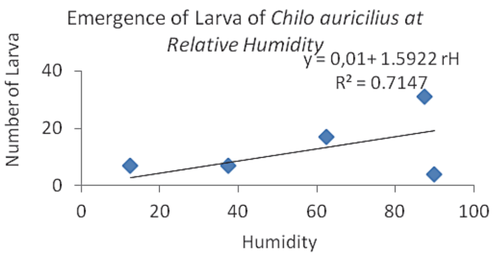

Fig. 3. Correlation between humidity and larvae emergence

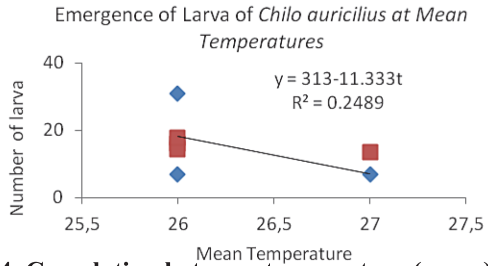

Fig. 4. Correlation between temperature (mean) and larvae emergence

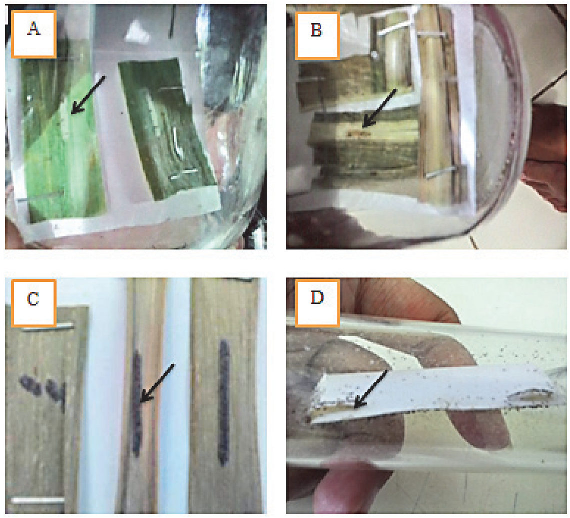

Fig. 5. A. *T.japonicum* is ovipositing the borer eggs; B. Sign of borer eggs to emerge the Instar-1; C. parasitized eggs of cane borer; D. Instar-1 larva emerged

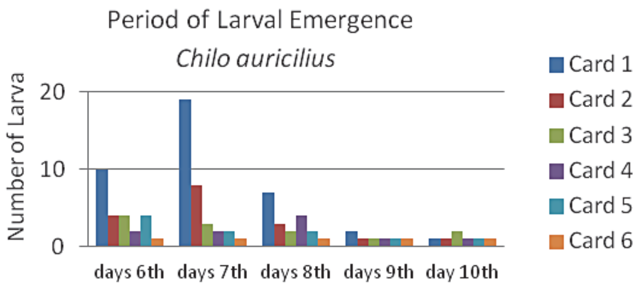

Fig. 6. Period of larva of *Chilo auricilius* emergence

ers are 100 (one hundred). Cards are prepared measurement 8.5 cm x 4.5 cm, card is cut and put into glass jar. And then eggs of *Corcyra cephalonica* which were parasitized with *Trichogramma japonicum* in accordance with the treatments arrangement are put into the glass jar (which contain 100 eggs of stem borers. Then put them into flask (the number of each is in accordance with the treatments arrangement). Count the number of eggs of *C. auricilius* that are parasitized and that are not parasitized. Count the larva of instar-1 that emerges out of the eggs of *C. auricilius*, and count the length or duration of process of parasitizing of the eggs of *C. auricilius*. Data were analyzed with Anova and Regression to see their correlation and the possible predict.

Results and Discussions

Meteorological Effects

The most influencing meteorological elements to the experiment were humidity and temperature. Results are presented in Figures 2, 3, and 4. There was correlation between meteorological (temperature and humidity) and larva emergence of *Chilo auricilius.*Elements of meteorology influenced the life of *Trichogramma japonicum* and the stem borer (*Chilo auricilius)* as well.

Cox et al. (1981) reported humidity limits for complete development of *C.cephalonica* rom egg hatch to adult emergence were about 70% RH, highest survival and most rapid development occurred at 30°C–32.5°C and 70% RH. Development was completed in the range 15%–80% RH, but few adults emerged at 15% RH and none at 90% RH unless a mould-inhibitor was present in the food.

Sapdi, (2008) reported that the activities of parasitoid *Trichogramma japonicum*were higher at the temperature 25°C–27°C, and (Bayram et al., 2004) revealed that in general parasitoid *Trichogramma japonicum*prevailed best at temperature of 20°C–30°C. Oztemzi and Kornofior (2007) reported with respect to parasitismrate, flood irrigation is a better system than sprinkler irrigation. Sprinkler irrigation directly increases humidity of the atmosphere.

Djuwarno and Wikardi (1997) stated if *Trichogramma japonicum* get mal-nutrition or unsuitable nutrition, it will not develop a healthy larva and stop in pre-nymph stage. And the thickness of egg shell will also influence on the ability of newly adult wash to oviposit their eggs. Indirectly this case will cause the mortality of pre-adult of *Trichogramma japonicum.*

Figures 3 and 4 show number of *Chilo auricilius* larva was strongly correlated with humidity (R^2=0.7147) and less strong with temperature (R^2=0.2489). (Sattar et al., 2016) stated that temperature rise more than 40°C in month of June and relative humidity below than 40% that direct hit on borer population.

Percentage of parasitized sugarcane borer eggs

Analysis result presented in Table 1 shows analysis of percentage of parasitized sugarcane stem borer *Chiloauricilius,* there was only treatment P_6 different significantly.

Table 1
Percent of Parasitized Sugarcane Borer Eggs (*Chilo auricilius*)

Treatments	Percent of Parasitized Egg *Chilo auricilius* (%)
P_1(1 Card)	53.00 a
P_2(2 Card)	64.33b
P_3 (3 Card)	72.33 b
P_4 (4 Card)	76.67b
P_5 (5 Card)	77.67b
P_6 (6 Card)	95.33 c

Note: Differences between means at P ≥ 0.05 are indicated by different letters

Tabel 1 show the percentage of parasitized borer eggs as stated by (Rauf, 2000) that parasitism rate *Trichogramma japonicum* were 68.4% up to 94.8%, and the percentages were classified as high. The treatment P_1-P_6 show that the more parasitized eggs of *Corcyra cephalonica*applied the higher percentage of parasitized stem borers. The most effective and highest parasitism of *Trichogramma japonicum is the treatment* P_6 with 95.33%. It means the parasitism rate of *Trichogramma japonicum* against the stem borers *Chilo auricilius* eggs was high and affective.

The percentage level of effectiveness of *Corcyra cephalonica* was more or less the same with the levels reported by previous studies of *Trichogramma* parasitoid effectiveness. In this case reported application of *Trichogramma* parasitoid could lower the stem borer infestation to 1.25% level (Saljogi and Walayati, 2013). Ahmad et al. (2012) quoted some reports that *Trichogramma* is an important egg parasitoid with effective control against of the sugarcane stem borers worldwide, in Canada and the former USSR it reduced the damage up to 70% to 92% on sugarcane, and in Asia, it reduced the incidence of pests in sugarcane by early shoot borer from 43% to 82%. While Goebel et al. (2011); Goebel et al. (2013) reported that *Trichogramma* released could drop the stem borers infestation to minimum level of 9.8%, though it is higher than insecticide dropping down to level 4.6% but it is still lower than untreated control dropping to level of 14.4% stem borer infestation. Padmasri and Sudh rani (2014) reported the decrease of infestation of cane borers to 70.70% in 2011, and 53.87% in 2012.

Percentage of the Sugarcane Borers Chilo auricilius Larvae Emerged

There was significant different in number of the borers (*Chilo auricilius)* larva emerged as presented in Table 2.

Table 2
Percentage of number of the stem borers larvae emerged (*C.auricilius*)

Treatments	Number of *C. auricilius*larvae emerged (%)
P_1 (1Card)	42.33 c
P_2 (2 Card)	26.67 b
P_3 (3 Card)	14.00b
P_4 (4 Card)	7.33 a
P_5 (5 Card)	5.67 a
P_6(6 Card)	3.00 a

Note: Differences between means at P ≥ 0.05 are indicated by different letters

The treatment of P_6 showed less number of larva emerged as a result of more eggs were parasitized as shown in Table 1 and Table 2. Percentage of larva of borers emergence was 3% indicated that the rice moth (*C.cephalonica*) applied was suitable and affective as intermediate host for *T. japonicum* parasitoid life in absent of the stem borer eggs.

Figure 5 illustrates the dynamic of *T.japonicum* : ovipositing eggs , emergencing, Parasitizing, Eggs of Cane Borer.

Number of *C. cephalonica* affected number of larvae of *C. auricilius* emerged was also reported by Susniahti and Susanto, (2005) noted that number of larvae developed in the second generation was affected by *C. cephalonica* eggs age.

Parasitism of parasitoid was influenced with the interaction between parasitoid and its host. Detailed that the interaction depended on the egg age, process of host selection by parasitoid, and quality of host egg nutrition (Susniahti and Susanto, 2005).

Period of Chilo auricilius Larva Emergence

Egg of *Chilo auricilius* survived from parasitoid of *Trichogramma* are white (milky white), then turn violet and more transparent. After hatching the egg outer shell turn red in colour. Eggs usually hatch in the morning, and the 2 cm long of larva emerge. The size captive of head is bigger than the body.

Figure 6 shows the period of Larva of *Chilo auricilius* Emergence. The first instar larva of sugarcane borers began to emerge at the sixth days till the 10^{th} day. The biggest number of emergence was at the seventh days. The appearance of the borer larvae is shown in Annex.

Noted the number of cane borers emerged or hatched from eggs of *Chilo auricilius* is depended on the egg resistances to parasitoid. Beginning from the development of egg to embryo, mitoses, blastulation and gastulation phases. Embryo developed within 72 hours. At the same time embryonal cells and organ are developing inside the egg, the parasitoid larva are also developing. They compete each other in nutrition. Physiological process of an egg goes so rapidly. Nutrition is utilized to supply energy for the metabolism of both parasitoid and the borer (Susniahti and Susanto, 2005).

As it was illustrated in Figure 5 and 6 means the period emergence of cane borers was in accordance with the report (Anderson and Nguyen, 2012) that the larva of *C. auricilius* emerged at the 5^{th} or 6^{th}days. Sattar et al. (2016) explained that egg to larval development of *Trichogramma* spp. was completed within the period of 5-7 days. However there were some eggs hatching later caused by some factors as the influences of parasitoid and competition between the parasitoid and the host (sugarcane borers) resulted in late emergence of larvae. Ayvasi at al. (2008) reported that increased parasitoid density caused an increase in the rate of parasitism, and increasing parasitoid density also caused a reduction in the rate of stem borer emergence.

Conclusion

The eggs of rice moth (*Corcyra cephalonica)* parasitized with *Trichogramma japonicum* was suitable to be the host of parasitoid which was proved with the affective parasitism on stem borers *(Chilo auricilius*). Treatment P6 at 900 eggs of *Corcyra cephalonica* caused the lowest emergence of stem borer larva (3%) and th e highest parasitized eggs (95.33%.). Sugarcane planters and group of farmers may keep *T. japonicum* all the time by mean of rearing the rice moth as the intermediate host in the time or period when the sugarcane plant is not available in the plantation (period when the cane is harvested). This laboratory study should be adjusted in various environmental characteristic.

Acknowledgements

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