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

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Abstract


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 (R² = 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. Female of *Trichogramma* spp will oviposit their eggs in *Corcyra cephalonica* eggs as the alternative host.

Reported there is significant influence of rice moth *Corcyra 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. Mehen-dale 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 subdistrict. 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* in the 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 day-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*)
- \( P_5 \) = Card 5 (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 auricilius egg (%)

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

\[
P = \frac{A}{B} \times 100\% \quad \text{Pabbage and Tandiabang, (2011)}
\]

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 Card 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. cephalonica 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

Eggs 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* (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 Kornofi (2007) reported with respect to parasitism rate, 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 (R²=0.7147) and less strong with temperature (R²=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 *Chilo auricilius*, there was only treatment P₆ different significantly.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Percent of Parasitized Egg <em>Chilo auricilius</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁ (1 Card)</td>
<td>53.00 a</td>
</tr>
<tr>
<td>P₂ (2 Card)</td>
<td>64.33b</td>
</tr>
<tr>
<td>P₃ (3 Card)</td>
<td>72.33 b</td>
</tr>
<tr>
<td>P₄ (4 Card)</td>
<td>76.67b</td>
</tr>
<tr>
<td>P₅ (5 Card)</td>
<td>77.67b</td>
</tr>
<tr>
<td>P₆ (6 Card)</td>
<td>95.33 c</td>
</tr>
</tbody>
</table>

*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 P₁–P₆ 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 P₆ 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 japonica* 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 Sudhrani (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.
The treatment of P₆ 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: ovi-positioning 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 infl  uenced 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 10th 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 5th or 6thdays. 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 borer) 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 (Coreyra 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 Coreyra cephalonica caused the lowest emergence of stem borer larva (3%) and the 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.

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