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Preference Testing of *Trichogramma japonicum* on Eggs of *Corcyra cephalonica* (Lepidoptera: Pyralidae) Cultured on Several Legume Feed Media

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Abstract : *Trichogramma japonicum* is one of the biological agents that can suppress the development of insect pests, especially stem borer pests on rice plants. As a parasitoid, *T. japonicum* is very important because it has a broad host search power. *T. japonicum* is very easy to propagate in the laboratory using an alternative host, *Corcyra cephalonica*. The *C. cephalonica* is a warehouse pest that has a wide host. The type of host or food of *C. cephalonica* will determine the population and egg quality of *C. cephalonica* which will be used as a host for the parasitoid *T. japonicum*. Nuts are a good alternative host for the development of *C. cephalonica* because they contain good nutrients for growth and eggs to be produced. The types of legumes used were red beans, green beans, soybeans and rice bran as controls. The eggs produced by *C. cephalonica* have variations in terms of egg size where those using red beans as feed have relatively larger length and width of eggs compared to other types of feed. In terms of the level of preference for the parasitoid *T. japonicum*, eggs from *C. cephalonica* cultured on kidney beans were preferable to eggs cultured on green beans, soybeans and rice bran. This is because the egg size is relatively large which can support the nutritional content needed by the parasitoid.
Keywords : *Trichogramma japonicum,Corcyra cephalonica*.parasitoid.

1. Introduction

The use of parasitoids in rice plants is the most effective and has a good future for use. Egg parasitoids, especially those found in eggs. Rice stem borer pests consist of *T. japonicum*, *Telenomus* sp. and *Tetrastichus* sp. Parasitoid propagation technique using alternative hosts is one of the efforts that can be done to explore the types of parasitoids that can be used as a form of control. The most widely used alternative host to reproduce the parasitoid *T. japonicum* is the insect egg *Corcyra cephalonica*. Many studies have reported on the use of *C. cephalonica*. Provision of feed types to alternative hosts can affect the ability of *T. japonicum* in parasitizing activities[1]. According to[2]that the host food, mung bean, contributed to the fitness of the parasitoid *Trichogrammatoidea armigera* where the parasitoid development was maximized, capable of producing high parasitization, high reproduction and parasitoids able to maintain a longer life of 96 hours.

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In general, insects have the same nutritional needs as other animals. Nutritional balance is very important in insects. Insects respond to nutritional imbalances in three ways: 1) insects can change the total amount of food ingested, 2) insects can move from one food to another with a different nutritional balance, and 3) insects can regulate the effectiveness of nutrients i.e. the balance of nutrients such as carbohydrates, proteins, fats, vitamins and amino acids associated with the natural diet of insects. Predatory insects have a high requirement for amino acids as well as carbohydrates, where this form of nutrition is taken from the protein content of the prey. Herbivorous insects in general require almost the same amount of protein, amino acids and carbohydrates as Orthoptera, Coleoptera, and Lepidoptera[3]. Insect nutritional needs can change at any time, depending onInsect nutritional requirements can change over time, depending on growth, reproduction, and migration. Usually insects in the early larval stage require a higher nitrogen content than in the late stages. The element of feed (nutrition) affects the life of insects. For insects, carbohydrates (sucrose, fructose) are the largest source of energy for the needs of the reproductive system and longevity. Protein is necessary for the growth and development of insects. Fats, fatty acids and sterols are needed by insects for energy supply and wing development. Some types of insects use pure fats such as linoleic acid and linolenic acid. Order Diptera requires linoleic and linolenic acids. Even in small amounts, vitamins are needed for insect life[4,5].*T. japonicum* is more easily propagated in the laboratory using the host *C. cephalonica. C. cephalonica* feed will affect the quality of parasitoids to be propagated[6,7].

Replacement hosts must meet the requirements, namely easy to maintain and provided in the laboratory. In addition, replacement host breeding must be relatively cheaper than natural host breeding[1]. The use of substitute hosts for *C. cephalonica* can affect the fitness and development of the later *T. japonicum* parasitoid. Until now, the replacement feed for *C. cephalonica* is still being developed to obtain the parasitoid *T. japonicum* with the maximum parasitization level. To achieve the above objectives, the research objective was to analyze the preference of the *T. japonicum* parasitoid on *C. cephalonica* eggs cultured from several feed media of legumes.

2. Experimental

This research was carried out at the Biological Agent Laboratory, Center for Food Crops and Horticulture Protection, Department of Agriculture and Livestock, North Sulawesi Province. Types of feed provided are rice bran, soybeans, green beans and red beans. At the initial stage, green beans, kidney beans, and soybeans are ground into fine grains. This is done so that *C. cephalonica* larvae can easily consume the feed. In order to get an even smoothness, the feed media that has been ground is then homogenized using the same sieve. Media Feed the nuts that have been mashed earlier then place in a plastic container with a media thickness of 2.5 cm.

a. Harvesting Rice Stem Borer Eggs that have been parasitized by *T. japonicum*

Harvesting of rice stem borer eggs was carried out in lowland rice plantings that were 2 weeks after planting, because this made it easier to find stem borer eggs. Egg collection was carried out at the center of rice cultivation in North Sulawesi. In this study, rice stem borer eggs were taken from lowland rice planting locations that have a high intensity of rice stem borer attacks, namely in Wineru Village, Poigar Subdistrict, Bolaang Mongondow District. From the results taken, the eggs of the stem borer were taken attached to the midrib of the leaves of rice fields. Rice stem borer eggs were collected as much as possible to obtain *T. japonicum*. Rice stem borer eggs were taken several times to meet the research stock for parasitoid testing in the laboratory.

b. Egg propagation of *C. cephalonica*

In the early stages of this activity, *C. cephalonica* pests were taken from rice bran shelters. Imago or larvae taken from rice bran shelters are then cultured in the laboratory. Breeding was carried out in a maintenance box measuring 60cmx30cmx20cm, at the top of the box is given a fine mesh lid with the aim of air circulation from insects *C. cephalonica*. After *C. cephalonica* became imago, it was transferred to a PVC pipe with a diameter of 4 inches and a height of 20 cm as a spawning site. The top and bottom of the PVC pipe are covered with gauze, at the bottom of the PVC pipe a petri dish is given as a place to hold *C. cephalonica* eggs and the top is not covered so that insects lay eggs on the top of the tube. The eggs collected were taken at the same age and then used in the treatment of feed media.

The types of feed provided are rice bran, soybeans, green beans and red beans. At the initial stage, green beans, kidney beans, and soybeans are ground into fine grains. This is done so that *C. cephalonica* larvae can easily consume the feed. In order to get an even smoothness, the feed media that has been ground is then homogenized using the same sieve.

Media Feed the nuts that have been mashed earlier then place in a plastic container with a media thickness of 2.5 cm. Each feeding treatment was invested in 1 day old *C. cephalonica* eggs. Observations were made on the time of emergence of imago. After becoming an imago, *C. cephalonica* was inserted into a 4-inch diameter PVC pipe to get eggs.

c. Preference testing of *T. japonicum*

Testing of host preferences for *T. japonicum* will use *C. cephalonica* eggs obtained from the breeding of legumes and rice bran as a control. After the *T. japonicum* parasitoid was propagated, it was continued with the separation of male and female imago aged 0-1 hours. The method of separating male and female imago is carried out under a microscope by distinguishing them based on the shape of their antennae.

The eggs produced from each imago that have been bred on several types of feed are then arranged on the 'pias' based on the type of feed, and put into a test tube with a diameter of 1 cm. then counted as many as 50 grains and placed in a bowl measuring 0.5 cm x 0.5 cm. eggs of *C. Cephalonica* are evenly sprinkled and glued using paper glue. Then the four treatments based on the type of feed were glued to each other and put into a test tube with a diameter of 1 cm in 40 replications. The process of setting the float in the test tube is arranged randomly and varies for each tube. The tube wall was given a thin streak of diluted honey liquid which acted as parasitoid feed. Each pair of Imago was then released into each test tube containing *C. cephalonica* eggs produced from breeding in each type of feed. The test tube was then covered with a black cloth. This experiment was designed to determine the level of preference of the parasitoid on *C. cephalonica* eggs cultured from several types of legumes and bran feed. Then observed the behavior of the parasitoid when laying eggs and observed the number of eggs that were parasitized.

Observations were made every day after the parasitoids were inoculated. Observations were made when the eggs were parasitized. On the day before the parasitoids came out of the eggs, the number of parasitized eggs was calculated in each group of eggs in the test tube. After that, the parasitized eggs were separated into a 1 cm diameter test tube. After the parasitoid imago came out of the parasitized egg, the number of male and female parasitoids was counted again.

d. Statistical Analysis

The things observed in this experiment were the percentage of parasitization of *T. japonicum* on *C. cephalonica* eggs cultured on several types of legume and rice bran feed, counting the parasitized eggs in each egg group,, counting the parasitized eggs in each egg group, measuring the length and width of the imago. The data obtained from the results of this study were analyzed by analysis of variance and descriptive. If there is a significant difference between treatments, then proceed with the smallest significant difference test.

The formula to calculate the percentage of *T. japonicum* parasitoids in *C. cephalonica* eggs is as follows:

$$P = \frac{a}{b} \text{x } 100\ \%$$

P=Percentage of parasitization
a= Number of parasited *C. Cephalonica*eggs
b=Number of *C. Cephalonica*eggs used

3. Results and Discussion

*C. cephalonica*was able to live its life on several media of legumes. This can be seen in the development process from egg to imago and is able to copulate and produce new offspring for the survival of the next generation. Legumes media as a place to live of *C. cephalonica* can actually affect the population, this is as stated by[2] that insect food media affects the development of the imago population. Food is a source of nutrition that is used to live and develop. Therefore, the selection of a species' host can be seen from the food factor as a source of nutrition for a species in population growth or in multiplying and continuing its offspring. The development of *C. cephalonica* consists of four stages namely egg, larva, pupa and imago, where the larval phase is the longest phase of the insect's life cycle. Eggs of *C. cephalonica* are placed on the media which will later become the feed for the larvae. The phase of insect life depends on the temperature and humidity of the warehouse or research site[8,9]. The development of *C. cephalonica* from egg to the emergence of imago is different in each treatment. The range of development of *C. cephalonica* from egg to imago for peanut green 28-43 days, soybeans 31-48 days, red beans 33-50 days and bran 22-35 days.The difference in the length of life of *C. cephalonica* can be determined by the thickness of the feed, the composition of nutrients and the range of temperature and humidity. The development of *C. cephalonica* determined by the carbohydrate and protein content in the feed medium[10,4]. The color of the eggs produced by *C. cephalonica* did not differ between treatments and was generally almost the same, namely yellowish white.

Table 1. Length and width of *C. cephalonica* eggs in various feed media

No.	Feed Media	Size (mm)	
		Length	Width
1	Red Bean	0.55±0.04	0.36±0.015
2	Soybean	0.55±0.024	0.34±0.022
3	Green Bean	0.54±0.026	0.35±0.025
4	Rice bran	0.52±0.039	0.34±0.025

Information from Table 1 were the length and width of *C. Cephalonica* eggs which are relatively larger in red beans, namely 0.55 mm and 0.36 mm wide, while the relatively small egg sizes are found in bran 0.52 mm long and 0.34 mm wide. The results of the length measurement and egg width of some legumes and bran feeds became one of the main factors in the study of the parasitoid *T. japonicum* because eggs were the feed medium for the parasitoid. The amount of nutrient content in eggs depends on the size of the egg.

The results of this experiment showed that the eggs in each test tube could be parasitized by the parasitoid *T. japonicum*. From the observations, it can be seen that before the parasitoid *T. japonicum* parasitized the eggs of *C. cephalonica*. First, the parasitoids orient by walking on the surface of the egg, then with their antennae groping the surface of the egg, then the parasitoids examine the eggs again with the ovipositor and place their eggs on the host *C. cephalonica* so that parasitization occurs by parasitoids.

Not all eggs that are examined by the parasitoid are then laid their eggs but the parasitoid will walk on all surfaces of the eggs and examine them and on eggs that he feels are suitable then he will transfer eggs. As reported by[2] that the parasitoid left the host after groping with the antennae and checking with the ovipositor whether the egg was parasitized or unsuitable. However, often the parasitoid only checks with the antenna and not with the ovipositor on the surface of the host egg. As stated in[5] that the parasitoid *Trichogrammaauosum* in receiving the host through 3 stages, namely (1) making antenna contact with the host (2) penetration of the ovipositor (3) eating host. The parasitoid *T. japonicum* penetrates and lays eggs on an idiobiont host so that there can be competition between parasitoid larvae and *C. cephalonica* larvae for survival, but for this experiment there was no competition because *C. cephalonica* eggs had been irradiated with ultra violet (UV).

Table 2. Percentage of preference level of parasitoid *T. japonicum* to host *C. cephalonica*

No.	Feed media	Percentage of parasitized eggs	Notations
1.	Soybean	3,1	a
2.	Rice bran	7.3	a
3.	Green Bean	9.65	a
4.	Red Bean	38.55	b
			LSD 5 % =7.01

Observational data regarding the level of preference of the parasitoid *T. japonicum* over the host *C. cephalonica* showed that *C. cephalonica* eggs were more favored on red bean diets. The table shows that the highest percentage of *T. japonicum* parasitization was found in the treatment of *C. cephalonica* eggs from red bean diet, namely 38.55%, followed by treatment with green beans 9.65%, then bran 7.3% and soybeans 3.1%. The parasitoid *T. japonicum* prefers to oviposition the eggs of *C. cephalonica* cultured on red bean media in terms of egg size, and red bean diet looks wider on average than those cultured from green beans, soybeans and bran (Table 2). The results of the variance between the four treatments showed a significantly different effect, which was then carried out by a 5% LSD test which showed a very significant effect between the treatment of *C. cephalonica* eggs cultured on soybeans, bran, green beans and *C. cephalonica* eggs cultured with red beans on preference for the parasitoid *T. japonicum.*

Parasitoid behavior in host discovery can be determined by host size. The small size of the host is less favorable for the parasitoid to lay eggs because it is related to the success of the development of the parasitoid. In a smaller host the availability of nutrients is less adequate than in large host eggs[1]. The high host density makes it easier for parasitoids to find the host, resulting in increased parasitization[11,4].

In addition to physical factors in finding a parasitoid host can use chemical stimuli called kairomone. The compounds produced by the host to attract the host are volatile which are then emitted to elicit a response from the parasitoid, but this is highly dependent on the parasitoid in capturing the stimulus from the host. Conforming to[12] that female parasitoids from *Trichogammatidae* respond to host stimuli based on hydrocarbon chemical compounds. *C. cephalonica* eggs cultured on kidney beans were larger in size with the assumption that the hydrocarbon content in the eggs was more than that in *C. cephalonica* eggs cultured on green beans, soybeans and bran.

Table 3. Measurement of length and width of male and female imago of *T. japonicum*

No.	Feed media	Male (mm)		Female (mm)	
		Length	Width	Length	Width
1.	Rice bran	0.362±0.027	0.163±0.013	0.393±0.016	0.169±0.010
2.	Green bean	0.388±0.029	0.176±0.011	0.419±0.024	0.184±0.011
3.	Soybean	0.376±0.021	0.166±0.014	0.403±0.019	0.180±0.010
4.	Red bean	0.389±0.028	0.181±0.011	0.432±0.033	0.187±0.009

The results of the average length and width of male and female imago can be seen in Table 3.The results of the study on measuring the length and width of male and female imago of *T. japonicum* showed that the average body length of males and females was still relatively larger, seen in the parasitoid *T. japonicum* which parasitized the eggs of *C. cephalonica* which was bred on red bean diet, which was 0.389 mm male. and 0.432 mm female then followed by Mung Bean 0.388 mm male and female 0.419 mm, male soybean 0.376 mm and female 0.403 mm and male bran 0.362 mm and female 0.369 mm. Likewise with the size of the body width of the parasitoid *T. japonicum*, the average male and female average size is relatively large in *T. japonicum* which parasitizes *C. cephalonica* eggs bred on red bean feed, female 0.181 mm and female 0.187 mm, then male green beans 0.176 mm and female 0.184 mm, male soybean 0.166 mm and female 0.180 mm and male bran 0.163 mm and female 0.169 mm. Egg size is very influential on parasitoid development because the relatively larger egg size will provide sufficient food for parasitoids to develop inside the host egg. The larger the egg size will affect the size of the parasitoid, and the relatively large size of the parasitoid will affect the parasitoid parasitoid[12].

4. Conclusions

Insect *C. cephalonica* is able to reproduce on red bean, soybean, green bean and rice bran feed media. Red bean media as feed for *C. cephalonica* can produce quality eggs for the development of the parasitoid *T. japonicum*. *C. cephalonica* eggs cultured on red bean media had relatively larger lengths and widths (0.55 mm long and 0.36 mm wide) compared to those cultured on green beans, soybeans and rice bran media.

C. cephalonica eggs cultured on red bean media were highly favored by the parasitoid *T. japonicum*. The relatively large size and shape of the eggs made the parasitoid choose to lay eggs on *C. cephalonica* eggs cultured on red bean feed media, compared to *C. cephalonica* eggs cultured on green bean, soybean and bran feed media. The length and width of the male and female imago of *T. japonicum* showed that the average body length of males and females was still relatively larger, seen in the parasitoid *T. japonicum* which parasitized the eggs of *C. cephalonica* bred on red bean diet, the males were 0.389 mm and females 0.432 mm.

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