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EFFECT OF HOST-LARVAL DIET ON THE HOST ACCEPTANCE AND HOST SUITABILITY OF THE EGG PARASITOID *Telenomus remus* NIXON (Hymenoptera: Scelionidae) ON *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae)

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ABSTRACT

Effect of host-larval diet on the host acceptance and host suitability of the egg parasitoid* Telenomus remus *Nixon (Hymenoptera: Scelionidae) on* Spodoptera frugiperda *J. E. Smith (Lepidoptera: Noctuidae). The life history of parasitoids is an important factor that can determine their ability to attack a host. The type of food consumed by the host can affect the ability of parasitoids such as host searching behavior, host suitability and host acceptance. In this research, we evaluate the effect of the *S. frugiperda* larvae diet on its suitability of the eggs produced by the adults for the egg parasitoid *Telenomus remus*. The research was studied on two types of egg masses of *S. frugiperda* that obtained from the moths that fed with natural or artificial diet during their larval stages. Parasitoid was reared from both types of hosts. An egg mass consisting of 50 *S. frugiperda* eggs from both types of hosts was exposed to one egg parasitoid female for 24 hours. *S. frugiperda* eggs then were reared until the parasitoid adult emerged. Each experiment was repeated 20 times. Host acceptance was observed through the host parasitism rate and its parasitization. Meanwhile, the host suitability was observed through the sex ratio of the emerging parasitoids. The results showed that *S. frugiperda* eggs reared using artificial diet had a higher parasitism rate (99.33%) than those of natural diet (82.53%). In contrast, the level of parasitization of *S. frugiperda* eggs reared using natural diet was higher (78.30%) than those of artificial diet (48.34%) because the number of emerging *T. remus* from *S. frugiperda* eggs reared using natural diet was also higher than those of artificial diet. However, the sex ratio (F:M) of emerging *T. remus* from *S. frugiperda* eggs reared using both of diet was female biased.

Key words: biological control, fall army worm, mass rearing, parasitoid

INTRODUCTION

As a part of tritrophic interaction between plants-herbivores-parasitoids, plant as a source of diet for herbivores can influence parasitoid reproductive traits as their natural enemy such as life span, fecundity, fertility, sex ratio, mating and host searching behavior as well (Benelli *et al.*, 2017). Kuramitsu *et al.* (2019) reported that larval parasitoid *Cotesia kariyai* showed different host searching behavior responses on different host plants of *Mythimna separata*. The different host plant also affected the parasitism rate of *M. separata* by *C. kariyai* (Kuramitsu *et al.*, 2016). It also reported in another system that egg parasitoid *Trichogramma minutum* shows the different host acceptance behavior in the host eggs that obtained from adults reared using natural and artificial diet (Song *et al.*, 1997).

An easy parasitoid rearing method is an essential factor in biological control (Wang *et al.*, 2019). The important factors determining the success of parasitoid rearing are the availability and suitability of the diet used (Urrutia *et al.*, 2007), the microclimate, including temperature, humidity, and light (Cohen, 2018). Furthermore, superparasitism can also affect the success of parasitoid rearing in the laboratory (Tormos *et al.*, 2012). The artificial diet is generally used to avoid cannibalism in certain species and to gain convenience in multiplying parasitoid hosts (Susrama, 2018). The use of artificial diet is an effort to simplify the process of parasitoid rearing. The use of artificial diet can also be a solution when the food (natural diet) of the host insects is not available.

Spodoptera frugiperda (Lepidoptera: Noctuidae) is a new pest species of armyworm in Indonesia. *S. frugiperda* had been reported to spread in several areas in Indonesia including Aceh, North Sumatra, West Sumatra, Riau, Jambi, Bengkulu, South Sumatra, Lampung, Banten, West Java, Central Java, and West Kalimantan (BBPOPT, 2019). *S. frugiperda* has massive attacks with a high spread rate in several regions of the world, such as America (Sparks, 1979), Africa (Goergen *et al.*, 2016), and Asia (CABI, 2019). Maize, an important commodity in many tropical countries, is one of the hosts for this pest (Kuate *et al.*, 2019).

Biological control more considers the effectiveness of control and environmental impacts compared to other control methods (Norris & Kogan, 2003). Biological control agents that can be used as natural enemies for *S. frugiperda* are the egg parasitoids such as *Telenomus* sp. and *Trichogramma* sp. (Shylesha *et al.*, 2018). Kenis *et al.* (2019) reported the use of egg parasitoid *T. remus* to control the population of *S. frugiperda* in Africa by 2016. *T. remus* is an existing egg parasitoid species in Indonesia for a long time (Buchori *et al.*, 2017). The results of previous studies show that *T. remus* can be used as a natural enemy of *S. frugiperda* (Sari *et al.*, 2020). *T. remus* has never been reported to be directly associated with the eggs of *S. frugiperda* in Indonesia. However, several studies in other countries have shown that *T. remus* is a potential biological agent for *S. frugiperda* (Kenis *et al.*, 2019; Liao *et al.*, 2019). As a preemptive biological control method, a study on the rearing method of the parasitoids is necessary. This study can support the success of the release of parasitoids to the field to control the population of targeted pests. Considering that diet is a major factor in determining the success of parasitoid rearing in the laboratory, this research was conducted to evaluate the effect of *S. frugiperda* larvae diet i.e., natural, and artificial diet on its suitability of the eggs produced by the adults for the egg parasitoid *T. remus* rearing.

MATERIALS AND METHODS

Research Site. This research was done during June to September 2020. Field activity was carried out in corn field around Dramaga 6°32'57.57" S, 106°44'33.32" E, Bogor, Indonesia and laboratory activity was carried out in the Laboratory of Biological Control, Department of Plant Protection, IPB University.

***Spodoptera frugiperda*.** The *S. frugiperda* used were the result of field collections at the research site. Larvae (all stages) obtained from the field were maintained and reared in an insulated rearing container (35 × 27 × 7 cm). The larvae were placed individually in each separated section of the container to avoid cannibalism. Baby corn was given as a food source (Sari *et al.*, 2020) every day until the larvae entered the pre-pupal stage. Shortly before becoming pupae, the insects were kept in a sterile sandy container as a medium for pupation. After the pupae formed, the pupae were taken and placed in a tubular adult insect rearing container (diameter = 15 cm, height = 30 cm). Adult insects were fed with a 25% honey solution. Rearing was carried out to get the first generation. The eggs that have hatched into larvae produced from the first generation were reared and reproduced using the same method. For experimental purposes, the larvae were reared using baby corn as a natural diet and an artificial diet.

Table 1. Composition of artificial diet for *S. frugiperda*

Material	Volume
Agar	24 g
Red bean	125 g
Wheatgerm	100 g
Yeast	62.5 g
Casein	50 g
Ascorbic acid	6 g
Sorbic acid	3 g
Methyl paraben	5 g
Vitamin mix	10 g
Tetracyline	0.125 g
Aquades	3000 mL

Artificial Diet. The artificial diet used in this study was prepared following the reference by Sari (2020) using the ingredients presented in Table 1. The kidney beans were blended and drained with 1000 mL of distilled water. Ingredients such as wheat germ (Dehealth Supplies, Indonesia), yeast (Lesaffre, France), casein (Sigma-Aldrich, Germany), ascorbic acid (Sigma-Aldrich, Germany), sorbic acid (Sigma-Aldrich, Germany), and methylparaben (Sigma-Aldrich, Germany) were then added to the kidney bean mixture. Agar was cooked in 1000 mL of distilled water for two minutes. The cooked agar was then mixed into the kidney beans mixture. Vitamin-mix (Fisher Scientific, Finland) and tetracycline (Novapharin, Indonesia) were added. The dough made was then poured into a storage container (20 × 20 × 10 cm) and stored in the fridge.

The diet was given in small pieces (2 × 2 cm). The larvae that were fed with the artificial diet were reared in Petri dishes (diameter = 86 mm). This was important to maintain the humidity of the artificial diet given. When the diet dried, it was replaced with a new one.

Telenomus remus. The parasitoids of *T. remus* were obtained by collecting *S. frugiperda* eggs at the research site. *S. frugiperda* eggs were reared in the laboratory. *S. frugiperda* eggs were then incubated in a tube (diameter = 2 cm, height = 10 cm) until the adult parasitoids emerged. The emerging parasitoids were fed with a 25% honey solution. After two days of the emergence, the mated female parasitoids were re-exposed to *S. frugiperda* eggs which reared using a natural diet in a tube (diameter = 2 cm, height = 10 cm). The parasitized eggs of *S. frugiperda* were then reared until the emergence of the next generation of adult parasitoids. The parasitoids were then used for experimental purposes.

The Parasitism of *T. remus* on the Eggs of *S. frugiperda* Reared Using Artificial Diet. The eggs of *S. frugiperda* obtained from the moth that had been fed by both diets (a total of 50 eggs) were placed on a piece of paper (1 × 5 cm). The paper was then put into a rearing tube (diameter = 2 cm, height = 10 cm) (Figure 1). *S. frugiperda* eggs were then exposed to a mated female of *T. remus* for 24 hours. The parasitized eggs of *S. frugiperda* were then reared until adult *T. remus* emerged. Each experiment was repeated 20 times. In total, 2000 eggs of *S. frugiperda* were used in this study, consisting of 1000 eggs of *S. frugiperda* reared using artificial diet and another those of using the natural diet. The variables observed included the number of parasitized eggs (parasitism rate), the success of parasitism (level of parasitization), the number of emerging parasitoids, and parasitoid sex ratio. Sex ratio was determined by calculating the number of male and female (Figure 2) progeny emerged. The calculation of the parasitism rate was using the following formula:

$$PR = \frac{n}{N} \times 100\%$$

Figure 1. Experimental set up.

PR = Parasitism rate;
n = Number of parasitized eggs;
N = Total eggs.

The calculation of the evel of parasitization was using the following formula:

$$P = \frac{a}{A} \times 100\%$$

P = Level of parasitization;
a = Number of hatched parasitized eggs;
A = Number of parasitized eggs.

Parasitism rate can be observed by the presence of black spots on the eggs, while level of parasitization can be observed by the number of emerging adults from parasitized eggs.

Statistical Analysis. The data of the number of parasitized eggs, parasitism rate, level of parasitization, and the number of emerging parasitoids reared with natural and artificial diets were analyzed using paired *t*-test. Parasitoids that did not emerge from the parasitized eggs were not included in the analysis (N = 5). Data analysis was performed using R Statistic version 3.6.3. (R Core Team, 2013).

RESULTS AND DISCUSSION

The results showed that the type of diet used greatly affected the ability of *T. remus* to parasitize the eggs of *S. frugiperda*. It was indicated by the parasitism rate and level of parasitization as presented in Figure 3. The eggs of *S. frugiperda* reared using artificial diet

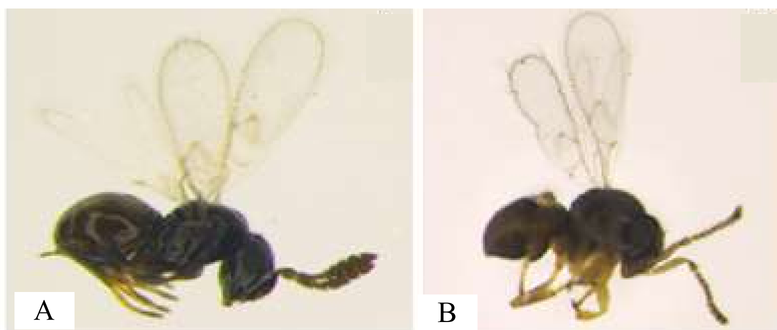

Figure 2. *Telenomus remus* adult. (A) Female and (B) Male.

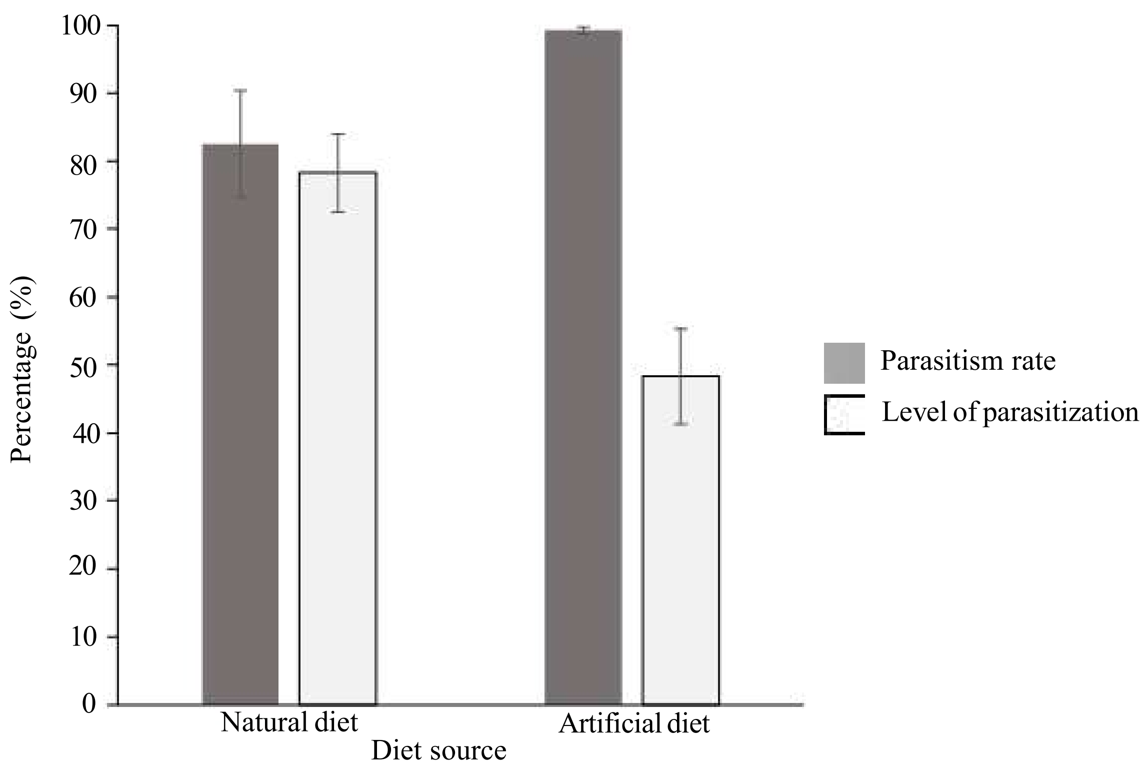

Figure 3. The parasitism rate and level of parasitization of *T. remus* on *S. frugiperda* eggs reared using natural and artificial diet.

had significantly higher parasitism rate than those using natural diet (paired *t*-test, P = 0.025, N = 15). However, the level of parasitization of *T. remus* on the parasitized eggs of *S. frugiperda* reared using natural diet was higher than those using artificial diet (Paired *t*-test, P = 0.001, N = 15). There was a considerable difference (50.99%) between the parasitism rate and the level of parasitization on *S. frugiperda* eggs reared by artificial diet. In contrast, the difference between the parasitism rate and the parasitization on *S. frugiperda* eggs reared by natural diet was only 4.23%. This result is closely related to the number of emerging parasitoids from the parasitized eggs of *S. frugiperda* reared using both diet (Figure 4). About 9–50 of the 50 *S. frugiperda* eggs reared using a natural diet exposed to *T. remus* were successfully parasitized, resulting in 50 (mode data) emerging parasitoids. Meanwhile, 47–50 *S. frugiperda* eggs reared using the artificial diet were successfully parasitized, resulting only 27 emerging parasitoids (mode data). This may occur due to the differences in the nutritional content of *S. frugiperda* eggs. The natural diet may have better nutritional content than the artificial one. The eggs of *S. frugiperda* reared using the natural diet contained sufficient nutrients for *T. remus*, so that more parasitoids emerged from the eggs (Paired *t*-test, P = 0.01, N = 15). Similar finding was also reported by Uçkan & Ergin (2002) that the proportion of adult female and male of *Apanteles galleriae* emerged was different due to the difference in the quality of host diet tested. In addition, the self-defense system of the host, such as parasitoid encapsulation, can also play a role in inhibiting the process of parasitization. Encapsulation causes mortality in parasitoids in the host due to the lack of oxygen and nutrients (Blumberg, 1997a). The presence of encapsulation was not observed in this study. Therefore, it can be an interesting topic for further investigation since encapsulation is another determining factor in the mass rearing of parasitoids in the laboratory and a determinant of the sustainability in biological control programs in the field (Blumberg, 1997b).

The incidence of superparasitism was also observed in this study. One of all experimental replications, superparasitism was observed in the eggs of *S. frugiperda* that were reared using the natural diet. Eighteen of the 50 *S. frugiperda* eggs exposed were successfully parasitized, resulting in 40 emerging parasitoids. Under these conditions, the parasitization was calculated to be 100%. Superparasitism in parasitoids can have a negative effect on the development of parasitoids, such as on the size and number of eggs laid and the sex ratio of the offspring produced (Godfray, 1994). Husni *et al.* (2011) stated

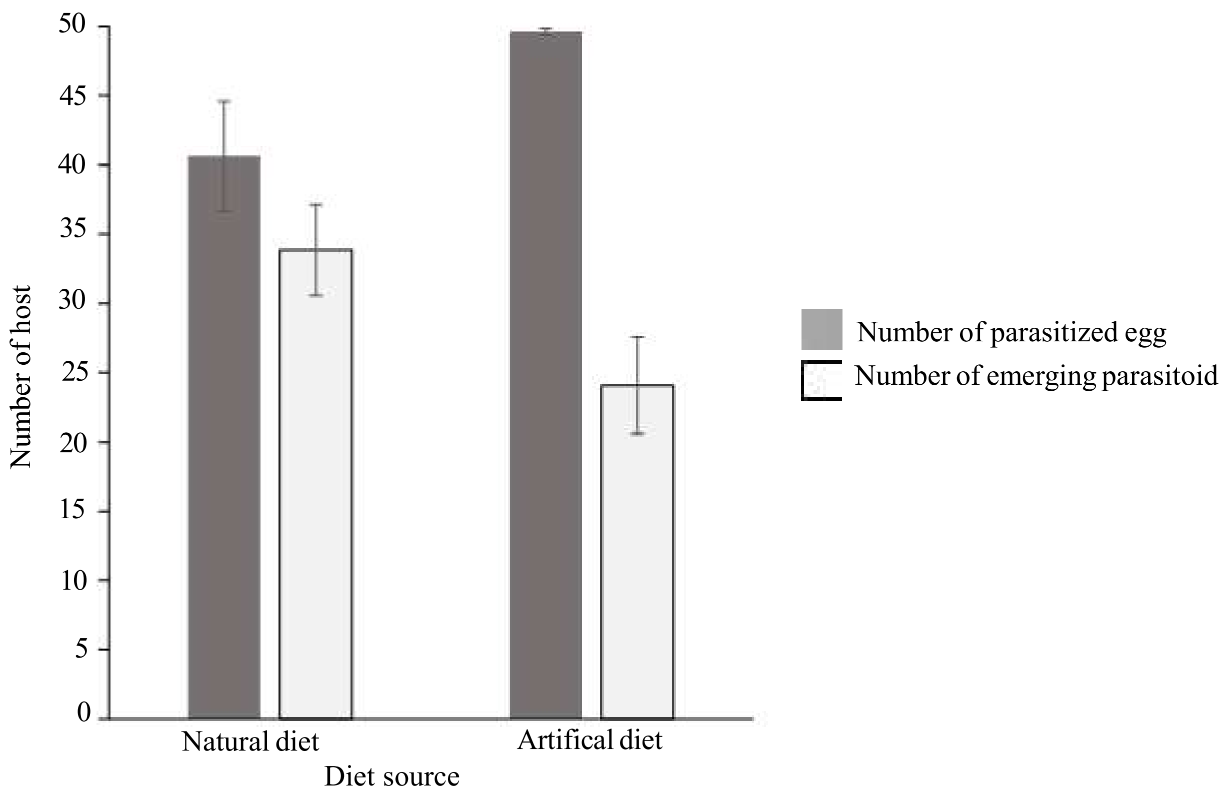

Figure 4. Number of parasitized eggs and the number of emerging *T. remus* from *S. frugiperda* eggs reared using natural and artificial diet.

that the number of offspring of the parasitoid *Tetrastichus brontispae* decreased along with the increasing frequency of superparasitism in the host coconut hispine beetle *Brontispa longissima*. Pabbage & Tandiabang (2007) also reported s similar interaction between the parasitoid *Trichogramma evanescens* and the pest *Ostrinia furnacalis*. Superparasitism is a defense strategy of the parasitoids against the host defense reactions in the form of encapsulation that may occur (Luna *et al.*, 2016).

Although the number of emerging parasitoids from the eggs of *S. frugiperda* reared using the artificial diet was lower than those of the natural diet. The sex ratio of *T. remus* progenies reared using both of diet was female biased (Figure 5). Sex ratio is one of the important characteristics in parasitoid biology, which has an important role in controlling pest populations and the stability of host-parasitoid interactions in the field (van Baalen & Hemerik, 2008). The high number of female parasitoid progenies can determine the sustainability of biological control due to their role in parasitization, thereby indirectly controlling the pest population as host insects. Senrayan & Annadurai (1991) said that sex ratio of adult parasitoids is not affected by host diet. Sex ratio of parasitoid is more affected by adult female diet. For example, sex ratio of *Trichogramma ostriniae* is female biased when the adult female fed with aphid honeydew compared to unfed female (Fuchsberg *et al.,* 2007). Similary, sex ratio of *Dolichogenidea tasmanica* is shifted to equal sex ratio when female fed on flowers compared to male biased in the absence of flower nectars for female (Berndt & Wratten, 2005). However, Uçkan & Ergin (2002) reported that sex ratio of *A. galleriae* is affected by the type of host diet. This may vary according to the nature and environmental condition because there are a few factors affecting parasitoid's sex ratio.

CONCLUSION

The type of host diet affects the parasitism rate and level of parasitization of *T. remus*. The eggs of *S. frugiperda* that were reared using the artificial diet had a higher parasitism rate than those reared using the natural diet. The level of parasitization between the two diet types showed the opposite. However, the sex ratio of *T. remus* progenies emerging from the eggs of *S. frugiperda* reared using both of diet was female biased. Although the success of parasitism of *T. remus* reared using artificial diet was low, this result indicate that the artificial diet has great potential in the mass rearing of *T. remus* as a parasitoid for *S. frugiperda* eggs, a new species of armyworm in Indonesia.

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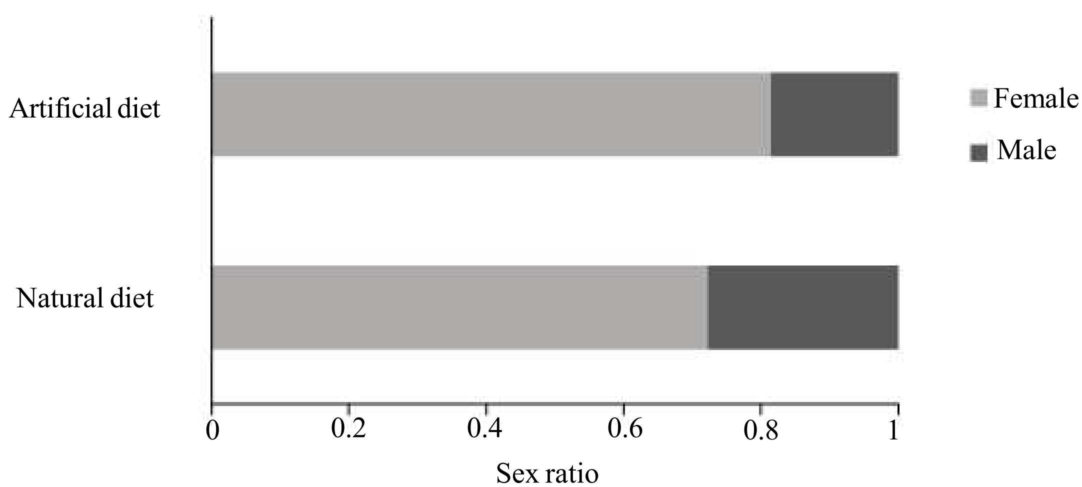

Figure 5. Sex ratio of *T. remus* progenies emerge from S. *frugiperda* eggs reared using natural and artificial diet.

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