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BIOLOGY OF *Tetrastichus brontispae* (FERRIERE) (HYMENOPTERA: EULOPHIDAE), THE COCONUT LEAF BEETLE PARASITOID

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Abstract. *Biological control using parasitoids should be carried out to reduce the population of Brontispa longissima without pesticides. Tetrastichus brontispae is a larva-pupa parasitoid and is a gregarious parasitoid. Many factors can influence the biology of the parasitoid, which consists of biotic and abiotic factors. The existence of a host is one of the biotic factors that influence the life of parasitoids. The study aimed to study various biological parameters of the T. brontispae parasitoid which included the shape, size, color, and length of each egg, larva, pupa, and imago stage as well as personality, sex ratio, and the number of hosts parasitized by female T. brontispae imago. The research was carried out in the form of an experiment using a completely randomized design. Experiments for biological observations of female T. brontispae parasitoids consisted of 4 treatments and 10 replications. The period of development, offspring, and sex ratio of the parasitoid Tetrastichus brontispae were studied using young pupa Brontispa longissima as hosts. Maintenance is carried out by inserting the parasitized larvae or pupa into the test tube provided. T. brontispae is a gregarious endoparasitoid. The development period from egg to imago is 18.95 ± 0.75 days. The length of life of the imago was significantly different between females (9.7 ± 0.51 days) and males (4.5 ± 0.52 days)—honey in increasing lifespan and enhancing T. brontispae offspring. The offspring of a female is 17.50 ± 1.08, with a female proportion of 60.12%. Imago T. brontispae fed honey had a longer and higher actual life span than without honey. Apart from the honey feed, the presence of a host can also affect T. brontispae.*
Keywords: *integrated pest management; cocos nucifera*

1. Introduction

Based on the coconut leaf beetle, *Brontispa longissima* Gestro (Coleoptera: Chrysomelidae) is one of the main pests destroying coconut shoots first reported from the Aru Islands (Maluku Islands) in 1885. This species was first described from the Aru Islands and is thought to have originated in New Guinea and the Bismarck Islands. *B. longissima* has spread to more than 25 countries in Asia, Australia, the Pacific Islands, and several regions in Indonesia (CABI, 2016; Li *et al*., 2021). Initially, this pest did not cause severe problems and was limited to certain areas. However, due to its high mobility and environmental factors, it eventually spread to almost all coconut plantations in Indonesia (Wiratno & Rohimatun, 2012; Li *et al*., 2021).

B. longissima larvae and eggs feed on the youngest coconut leaf tissue. Affected leaves dry up, the plant experiences stunts and reduced fruit production. Prolonged attacks on young plants can cause death (Asia Pacific Forest Invasive Species Network, 2013; Zou *et al*., 2020). Damage caused by the pest *B. longissima* reduces production by 30-40% per tree and causes losses of US

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$ 40 million annually (Ayri & Ramamurthy, 2012; Takano *et al*., 2012). The research results from Yudha (2015) stated that the percentage of *B. longssima* pests in Solok Regency was 58.91%, and the rate of plant stems attacked 39.82%. In addition, population sex ratios have implications in the management of this pest (Acevedo *et al*., 2014). The level of damage caused by *B. longissima* attacks varies from light to heavy attacks.

To control these insect-pest, farmers are solely dependent on chemical sprays. Injudicious and indiscriminate use of chemical sprays leads to pest resistance resurgence and destroys the natural Enemy's population in the coconut field. The use of chemical pesticides in coconut pest control efforts raises serious concerns for the economy and health of farmers, local communities, and consumers. Henceforth to control these insect pests, using IPM- Integrated Pest Management strategy is more applicable because IPM is a pest population management system utilizing all suitable technologies in a compatible manner to maintain the pest population below the economic injury level (Abhishek & Dwivedi, 2021). The negative impact that is also caused by the use of chemical pesticides on natural ecosystems is the decreasing population of birds that land on coconut trees, even though birds are natural enemies of pests that can act as predators to reduce coconut pest populations.

One of Integrated Pest Management strategies that can be applied is biological control. Biological control is an effort to exploit and use natural enemies to control pest populations by utilizing parasitoids, predators, or pathogens. Biological control using parasitoids, predators, and fungal entomopathogens has been shown to reduce the population of *B. longissima* (APFISN, 2013). One of them is the parasitoid *Tetrastichus brontispae* (Ferriere) which has played a vital role in controlling the pest *B. longissima* in several countries, including Indonesia (Kalshoven, 1981; Duan *et al*., 2012). Currently, the control method that is considered the most effective is using biological control agents and has been proven to reduce the population of *B. longissima* in the field in 2008 in the Ende district, namely by using *T. brontispae* (Nere, 2011). Biological control of *B. longissima* using the parasitoid *T. brontispae* and the fungus M. anisopliae are potential natural enemies in suppressing the population of *B. longissima* pests (Bursatriannyo, 2014).

Tetrastichus sp. is a cosmopolitan insect with hosts generally of the order Coleoptera and Hymenoptera, sometimes parasites of Lepidoptera and Diptera. These parasitoids attack the host at the larval or pupa stage and is a gregarious parasitoids (Thi *et al*., 2009; Liansheng *et al*., 2014). The parasitization power of *T. brontispae* in the laboratory is very high, namely 60-90 percent (Chin & Brown, 2000). Liu *et al*. (2022) showed that the peak emergence time of parasitoids was within the first two h after the first parasitoid emerged. Most host pupae had one or two emergence holes, mainly on the third to sixth abdominal segments. The average number of emerged adults

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was 23.51 ± 9.08 (Liu *et al.,* 2022). Once it leaves the host pupa, the courtship and mating behaviour of *T. brontispae* were observed within 1–2 min after a male meets a female, and the mating process was usually short. The biology of a parasitoid is fundamental to know in taking effective and efficient action in pest management (Liu *et al.*, 2022).

Many factors can influence the biology of the parasitoid, which consists of biotic and abiotic factors. The existence of a host is one of the biotic factors that influence the life of parasitoids. The presence of parasitoids in the field is strongly influenced by the presence of the host. Conditions of rainfall, temperature, humidity, and availability of feed are among the abiotic factors that can affect the biological life of parasitoids (Qian *et al.*, 2006; Ikhsan *et al*., 2020).

There have been found *B. longissima* parasites by *T. brontispae* in West Sumatra coconut plantations, but *B. longissima* attacks still increase. Complete information about the biology of *T. brontispae* and its preference for the stage and age of *B. longissima* larvae and pupae is needed so that an appropriate biological control technology can be designed to control *B. longissima* by utilizing the parasitoid *T. brontispae.* This study aims to study various biological parameters of the parasitoid *Tetrastichus brontispae*.

2. Methods

2.1. Place and Materials

The research was conducted at the Bioecology Laboratory of the Department of Plant Pests and Diseases, Faculty of Agriculture, Andalas University. The materials used in this study were *Brontispa longissima*, the parasitoid *Tetrastichus brontispae*, coconut leaf as a food ingredient for *B. longissima*, and honey as a food for the parasitoid *T. brontispae*. The tools used consisted of a plastic box measuring 26x19x8 cm, a test tube, tweezers, a thermometer, gauze, rubber bands, a soft brush, scissors, a microscope, label paper, a camera, white and black paper, distilled water, and stationery.

2.2. Provision of Host Insects (Larva and Pupa of *B. longissima*)

Larvae and pupae of *Brontispa longissima* were obtained by collecting eggs and *B. longisssima* in Nagari Panarian, Gunung Talang District, Solok Regency. The eggs and imago collected are then taken to the laboratory to be raised and cultured. Eggs and imago are presented separately. Eggs attached to coconut leaves are kept in plastic boxes measuring 26 x 19 x 8 cm with a perforated lid and gauze. After the eggs become larvae, the coconut leaves are replaced as food ingredients once in 3 days by moving the Pet larvae into new leaf folds. Coconut plant is obtained from coconut plants around Pauh District, Padang City. This maintenance is carried out until the host insect is ready to be applied in the experiment.

Several pairs of *B. longissima* imago from the land were put in a plastic box measuring 26 x

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19 x 8 cm filled with coconut leaves, a perforated cover, and gauze. Replacement of coconut leaves is done once every three days, the used leaves from the maintenance of imago are placed in a different box, and the imago is given a new fresh leaf. In the leaves of the former imago rearing, several *B. longisssima* eggs were raised until they hatched into larvae and were ready to be applied for experiments. To find out the temperature of the research room, a thermometer is hung in the research room.

2.3. Propagation of Parasitoid *Tetrastichus brontispae*

The parasitoids used in this experiment were collected from the same site as the host insects. *T. brontispae* parasitoids were obtained by collecting larvae and pupae of parasitized *B. longissima*. Larvae parasitized by *T. brontispae* have symptoms on their bodies that become hardened and look dry. Maintenance is carried out by inserting the parasitized larvae or pupa into the test tube provided. Symptomatic *B. longissima* was left until *T. brontispae* imago appeared, then allowed to copulate for 24 hours to be ready for use in the experiment. The *T. brontispae* parasitoids were given fed in 10% honey solution, smeared on paper, and then put in a hanging position in the test tube.

2.4. The development of the prepubescent stage of *T. brontispae*.

In a test tube, ten young pupae of *B. longissima* and coconut leaf were treated with six populated female *T. brontispae* imago (age one day). This treatment was made as many as 44 replications. The parasitization continued for 3 hours (09.00-12.00 WIB). After that, the *T. brontispae* imago was removed from the test tube, while the *B. longissima* pupa remained in the test tube. Of the 44 test tube replications, two tubes were taken per day for host surgery, which was carried out until the parasitoids appeared.

Observations were made by carefully dissecting the *B. longissima* pupa applied under a microscope. The developments observed included the shape, size, and duration of each parasitoid egg, larva, and pupa stage. The pre-adult stage of parasitoids was observed from the first day of egg-laying until the parasitoids appeared. Observation data were based on 100 prepubescent individuals of *T. brontispae* obtained from 6-9 parasite hosts.

2.5. Long life and meridians.

A pair of *T. brontispae* imago that had just emerged from the host were inserted into a test tube containing three young *B. longissima* pupae, placed in a fold of coconut leaf. Every 24 hours, the host pupa was replaced until the parasitoids died. Eggs laid every day were examined by dissecting the *B. longissima* pupa. The life span of the imago is calculated from the time it appears until it dies. Offsprings are calculated by adding up all the eggs laid by a female during her life. The experiment was carried out with two treatments: the treatment of parasitoids that were not given honey feed and those that were given honey feed. Each treatment consisted of 10 replications.

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2.6. Sex ratio.

A pair of *T. brontispae* imago that had just emerged from the host were inserted into a test tube containing three young *B. longissima* pupae, placed in a fold of coconut leaf. Every 24 hours, the host pupae are replaced until the parasitoids die. The pupae treated with the parasitoids were nurtured until the parasitoids appeared. The number of parasitoid images that appeared and their sex was recorded. The experiment was carried out with ten replications. The sex ratio is expressed as the percentage of females.

3. Results and Discussion

3.1. Results

3.1.2. Pre-adult development stage

The size and duration of the pre-adult development of *T. brontispae* are presented in Table 1. The eggs are elliptical (oval), white and transparent (Figure 1a), laid in the host's body. The incubation period for *T. brontispae* eggs lasts about 2-3 days.

Table 1. Body size and duration of pre-adult development of *T. brontispae* parasitoids (x ± SD)

Phase	N	Size		N	Duration (days)
		Long (mm)	Wide (mm)		
Egg	100	0.20 ± 0.01	0.06 ± 0.00	100	2.09 ± 0.29
Early instar larvae	100	0.43 ± 0.14	0.09 ± 0.03	100	2.82 ± 0.41
Late instar larvae	100	0.91 ± 0.19	0.25 ± 0.07	100	4.19 ± 0.39
Pupa	100	1.14 ± 0.13	0.38 ± 0.03	100	9.85 ± 0.36
Total pre-adult	-	-	-	100	18.95 ± 0.75

In general, larvae are classified as early instars and advanced instars, distinguished by body size and color. The early instar larvae (Figure 1b) are slightly brown and transparent, while the late instars (Figure 1c) are reddish-brown. Besides, advanced instars are larger. Early and late instar larvae development time was 2.82 ± 0.41 and 4.19 ± 0.39 days, respectively (Table 1).

The newly formed *T. brontispae* pupae were characterized by the appearance of the eye shape and segmentation of the *T. brontispae* body. The body was clear white, then turned brown, and the compound eyes were red. At the end of the pupal period, the whole body turns black (Figure 1d). The pupal stage lasts 9.85 ± 0.36 days.

Pre-adult development of *T. brontispae* from egg to pupa takes place in the host's body. The time taken from the eggs were laid until the imago appeared (pre-adulthood) was 18-20 days, with a mean of 18.95 ± 0.75 days (Table 1). Waterhouse and Sands (2001) state that the life cycle of *T. brontispae* ranges from 16-21 days.

The results obtained for pre-adult development indicate the period required for one generation of *T. brontispae*. This information is vital, especially about the development of *T. brontispae*

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populations and specific purposes such as propagation in the laboratory and release in the field.

3.1.2. Long life and meridians

Female imago lives longer than males. In the treatment without honey feed, male imago (2.4 ± 0.52 days) while the female (3.7 ± 0.48 days) and in the treatment with male imago honey feed (4.5 ± 0.52 days) while the female (9.7 ± 0.51 days). Without honey feed, during its life, a *T. brontispae* was able to lay eggs between 9-12 with an average of 10.90 ± 1.37, and with honey feed, *T. brontispae* could lay eggs between 16-19 with an average of 17.50 ± 1.08. The oviposition period lasts about two days, then *T. brontispae* undergoes post-oviposition until death. This suggests that *T. brontispae* is a proovigenic parasitoid. (Figure 2).

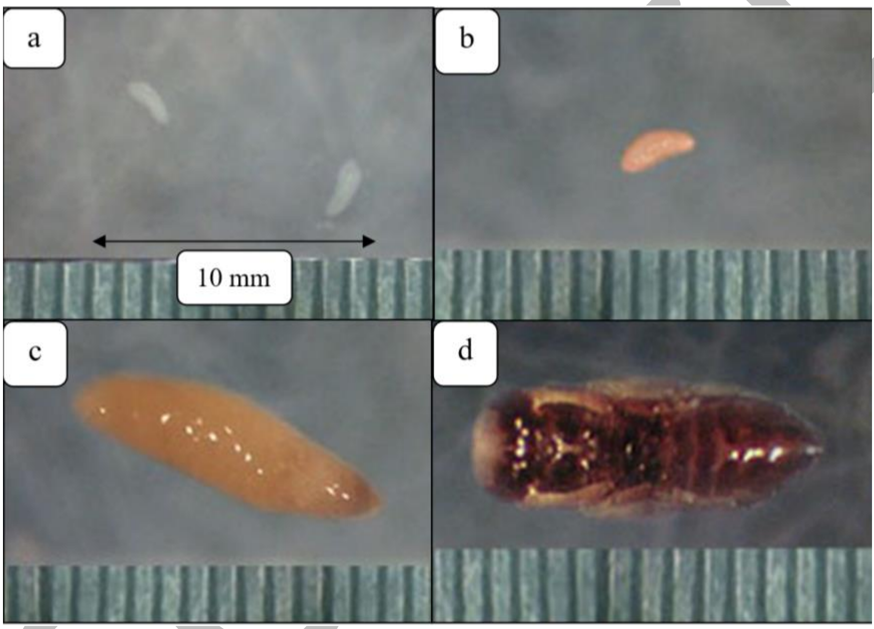

Figure 1. Pre-adult stage *T. brontispae* a. Eggs, b. Early larvae, c. Larva continued, d. Pupa

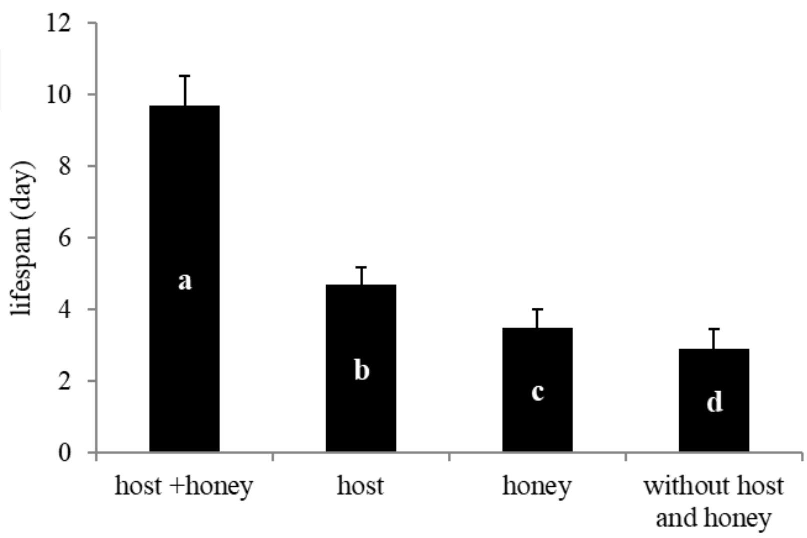

Figure 2. The lifespan of female *T. Brontispae* imago on the various provision of hosts and honey

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3.1.3. Sex ratio

The sex ratio is expressed in the sex factor (% female). Based on 173 imago tails that emerged from 10 cows, the percentage of females that appeared was between 55.56% and 66.67%, with an average of 60.12% ± 3.60 (Table 2). The percentage of female *T. brontispae* parasitoid obtained in the test was more than the percentage of males. The higher proportion of female than male progeny revealed that *T. brontispae* could be effective in controlling the host pest population in the field. Female parasitoid individuals are insects that can work effectively to reduce pest populations in the area because female individuals can produce and lay eggs or parasitize their hosts.

Table 2. Length of life and superiority of *T. Brontispae*

Treatment	N	Long life (x ± SD)		Parasitisation			Offspring		
		Male	Female	Min.	Max.	(x ± SD)	Min.	Max.	(x ± SD)
Without honey	100	2.4 ± 0.52	3.7 ± 0.48	1	2	2.10 ± 0.57	9	12	10.90 ± 1.37
Honey feed	100	4.5 ± 0.52	9.7 ± 0.51	1	3	1.40 ± 0.52	16	19	17.50 ± 1.08

Table 3. *T. brontispae* sex ratio

Parameter	N	Min.	Max.	Average ± SD
Progeny	173	16	19	17.30 ± 0.95
Male	173	6	9	7.10 ± 0.99
Female	173	9	12	10.20 ± 0.92
Percentage of females (%)	173	55.56	66.67	60.12 ± 3.60

3.2. Discussion

Tetrastichus brontispae is a larval pupa parasitoid and a gregarious parasitoid. This parasitoid attacks the late instar larvae and pupae, 1-2 days old. Gregarius parasitoid is a type of parasitoid that can grow and develop more than one individual parasitoid in one individual host. In gregarious parasitoids, superparasitism (laying of eggs or some eggs on a host that other parasitoids of the same family have parasitized) affects egg size and the number of eggs laid (Husni *et al*., 2011). The results revealed that an increased host density resulted in no increased parasitism of *B. longissima* by *T. brontispae*; the optimal host density was three host pupae per parasitoid when considering the costs for mass rearing. Moreover, parasitoid age was crucial for effective parasitism and affected the emergence rate. Although 2-h to 4-day-old parasitoids successfully parasitized the host pupae, younger parasitoids (within 2-day-old) presented higher parasitism capacity than older parasitoids (Liu *et al.* 2014).

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Availability of nutrients in the host dramatically affects the development of pre-adult parasitoids. Chou (2009) stated that the development period of *T. brontispae* eggs, larvae, and pupae were 3.1, 5.0, and 9.3 days, respectively. The development of *Tetrastichus* sp. is also influenced by abiotic factors, such as temperature and humidity. According to Sidauruk *et al.* (2013), *T. brontispae* requires a temperature of 25-30^{0}C with a humidity of 70-75%. Exposure to low temperature (2 ºC) for 4, 24, and 48 h hurt biological performance (survival, parasitism rate) of *T. brontispae* with immature parasitoids (eggs, larvae, and pupae) being more sensitive to low temperature than adults (Lu *et al.* 2008).

After leaving the host, the parasitoid *T. brontispae* immediately copulated. One day after the parasitoid copulation, the female parasitoids can lay eggs. When trying to spawn, the *T. brontispae* imago first approaches the host. The signs used include chemical compounds on the surface of the host's body and physical signs such as the size, shape, age, or texture of the host. Once it leaves the host pupa, the courtship and mating behaviour of *T. brontispae* were observed within 1–2 min after a male meets a female, and the mating process was usually short (Liu *et al.*, 2022).

When the female *T. brontispae* parasitoid was first exposed to the host, the female did not directly approach the host. Parasitoids are seen walking around the host. After five to ten minutes, some parasitoids begin to approach the host but have not yet made oviposition. The parasitoids begin to mount the host's body and make oviposition in the next few minutes. At the time of treatment, the female parasitoid *T. brontispae* tended to wait to get a chance to lay eggs so that oviposition did not co-occur. In treatment with one female parasitoid, the laying process in the host body can occur well, and the opportunity to develop and emerge as an adult parasitoid is greater. Liu *et al.* (2022) said that the duration of oviposition was 16–20 min. The number of emerged adults had nothing to do with oviposition duration. Every segment of host pupa could be parasitised except the first and the last segments. When ovipositing, the female will choose to parasitise among multiple pupae

Symptoms of the parasitized host change color to black, and then the host does not move. Moore (2007), which states that in the body of the host, these larvae continue to parasitize the host so that the host loses turgor, becomes dark, and rot. Furthermore, the host's body can be broken, and when it dries, the integument changes color. It was also found that the parasitized host would die on the 3rd to 6th day after being infested. It was also seen that the abdomen was growing because it contained *Tetrastichus* sp larvae (Sidauruk, 2013, Ikhsan *et al.*, 2016).

A female *T. brontispae* can parasitize 1-3 hosts during its lifetime. The highest oviposition and meridian is a day after emergence from the host. The research results by Thi *et al.* (2009) stated that *T. brontispae* could parasitize 1-4 *B. longissima* birds during their lifetime, with an average parasite of 2.7. The optimal time to parasitize *T. brontispae* against *B. longissima* is 24 hours after

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the appearance of an adult (Pundee, 2009). Females and males were capable of mating more than once in their lifetime, and antennae appeared to be an essential communication tool during their courtship behavior. When multiple adults were together, the apparent competition was observed between males. When ovipositing, the female will choose to parasitise among multiple pupae (Nguyen *et al*., 2012; H. Liu *et al*., 2022).

The results showed that *T. brontispae* keridian ranged from 9-19. This is consistent with Waterhouse and Sands (2001) statement that one parasitized pupa can produce 8-20 parasitoids. Apart from the feed, the presence of a host also affects the lifespan of female *T. brontispae* imago. The parasitoid treatments with host + honey, only the host, only honey, and without the host and honey (given paper moistened with water) had a lifespan, and standard deviations were 9.7 ± 0.82, 4.7 ± 0.48, 3.5 ± 0.53, and 2.9 ± respectively. 0.57 days. The results showed that one day after the parasitoid copulated, the female parasitoid was able to lay eggs. From the observations, it can be seen that the age of *Tetrastichus sp.* males is shorter than females. The second or third day after infestation and after *Tetrastichus sp.* The parasitoid dies after the male finishes copulation (Sidauruk, 2013; Takasu *et al*., 2018).

It is hoped that the positive impact of the research results obtained can advance parasitoid conservation efforts in the field. The technique of environmental manipulation in coconut cultivation can be carried out by planting flowering plants because it is proven that honey can increase the life span and parasitoid properties of *T. brontispae*. The nectar in flowers is a source of food for parasitoid imago, which affects the length of life and the parasitoid quality (Wackers, 2001). Apart from being influenced by genetic factors, the life span of parasitoids is also influenced by the environment such as temperature, humidity, food sources (Uckan & Ergin 2003), and population density (Hooper *et al*., 2003; Tang *et al*., 2014).

Temperature is one of the leading environmental variables affecting development, reproduction, parasitoid longevity, survival, parasitic performance, and progeny production. The survival of *T. brontispae* decreases as the temperature increases. The increased temperature resulted in a decrease in adult survival and the survival of female parasitoids. Low temperatures (below 16 °C) and high temperatures (above 31 °C) reduce the parasitic power of *T. brontispae* (Liu *et al*., 2014, Ikhsan *et al*., 2020). This parasitoid attacks the old larval and young pupae stages of the coconut leaf beetle (*Brontispa longissima*). The parasitic power in the laboratory is 10% of late instar larvae and 60-90% of *Brontispa longissima* pupae. Natural parasites in West Kalimantan are 59.23% (Wagiman, 2015).

According to Sidauruk *et al*. (2013) obtained, a ratio of *T. brontispae* between males and females of 1: 1.5, then the results of research by Duan *et al*. (2012) obtained from 1 host 57 offspring obtained a male to female ratio of 1: 3. The laboratory temperature at the time of the

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study ranged from 24-300 C. Lu (2008) stated that temperature did not affect the sex ratio of parasitoid offspring. In addition, the sex ratio, the emergence rate, and the developmental time were not influenced by the host stages (K. Liu *et al.* 2016).

4. Conclusions

The development period from egg to imago is 18.95 ± 0.75 days. The length of life of the imago was significantly different between females (9.7 ± 0.51 days) and males (4.5 ± 0.52 days)—honey in increasing lifespan and enhancing *T. brontispae* offspring. The offspring of a female is 17.50 ± 1.08, with a female proportion of 60.12%. Apart from the honey feed, the presence of a host can also affect *T. brontispae*.

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