

BIOLOGY AND BEHAVIOR OF *Eochantecona furcellata* WOLFF (HEMIPTERA: PENTATOMIDAE) MASS REARED ON LARVAE OF *Corcyra cephalonica* (LEPIDOPTERA: PYRALIDAE) and *Tenebrio molitor* (COLEOPTERA: TENEBRIONIDAE)

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ABSTRACT

Eochantecona furcellata is a generalist predator. It has a wide prey range of lepidopterans infesting several pests on food crops and plantation crops. This research attempted to determine the biology of the predator *E. furcellata* with the given type of prey, namely *Corcyra cephalonica* and *Tenebrio molitor* larvae. In this study, *E. furcellata* was reared on *Asystasia* plants and given diet of 10 larvae of *C. cephalonica* and *T. molitor* each, and then observed its biological parameters. The results showed that *E. furcellata* given a *T. molitor* diet took a longer time to search and preyed on more larvae than those fed a *C. cephalonica* diet. The lifespan of the nymphal stage of *E. furcellata* when given two types of prey was not significantly different, but the lifespan of the male and female adults was significantly different. The mean age of both the male and female adults was shorter when prey of *C. cephalonica* than of *T. molitor*. The number of eggs produced, the number of eggs hatched and the percentage of eggs hatched by *E. furcellata* female were higher on *T. molitor* prey than on *C. cephalonica*. The provision of *T. molitor* prey as food in mass rearing of *E. furcellata* has a better impact on its biological aspects than *C. cephalonica* prey and is expected to support the natural enemy augmentation program for pest control in oil palm plantations.

Keywords: Biology, *Corcyra cephalonica*, *Eochantecona furcellata*, *Tenebrio molitor*

ABSTRAK

Eocanthecona furcellata ialah pemangsa umum. Ia mempunyai pelbagai mangsa yang luas seperti beberapa perosak pada tanaman makanan dan tanaman ladang. Selain itu, kajian ini cuba untuk menentukan biologi pemangsa *E. furcellata* dengan dua jenis diet yang berbeza iaitu larva *Corcyra cephalonica* dan *Tenebrio molitor*. *E. furcellata* ditenak pada tumbuhan *Asystasia* dan diberi mangsa kedua-dua 10 individu *C. cephalonica* dan *T. molitor* dan kemudian memerhatikan biologinya. Hasil kajian menunjukkan bahawa *E. furcellata* yang diberi diet *T. molitor*, mengambil masa yang lebih lama untuk mencari mangsa dengan lebih pantas dan bilangan larva yang dimangsanya adalah lebih banyak daripada yang diberi *C. cephalonica*. Jangka hayat peringkat nimfa *E. furcellata* apabila diberi dua jenis mangsa tidak berbeza secara ketara, tetapi jangka hayat dewasa jantan dan betina adalah berbeza dengan ketara. Umur purata kedua-dua imago jantan dan betina adalah lebih pendek dalam mangsa *C. cephalonica* daripada *T. molitor*. Bilangan telur yang dihasilkan, bilangan telur yang menetas dan peratusan telur yang ditetas oleh *E. furcellata* betina adalah lebih baik pada mangsa *T. molitor* berbanding *C. cephalonica*. Menyediakan mangsa *T. molitor* sebagai makanan dalam penternakan massa *E. furcellata* memberi impak yang lebih baik terhadap aspek biologinya berbanding mangsa *C. Cephalonica*, dan dijangka menyokong program menambah musuh semula jadi untuk kawalan perosak di ladang kelapa sawit.

Kata kunci: Biologi, *Corcyra cephalonica*, *Eocanthecona furcellata*, *Tenebrio molitor*

INTRODUCTION

Nettle caterpillars are pests that can reduce the production of oil palm plants. High infestation levels of leaf-eating caterpillars can significantly defoliate oil palm trees, resulting in yield losses of over 40% in the first and second years (Suparman et al. 2014; Pinnamaneni & Potineni 2023). There are four common species of nettle caterpillars (Lepidoptera: Limacodidae) in Indonesia to infest oil palms such as *Setothosea asigna*, *Setora nitens*, *Darna trima*, and *Parasa lepida*. Nettle caterpillars infest oil palm leaves and are able to consume the leaves until the leaves have holes till close to the leaf veins. A nettle caterpillars can consume 400 cm² of leaves during its lifetime (Gani et al. 2019). One of the predatory insects that can be able to control nettle caterpillars in oil palm plantations is the *Eocanthecona furcellata* Wolff (Hemiptera: Pentatomidae). *Eocanthecona furcellata* is a predatory stink bug that can be found in Southeast Asia, India, China, Taiwan and Japan, such as in cotton, bean, and vegetable fields (Tuan et al. 2016; Sarkar et al. 2021; Zhu et al. 2023). Since 1996, the predator *E. furcellata* has been mass-reared and used as a biological control agent in Taiwan against several pests including *Spodoptera litura* Fabricius (Lepidoptera; Noctuidae) and *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae) (Tuan et al. 2016)

Eocanthecona furcellata is a predator with a large prey range. This insect is known to prey on various leaf caterpillars including hairy caterpillars (*Galleria mellonella* L. (Lepidoptera: Gracillaridae) (Vanitha et al. 2018). In India, *E. furcellata* is an important predator of several pests species such as *Antheraea mylitta* (Barsagade & Gathalkar 2016), *Andrallus spinidens* and *Spodoptera frugiperda* (Jamil et al., 2021; Shylesha et al. 2018), *S. litura*, *Spilarctia obliqua* (Chaudhary et al. 2022), *Spilosoma obliqua* (Halder et al. 2020), *Maruca virata* (Tiwari et al. 2017).

In oil palm plantations, predatory stink bug from the order Hemiptera, especially *Sycanus* sp. and *E. furcellata* have been widely used in controlling oil palm leaf-eating

caterpillar pests such as nettle caterpillars and bagworms (Diratika et al. 2020; Rizali et al. 2018). Gani et al. (2019) and Susanto & Dongoran (2009) had reported the ability this predator to prey on each *E. furcellata* adult was one larva per day.

One method of biological control is augmentation. This is releasing several biological agents into the field to reduce pest populations below the control threshold. An important factor in spread of biological agents with the augmentation method is mass rearing of the biological agents. It requires a simple and effective mass rearing technique to create a variety of substitute prey for predatory insects. Therefore, this research was conducted to determine the predatory biology and behaviour of *E. furcellata* on two different diets *Corcyra cephalonica* (Lepidoptera: Pyralidae) and *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae in laboratory setting and it is envisaged that the best prey will facilitate the mass rearing of these predatory insects and enable their employment in oil palm fields for biological control.

MATERIALS AND METHODS

Sampling Location and Materials of Study

This research was carried out at the Research and Development Center Laboratory, PT. Nusa Pusaka Kencana, Bahilang Plantation (Asian Agri), Tebing Syahbandar District, Serdang Bedagai Regency, North Sumatra Province, Indonesia at an altitude of ± 30 meters above sea level. The material used in this research was predator imago *E. furcellata*, *T. molitor* larvae, *C. cephalonica* larvae, *Asystasia* plants, rice groats, ground corn, chayot, organdy cloth, clear mica plastic, cotton, containers measuring 34 x 24 x 12 cm, plastic containers with a diameter of 11.5 cm and a height of 12 cm, 5 x 3 cm polybag, a camera, stopwatch, petri dish, painting brush, scales, tweezers and other supporting tools. The data obtained from each parameter is recorded and then processed statistically using the t-test.

Mass Rearing of the Diets: *Tenebrio molitor* and *Corcyra cephalonica*

Tenebrio molitor, obtained from the previously maintained Asian Agri Research and Development Center in Bahilang Plantation, Tebing Syahbandar District, Serdang Bedagai Regency, North Sumatra Province. Larvae and adults of *T. molitor* were reared on 400 g of corn starch media in a rearing box measuring 34 x 24 x 12 cm and *T. molitor* needed water for its growth and development, in this study chayot was used as a source of water for *T. molitor*. The 300 grams of chayot has been cut into thin pieces is given to the larval and imago stages every day. The resulting eggs are transferred to another box, to prevent the imago from eating its own eggs and early instar larvae.

Corcyra. cephalonica obtained from the Asian Agri research and development center which has been maintained previously. Larvae, pupae and imago were reared on rice media in rearing boxes measuring 34 x 24 x 12 cm. When the imago is transferred to the laying box, the eggs produced were taken using a brush and put into a new rearing box with the same media.

Provision of Host Plants

Asystasia plants taken from oil palm plantations were planted in polybags and maintained for 1 week for the adaptation process in the polybags. These plants were then put into plastic containers and then predatory stink bugs were put into it and covered with gauze (Figure 1). *Asystasia* plants selected from the field must be truly healthy and not contaminated with pesticides and other chemical toxins. The characteristics of healthy plants are seen in the leaves that look fresh and not dull.

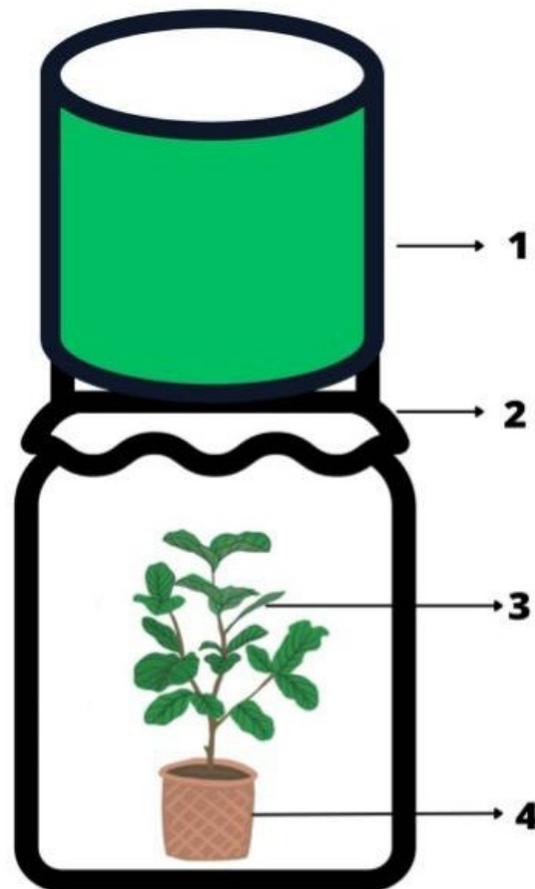


Figure 1. Maintenance container: (1). Barriers to prey larvae, (2). Organdy fabric, (3). *Asystasia* Plant, (4). Polybag

Sampling of the Predator *Eocanthecona furcellata*

Predatory stink bugs were collected from the field using an oblique method, namely collecting predatory stink bugs using hands or insect nets, then placing them in a plastic container that had been provided with air holes. The predatory stink bugs *E. furcellata* were taken directly from the field from oil palm leaves (Asian Agri plantation) in approximately 15 pairs of *E. furcellata* adults.

The predatory *E. furcellata* from the field were placed in a plastic container containing *Asystasia* plants in pairs and given diet of 10 *T. molitor* and *C. cephalonica* larvae every day in separate containers. The container was covered with gauze and the diet was placed on top of the gauze. A cardboard barrier was created and wrapped around the whole circumference of the container (the green part of Figure 1) to prevent the diet from escaping the gauze. *Asystasia* plants were replaced once a week along with cleaning the maintenance container. After laying eggs, the eggs were separated from the mother and stored in a petri dish.

Application of *Eocanthecona furcellata* to the Insect of the Test

Newly hatched *E. furcellata* Nymphs were placed in a plastic container containing *Asystasia* plants and given five prey larvae/day according to each treatment, after which the plastic container was covered with gauze. Prey was given every day. For the life cycle parameters of *E. furcellata*, nymphs were reared until they became adults to observe the speed of finding prey, predatory behavior, and the number of larvae preyed on. Insect maintenance was carried out from the first nymph and maintained until they became adults. Insects were maintained in

each separate container by inserting 1 individual *E. furcellata*. This experiment was repeated 15 times.

Other nymphs that have hatched at the same age are kept in a maintenance container until they become adults. Two pairs of male and female *E. furcellata* that are 1 day old are put into a plastic container filled with *Asystasia* plants and given 10 larvae/day as diets. These insects are kept until the adult dies. This experiment was conducted to see the fecundity of *E. furcellata* and was repeated 10 times. After the female *E. furcellata* lays eggs, the eggs are separated from the mother and kept in a petri dish. This is done until the female imago of *E. furcellata* no longer produces eggs.

Observation Variables

a. **Long time searching for prey**

The time needed for a predator to search for prey was calculated from the first time the larva was placed in the rearing container until the predator finds the prey larva. This observation was made for each nymph and imago at 07.00 AM - 07.00 PM.

b. **The Number of larvae preyed upon**

Observation of the number of larvae preyed on was carried out by observing how many predators preyed on the larvae being tested, starting from the first instar nymph until it became an adult. Observations start from at 07.00 - 19.00 WIB.

c. **Life cycle**

Observations on the life cycle were carried out by observing how long it takes for predators to complete each stage of their developmental stage until they become adult insects.

d. **Fecundity**

Nymphs were reared until the imago is 1 day old are paired according to treatment and repetition, then waited until they lay eggs. Observations of fecundity are carried out by counting the number of eggs produced by a female imago during her lifetime, starting from the time the female first lays her eggs until the female predator dies and calculating the percentage of eggs that hatch, which can be calculated formulas below:

$$\text{Egg Hatchability} = \frac{\text{The number of eggs hatched}}{\text{The number of eggs laid}} \times 100 \%$$

(Sugma et al. 2018)

RESULTS AND DISCUSSION

Long Time Searching for Prey

The ability of *E. furcellata* adult to find prey was faster than that of nymphs, both *C. cephalonica* and *T. molitor* prey (Table 1). The adult takes a faster time to find prey compared to the nymph stage. This is related to the relatively smaller body size of nymphs, slow feeding activity and predator saturation time after preying on one prey.

Table 1. Mean search duration for *Eocanthecona furcellata* for *Tenebrio molitor* and *Corcyra cephalonica* prey

| Stadium | N | Length of Search for Prey (Minutes) | | T-test (P-value) |
|------------------------|----|-------------------------------------|-------------------|---------------------|
| | | <i>C. cephalonica</i> | <i>T. molitor</i> | |
| 1 st Instar | 15 | - | - | - |
| 2 nd Instar | 15 | 177.3±4.3 | 165.2±2.0 | 0.009664 |
| 3 rd Instar | 15 | 111.8±3.7 | 106±2.5 | 0.257654 |
| 4 th Instar | 15 | 104.8±2.9 | 99.4±3.8 | 0.292611 |
| 5 th Instar | 15 | 98.3±2.0 | 97.2±2.9 | 0.792729 |
| Adult | 15 | 91.1±0.5 | 88.3±0.4 | 0.000165 |

If the $P < 0.05$, it means that the treatments are statistically different based on t-test.

The time of searching prey for *T. molitor* was faster than prey *C. cephalonica*. Nevertheless, the movement of *T. molitor* prey is slower and more silent and does not put up much resistance when it is preyed upon. In contrast, the larvae of *C. cephalonica* move more and squirm when *E. furcellata* inserts its stylet. In interactions between predators and prey, predators will take into account the energy they expend and the nutrients they will get from a prey, therefore the size of the prey largely determines whether the predator will handle it individually or in a group (Portalier et al. 2019). The research results of Susilo (2007) stated that several *E. furcellata* nymphs sucked their prey together if the prey's body size was larger.

The Number of Larvae Prey

Predators of *E. furcellata* start to prey on 2nd instar to adult stage (Table 2). In 1st instar, *E. furcellata* has not yet carried out predation. Nymphs that have just hatched from eggs still live in groups and gather near the eggs. This is in accordance with the results of research by Hideharu (2004), Tiwari et al. (2017) which stated that the first instar nymphs of *E. furcellata* that have just hatched are still in groups and are not yet actively feeding because they are still zoophytophagous.

Table 2. Mean number of *Corcyra cephalonica* and *Tenebrio molitor* larvae eaten by *Eocanthecona furcellata*

| Stadia | N | Number of Preyed Larvae (Tail) | | T-test (P-value) |
|-------------------------|----|--------------------------------|-------------------|-----------------------|
| | | <i>C. cephalonica</i> | <i>T. molitor</i> | |
| 1 st Instar | 15 | - | - | - |
| 2 nd Instars | 15 | 4.5±0.1 | 4.3±0.1 | 0.334282 |
| 3 rd Instars | 15 | 6.3±0.2 | 5.8±0.4 | 0.276934 |
| 4 th Instars | 15 | 9.6±0.5 | 6.7±0.3 | 0.638944 |
| 5 th Instars | 15 | 18.7±0.5 | 14.1±0.4 | 3.67x10 ⁻⁷ |
| Adult | 15 | 46.6±1.7 | 66.3±1.9 | 7.22x10 ⁻⁶ |

If the $P < 0.05$, it means that the treatments are statistically different based on t-test.

The number of larvae preyed upon by 5th instar nymphs and adult was statistically different. The difference in the number of larvae preyed on by 5th instar nymphs of *E. furcellata* in each treatment occurred due to larval size *C. cephalonica* smaller than larvae *T. molitor* so that 5th instar *E. furcellata* nymphs that were given *C. cephalonica* prey preyed on more larvae. The results of Sravika et al. (2020) stated the number of prey larvae 5th instar nymphs of *E. furcellata* with 2nd and 6th instar *Spodoptera frugiperda* larvae prey were 68±5.41 larvae and 38±1.03 larvae. As the predator's body size increases, the level of nutritional requirements will

increase, it needs for prey also increase. This is in accordance with the research results of Halder et al. (2020) which explains that the amount of prey required by *E. furcellata* increases in line with increasing instars and insect growth and development.

Table 2 shows that adult's prey more on *T. molitor* than *C. cephalonica*. The lifespan or duration of the adult stage of *E. furcellata* varies depending on the type of feed. In *Spilisoma obliqua* diet, the lifespan of *E. furcellata* ranges from 15 days for males and 21 days for females (Halder et al. 2020), while in *Plutella xylostella* diet it reaches 61.3 days for males and 62.8 days for females (Tuan et al. 2016). The longer the lifespan of the adult, the more prey it needs. Tuan et al. (2016) stated that the amount of prey consumed by adults is significantly greater than that of the nymph stage because the lifespan of the adult is longer than the lifespan of the nymph.

Life Cycle

Predatory stink bug *E. furcellata* undergoes incomplete metamorphosis. Its life stages are egg, nymph and adult. The nymph stage is passed through 5 instars. The life cycle of *E. furcellata* fed on *C. cephalonica* and *T. molitor* during the 1st and 2nd instar nymphs was not statistically different, but from instar 3rd to adult it was statistically different (Table 3). Tiwari et al. (2017) stated that *E. furcellata* takes more than 24 days before becoming an adult. The results of the study by Halder et al. (2020) found that the nymph passes through five instars within 22.25 to 27.25 days and develops into an adult with the prey larva *Spilosoma obliqua* (Walker).

Table 3. Mean life cycle of *Eocanthecona furcellata* from nymph to adult

| Stadia | N | Life Cycle (Days) | | T-test (P-value) |
|-------------------------|----|-----------------------|-------------------|------------------------|
| | | <i>C. cephalonica</i> | <i>T. molitor</i> | |
| 1 st Instar | 15 | 3.5±0.13 | 3±0.13 | 0.384 |
| 2 nd Instars | 15 | 4.5±0.13 | 4.3±0.12 | 0.334282 |
| 3 rd Instars | 15 | 4.9±0.09 | 4.1±0.13 | 0.003166 |
| 4 th Instars | 15 | 4.3±0.12 | 4.7±0.12 | 0.013517 |
| 5 th Instars | 15 | 7.8±0.11 | 7.6±0.16 | 0,000476 |
| Male Adult | 15 | 16.4±0.32 | 34.2±0.22 | 3.22x10 ⁻¹⁷ |
| Female Adult | 15 | 19.8±0.29 | 36.3±0.30 | 1.46x10 ⁻¹⁵ |

If the $P < 0.05$, it means that the treatments are statistically different based on t-test.

The adults of *E. furcellata* those given *T. molitor* prey, had a longer average lifespan compared to those given *C. cephalonica* prey. This shows that the type of prey provided has a significant influence on the lifespan of *E. furcellata*. Rustam et al. (2019) stated that the life cycle of female adults lasted 37.41 days while the life cycle of males lasted 35.22 days. Suyal et al. (2021) explained the age of the predatory *E. furcellata* adults on prey, *S. litura* larvae, 18.01±0.13 days and *C. cephalonica* larvae, the total maturity of predators was 15.81±0.26 days.

The adults of *E. furcellata* fed by prey *T. molitor* lived longer because the nutrients contained in the prey larvae were different. The nutritional content of *T. molitor* larvae includes 48.35% protein and 38.51% fat (Rumpold & Schlüter 2013) while *C. cephalonica* larvae contain less than 41% protein and 19.35% fat (Ye Xingqian et al. 1998). Bateman (1972) and Shah et al. (2022) said that types of feed that contain lots of protein, vitamins, water and carbohydrates can prolong the life of insects that eat them. This is in accordance with the results

of Galego et al. (1993) and Vanitha et al. (2018) who stated that the age of *E. furcellata* adults was strongly influenced by its feed source.

The instar stages of *E. furcellata* in this study consisted of 5 instars. (Figure 2). These results are in agreement with the findings of Suyal et al. (2021), but differ from those of Galego et al. (1993), who found that *E. furcellata* feeding on *Parasa lepida* larvae went through seven nymphal instars. The life span of adult females is longer than that of adult males. This is consistent with the research findings of Pillai & Agnihotri (2013) and Lenin & Rajan (2016) who found that the life cycle of adult female *E. furcellata* is longer than that of males.

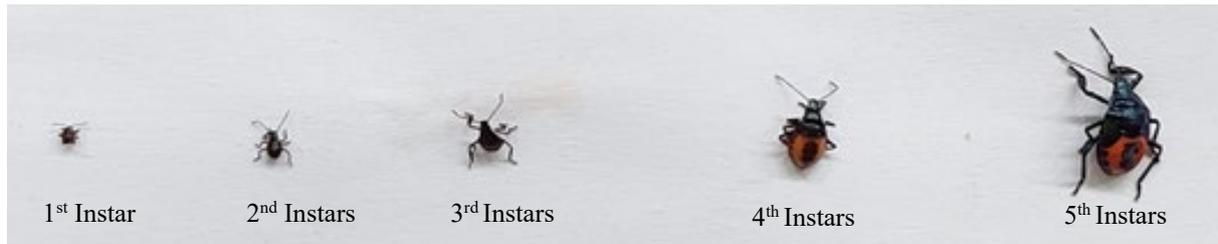


Figure 2 Long live immature stage of *Eocanthecona furcellata* nymph instars fed *Tenebrio molitor* prey

The differences between male and female *E. furcellata* can be distinguished based on their abdomen. The male adult has a tapered abdomen, while the female adult has a large and rounded abdomen (Figure 3). This is in accordance with the results of research by Rustam et al. (2019) and Lee et al. (2015) who stated that the distinguishing characteristic between male and female adults is that the size of the female adult is larger than that of the male.

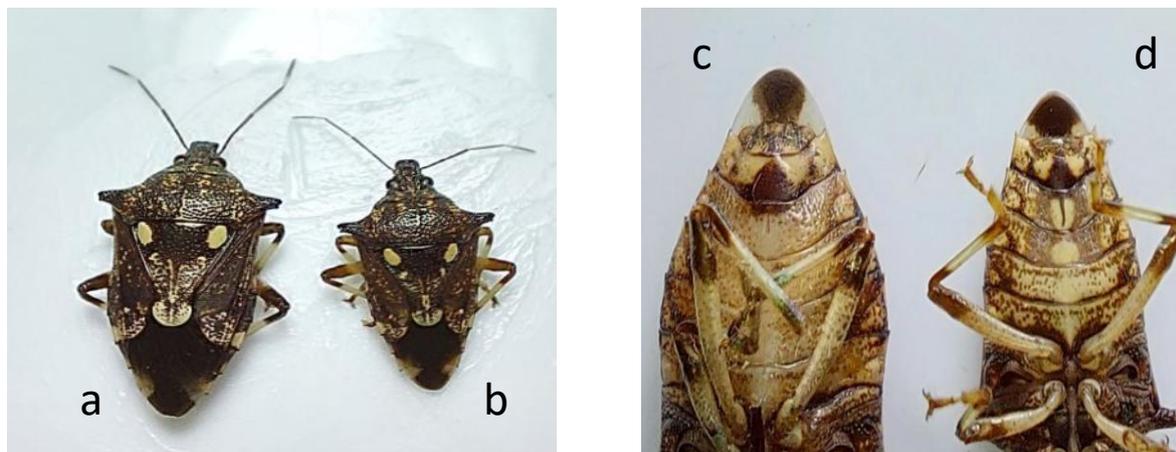


Figure 3. *Eocanthecona furcellata* adults: (a). Female, (b). Male (c). Female abdomen, (d). Male abdomen

Fecundity

Results of research on the period pre-oviposition, oviposition, post-oviposition, personality and hatching percentage of *E. furcellata* are presented in Table 4.

Table 4. Mean period of pre-oviposition, oviposition, post-oviposition, frequency and percentage of hatched eggs of *Eocanthecona furcellata*

| Parameter | Treatment | | T-test (P-value) |
|---------------------------------|-----------------------|-------------------|------------------------|
| | <i>C. cephalonica</i> | <i>T. molitor</i> | |
| Number of females (N) | 20 | 20 | |
| Preoviposition (days) | 3.5±0.22 | 14.3±0.21 | 1.04x10 ⁻¹⁰ |
| Oviposition (days) | 13.1±0.23 | 18.8±0.57 | 1.06x10 ⁻⁵ |
| Post oviposition (days) | 3.3±0.65 | 3.4±0.50 | 0,652501 |
| Number of eggs | 920 | 2134 | |
| Hatching eggs | 758 | 1879 | |
| Percentage of hatched eggs | 82.4±1.40% | 87.4±0.85% | 0.054529 |
| Percentage of eggs not hatching | 17.6% | 12.6% | |
| Average number of eggs | 46±1.50 | 106.7±5.08 | 6.42x10 ⁻⁵ |

If the $P < 0.05$, indicated the treatments are statistically different based on t-test.

The pre-oviposition and oviposition periods for *C. cephalonica* prey are shorter than for *T. molitor* prey. A short pre-oviposition period is beneficial for rapid growth, while a long pre-oviposition period is more beneficial for females to better mature their reproduction and increase fertility (Barilli et al. 2021). The results of the study by Dos Santos et al. (2022) showed that a longer pre-oviposition period allows females to select better quality resources, thereby increasing their reproductive success. The difference in the pre-oviposition period of *E. furcellata* may be influenced by the type of prey fed. The results of a study by Rustam & Syafa'ati (2023) showed that the pre-oviposition period of *E. furcellata* was 9.66 days when fed with *S. frugiperda* prey. This difference is related to differences in the nutritional value, size and physiological characteristics of the prey, which affect the health and reproduction of the predators. When predators are fed more nutritious prey, they tend to have a shorter pre-oviposition period because they can gather the energy and nutrients needed for reproduction more efficiently (Keerthi et al. 2020).

The average number of eggs, eggs that hatched and eggs that did not hatch, giving *T. molitor* prey, showed better results as they feed in the propagation process of *E. furcellata*. This is thought to be closely related to the nutritional content of *T. molitor*. The nutritional content present in *T. mollitor* is very suitable for the growth and development of *E. furcellata*, where this prey contains higher levels of protein and fat compared to *C. cephalonica* prey (Rumpold & Schlüter 2013; Ye Xingqian et al. 1998).

CONCLUSION

The ability of *E. furcellata* imago to search for prey is better than the nymph stage for two different prey. The predation process starts from the 2nd instar nymphs to the imago. *T. molitor* larvae are better used as alternative prey in the mass rearing of *E. furcellata* predators in the laboratory. This is supported by data on the ability to feed more during the nymph and imago periods, a longer life cycle, a greater number of eggs produced and a higher percentage of eggs hatched compared to the prey *C. cephalonica*. The success of mass rearing with alternative prey will open up greater opportunities to use this predatory stink bug *E. furcellata* to control the nettle caterpillars in oil palm plantations through a natural enemy augmentation programme.

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AUTHORS DECLARATIONS

Funding Statement

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Declarations

No ethical issue is required for this research.

Data Availability Statement

All data generated or analysed during this study are included in this published article.

Authors' Contributions

Maryani Cyccu Tobing (MCT), Johannes Surbakti (JS) and Widiastuty (W) conceptualized the research and designed the experiments. David Sinambela (DS), Tumpal Panjaitan (TP), participate in collecting research objects and supervising the implementation of research. Yohanes Samosir (YS), Ratna Rosanty Lahay (RRL) and Jonis Ginting (JG) participate in data collection and data processing. MCT, W and JS were responsible for writing the manuscript and interpreting the data. MCT, W is responsible for submitting the manuscript and revising the manuscript during the review process.

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