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Reproductive Performance of Black Soldier Fly (*Hermetia illucens*) With *Pennisetum purpureum* Extract

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Abstract
Black soldier fly (*Hermetia illucens*) is one of the biological agents for organic waste decomposition and this process produced insect biomass with significant economic value. Giving of juvenile hormone (JH) which is thought to be present in nappier grass (*Pennisetum purpureum*) can increase the number of BSF egg production. The purpose of this research is to determine the effect of nappier grass extract on the reproductive performance of *H. illucens* based on the number of eggs produced, the percentage of eggs hatched, and the sex ratio of BSF flies. The research was conducted experimentally using a completely randomized design (CRD). The number of eggs produced and the percentage of eggs hatched analyzed using non-parametric analysis with the *Kruskal-wallis* test and followed by the Mann Whitney test. Statistical testing using SPSS version 16.0. The sex ratio of BSF flies was analyzed descriptively. Phytochemical test analysis was carried out to ensure the content of compounds present in nappier grass extract (*P. purpureum*). The result shows that nappier grass extract has significant effect ($P<0.05$) on number of eggs produced and the percentage of eggs hatched based on *Kruskal-wallis* test. Based on the results of the Mann Whitney test, it was found that all treatments of nappier grass extract on the number of BSF egg production and the percentage of eggs hatched was significantly different ($P<0.05$). The highest egg production effect which the average number of egg production was 209 mg at 600 ppm. At 600 ppm had the highest percentage egg that hatched effect which the average of percentage egg that hatched was 88,759%. The optimum sex ratio for the cultivation was at 200 ppm, which is 60.42% female and 39.58% male
Key Words: Black Soldier Fly, Pennisetum purpureum, Reproductive performance

INTRODUCTION

Black soldier fly (*Hermetia illucens*) is a fly that has many benefits. This fly now many used as objects research because of its ability to consuming various types of organic waste (Kinasih *et al*., 2018). BSF larvae were able to reduce vegetable and fruit waste up to 63.9% (Nirmala, 2016), tuna head and offal waste up to 98.33% (Hakim *et al*., 2017), household waste in the form of cassava leaves up to 50.88% (Darmawan *et al*., 2017), and market organic waste up to 86.67% (Perkasa, 2019) and change it into the biomass the body can have protein content up to 50% (Bosch *et al*., 2014) and fatty acids close to 30% (Pang *et al*., 2020) of body weight. This body biomass potential as a raw material for production feed for aquaculture systems (Cummins *et al*., 2017), poultry farms (Onsongo *et al*., 2018), and mammals (Bosch *et al*., 2014) as well as raw materials biodiesel production (Pang *et al*., 2020). On the other hand, the resulting residue has potential as materials to improve soil quality (Beesigamukama *et al*., 2020).

A number of research shows there are levels maximum consumption of the larvae (Diener *et al*., 2009) and the conversion process is only done in stages larvae so that this activity is very dependent on the number of eggs that can be used for producing larvae. Naturally, female adults only mate and lay one egg times in their life is usually two days after the mating process is successful (Tomberlin & Sheppard, 2002). In condition this, then the stage of egg production that should be done artificially (Pastor *et al*., 2015) which allows eggs to be produced and collected in large numbers throughout years so that the waste treatment process can continuously (Macavei *et al*., 2020). Several studies have shown that the level of egg production is greatly influenced by the nutrition received by the parent when the larvae (Gobbi *et al*., 2013).

In the process of egg production there is one component that is a part an integral part of the adult female flies egg production process artificial environmental conditions, namely hormone, Juvenile hormone (JH) of course is a great candidate because it is a hormone that controls insect development (Gaubard , 2005), involved in regulating physiological processes such as metamorphosis and reproduction in most insects (Bede *et al*., 1999), increase pheromones but simultaneously suppress immune function (Rantala *et al*., 2003). Various types of plants have been known containing juvenile hormone, one of which is napier grass (*Pennisetum purpureum*).

P. purpureum or nappier grass which is usually used for animal food contains phenolic compounds such as alkaloids, flavonoids, saponins, terpenoids and tannins. The phenolic compounds have juvenile hormone activity that affects development insects that interfere with the molting process, or the process of change from eggs to larvae (Aradilla, 2009) and as a major gonadotropin regulating vitellogenesis, oogenesis, and other processes that are associated with reproduction (Wyatt, 1997).

In a study conducted by Al-Jorany & Al-Khazraji (2021) regarding the use of 800 ppm *Withania somnifera* extract containing alkaloids which can stimulate the multiplication of juvenile hormones against cotton leaf worm (*Spodoptera littoralis*) stated that the efficacy of *withania somnifera* ethanol extracts on the larvae of *Spodoptera littoralis* when the 2nd larvae instars were fed on food treated with 800 ppm extract concentration, The results pointed to increase the fertility rate of adults emerged from treated larvae and increased the number of eggs laid which rached 870 eggs/female While it reached 670 eggs/female in the control treatment. In a study conducted by Gujar & Palli (2016) stated that juvenile hormone affects the development of embryos in the eggs laid by females. The difference in the research that has been carried out is that in this study it used nappier grass extract which contains the same ingredients found in *Withania sommnifera* extract, then in this study a mixture of chicken feed was added and BSF larvae were used as test animals.

The purpose of this research is to determine the effect of napier grass extract on the reproductive performance of *H. illucens* based on the number of eggs produced, the percentage of eggs hatched, and the sex ratio of BSF flies. The results of this study are expected to provide scientific information on the reproductive performance of BSF by administering napier grass extract which can affect the number of eggs produced.

MATERIAL AND METHODS

The materials used in this study were BSF larvae, chicken feed in the form of pellets, nappier grass extract, attractant and dried banana leaves. The tools used for this study were container boxes, plastic cups measuring 6 cm x 6 cm x 9 cm, digital scales, tweezers, spoon, cages made of insect nets, eggier, plastic, rubber, stationery, handphone, measuring cup, tweezers, destilator, oven and spray. This research was conducted in September – November 2022 at the Green House of the Faculty of Biology and the Entomology - Parasitology Laboratory, Faculty of Biology, Jenderal Soedirman University.

The research was conducted experimentally using a completely randomized design (CRD) with 5 treatments and 7 replications. Treatment included:

T0: chicken feed + water

T1: chicken feed + napier grass extract 110 ppm

T2: chicken feed + napier grass extract 200 ppm

T3: chicken feed + napier grass extract 400 ppm

T4: chicken feed + napier grass extract 600 ppm

The independent variable is napier grass extract, while the dependent variable is BSF reproductive performance The research parameters observed were the number of eggs produced, the percentage of eggs that hatched, and the sex ratio of BSF.

a. Preparation of Nappier Grass Extract

Preparation of nappier grass extract refers to the method of Harborne (1987). After cleaning, cutting, and slicing napier grass, it is then dried using an oven at 50 - 60^{o}C for 6 days. After drying, the napiert grass is kneaded by hand until it is small. Chopped napier grass is soaked in a measuring cup with 95% ethanol in a ratio of 1: 2 (1 kg napier grass: 2 liters of ethanol) for 3 x 24 hours, then filtered. The maserate obtained was then concentrated in a destilator at a temperature of 40-50^{o}C, until the extract was obtained.

b. BSF Rearing

Container boxes, 6-day-old BSF larvae, feed, and napier grass extract were prepared. Each treatment containing 100 larvae placed onto container box. During experiments, the larvae were provided with 10 g wet/diet/larva/day with napier grass extract (110, 200, 400, 600 ppm) 10 mL/treatment/day. Treatment was done until larvae reach pupae.

Five cages made of insect nets were prepared. A total of 700 pupae from 5 treatments and 7 replications were made into one and put into each cage. Each cage was given an attractant in the form of fermented material and three eggier piles in each cage. Eggier is placed on top of the container containing the attractant. Inside the cage, dry banana leaves were also placed which were sprayed with water every day to drink the imagos.

c. Number of BSF Eggs in Each Cage

Observations were made by calculating egg spots and then converting them to the number of eggs produced every day in each treatment cage. The resulting eggs are then converted to get the number. Calculation of egg spots and number of eggs is carried out every day, starting from 08.00 to 14.00 WIB. Observations were made until all the flies in each cage died.

d. Calculation of the Percentage of Eggs that Hatched

During rearing the eggs hatch into larvae, the number of eggs produced per day is separated and the percentage of hatching is observed with maintenance until they hatch then the number of larvae is compared to the number of eggs. The total egg weight obtained every day in each cage is then put into the maintenance container, each data is entered into a container that has been given media in the form of fur then labeled and covered using a net. The eggs are not directly placed on the media, but are first given a paper mat so that the eggs do not directly interact with the growing media. The percentage calculation is converted to the weight of 3-day-old larvae, where the average weight of 3-day-old larvae is 0.05 g.

$$\text{Percentage of Eggs that Hatched (\%)} = \frac{\text{weight of 3 days old larvae}}{\text{number of eggs obtained x } 0{,}05}$$

e. BSF Sex Ratio

Observation of the ratio of males and females by observing dead flies or dead flies after reproduction (post term). Observations were made every day by counting dead flies and observing their sex in each treatment cage. After that, a comparison of the number of male and female BSF was recorded to determine the ratio

f. Data Analysis

Analysis of the number of eggs produced and the percentage of eggs that hatched were analyzed using non-parametric analysis with the Kruskal - wallis test, followed by Mann Whitney test. Statistical testing using SPSS version 16.0. The sex ratio of BSF was analyzed descriptively. Phytochemical test analysis was carried out to ensure the content of compounds present in Nappier grass extract (P. purpureum).

RESULTS AND DISCUSSIONS

According to Bilen *et al* (2013), the amount of egg production in insects is influenced by Juvenile Hormone (JH). JH influences the length of reproductive maturation in female insects. In most female insects, JH plays a role in mating, synthesis of sex pheromones, mating behavior and maturation of egg cells. JH released by the corpora allata modulate behavior related to female dispersal and reproduction. In the fat body of females, JH stimulates the synthesis of vitellogenin, and in the ovaries it facilitates the absorption of vitellogenin by increasing the patency of the follicular epithelium and then influencing the amount of egg production. In males, JH stimulates protein synthesis in the male accessory gland (mag). In some species, sex peptides produced by these glands are transmitted to the female during mating. This peptide then acts on CA and changes postcopulatory behavior in females and stimulates oogenesis (Hartfelder, 2000). Apart from that, according to Caruso *et al* (2014), there are several factors that influence egg production, namely physiological factors (low fertility, infertility, and nutritional deficiencies). Behavioral factors (low mating frequency, poor identification of nesting sites, and predation). Abiotic factors (lack of environmental stimulation), or technical factors (eggs laid outside the egg nest box and efficiency of egg collection. Results of analysis of the average of eggs that produced using Kruskal Wallis and Mann Whitney. The results of this analysis can be seen at. Table 1. following:

Table 1. The Average of eggs produced, percentage eggs that hatched and Sex Ratio of BSF

Parameter	Treatment				
	T0	T1	T2	T3	T4
Egg produced (mg)	67,39±18,55a	77,20±24,10b	95,39±44.92c	149,27±114.97d	209±161,10e
Number off eggs	3.275,15 - 3.315,58 eggs	3.751,92 – 3.798,24 eggs	4.635,95 – 4.693,18 eggs	7.254,52 – 7.344,08 eggs	10.157,40 – 10.282,80 eggs
Egg hatched (%)	84,56±4,91a	84,084±5,26a	84,671±4,50a	86,241±4.44b	88,759±4,33c
Larvae weight (gr)	138,48	157,7	196,26	312,81	450,78
Male	58.43	58.43	58.43	58.43	58.43
Female	41.57	41.57	41.57	41.57	41.57

Details: The different subscripts (a,b,c) indicate significant differences among the treatment within the number of egg produced ($P<0.05$) from the Mann Whitney Test follow up test analysis n = 350. Control/ T0 (chicken feed + water), T1 = (chicken feed + napier grass extract 110 ppm), T2 = (chicken feed + napier grass extract 200 ppm), T3 = (chicken feed + napier grass extract 400 ppm), T4 = (chicken feed + napier grass extract 600 ppm).

The research results showed that the percentage of eggs hatching, 0, 110, 200, 400, 600 ppm from the eggs produced from the first day to the tenth day, the average hatch rate was above 50%. At 0 ppm, the average egg hatching rate was 84.566% with a larval weight of 138,48 grams. At 110 ppm, the average percentage of eggs hatching was 84.08% with a larval weight of 157,70 grams. At 200 ppm, the average percentage of hatching eggs was 84,67% with a larval weight of 196 grams, 400 ppm obtained an average percentage of hatched eggs of 86.,4% with a larval weight of 312,81 grams and for 600 ppm an average percentage of hatched eggs was obtained of 88,75% with a larval weight of 450,78 grams (Table 4.1). Based on analysis using the *Kruskal-wallis* test, it can be seen that giving nappier grass extract has a significant effect on the percentage of egg hatching ($P<0.05$) (Appendix 5). Based on the results of the Mann Whitney test, it was found that the administration of nappier grass extract (0, 110, 200, 400, 600 ppm) on the number of BSF egg production was significantly different ($P<0.05$).

Based on the data obtained on the percentage of eggs that hatch, it can be concluded that nappier grass extract has an influence on the success of hatching BSF eggs. Where the extract contains JH which influences vitellogenesis in BSF females. Vitellogenesis is essential for egg development and embryo growth after oviposition in oviparous animals. Vitellogenesis in insect is characterized by the synthesis of vitellogenin (Vg) in body lipid (equivalent to vertebrate liver and adipose tissue), secretion into the hemolymph, transport through intercellular channels into the follicular epithelium and absorption of mature oocytes (Zheng *et al*., 2022). Juvenile hormone apart from functioning in the vitellogenesis process, JH also plays a role in oocyte development and maturation. JH is known in *H. virescens* to promote egg maturation. Kr-h1 expression is activated by JH through its hypothetical association with the cell membrane JH receptor via phospholipase C signaling. This signals the phosphorylation of Met and Tai by calcium/calmodulin-dependent protein kinase II (CaMKII), which then positively regulates their binding to JHRE, thereby activating the expression of target genes, such as Krh1, increasing egg hatching (Leyra *et al*., 2022).

In humidity conditions of more than 60% the egg hatch rate is 80%, then when the humidity is less than 60% the egg hatch rate is only 40% (Tomberlin et al., 2002). BSF eggs go through an incubation period of 72 hours or 3 days to hatch into larvae. In the next 22 – 24 days, the first instar larvae will develop until they become the sixth instar (Auliani *et al*., 2021). The development of the egg can be observed under a stereo microscope, the first changes that occur in the newly laid egg appear to be filled with yolk mass and within 24 hours embryogenesis has occurred, which can be seen, among other things, the segmentation of the larva's body, then within 48 hours the shape of the larva's body begins to appear. clear, there are red eye spots and mouth parts that are starting to become pigmented and within 72 hours clearer parts are visible such as spiracle channels that extend from the lateral spiracles to the posterior spiracles, as well as eye spots and mouth parts that appear increasingly clear, the movement of the embryo's body is also visible. The eggs hatch, the larvae emerge and immediately enter the feeding stage (Racmawati *et al*., 2010). The percentage of eggs that hatch is influenced by the fertility and fecundity of the imago. Fertility and fecundity of imago may be influenced by the food consumed during the larval phase, the physiological condition of the larvae, and the quality of the food consumed during the larval phase (Taufika *et al*., 2022). Results of analysis of the average percentage of eggs that hatched using Kruskal Wallis and Mann Whitney. The results of this analysis can be seen at. Table 1.

Based on the results of the calculation of the sex ratio obtained, it can be seen that in 600 ppm there were the highest number of females, namely 80,28%, then in 400 ppm there were 60,42% females, in 200 ppm there were 60,42% females, in 110 ppm there were 49,57 females, and in 0 ppm there were as many females. 41,57%. Then at 0 ppm, the highest number of males was obtained, namely 58,43%, then at 110 ppm, 50,43% of males were obtained, at 200 ppm, 39,58% of males were obtained, at 400 ppm, 32,15% of males were obtained, and at 600 ppm, the lowest number of males was obtained, namely 19,72% (Table 1).

Based on the sex ratio data obtained, it can be concluded that nappier grass extract has an influence on the BSF sex ratio. Where the extract contains JH which influences the sex ratio. At relatively high JH concentrations, molting stimulated by ecdysone will produce larval stages once again so that the product is larger larvae and a longer larval stage because it allows the larvae to feed on even more protein. JH inhibits metamorphosis. When juvenile hormone levels decrease, new ecdysone-induced molting can produce a developmental stage known as pupa. Inside the pupa, metamorphosis changes the anatomy of the larva into the form of an adult insect. The adult insect then emerges from the pupa. (Campbell, 2008). The size of the BSF larvae greatly influences the BSF pupae. BSF rely on their body size obtained from larval development to maintain their adult life size, larval development influences sex, adult wing size and ovary development in BSF (Miranda *et al*.,

2019). Adequate protein requirements lead to good growth and metabolism speed. Male BSF has a smaller body size than females. Based on the weight produced, female pupae weigh an average of 13% more than male pupae. According to Hoc *et al* (2019), the sex of the imago is influenced by differences in larval development time, females are heavier than males and take longer to develop. Adding juvenile hormone allows the larval phase to last longer, allowing the larvae to eat more food. According to Putra & Safa'at (2020), the weight and size of the pupa have a strong correlation with the sex of the imago. The female imago comes from a large pupa. Female pupae can be larger or smaller depending on the life history of the species, growth rate, and time spent during the larval period (Testa *et al*., 2018). JH also influenced fecundity and fertility of imago BSF. In *aedes aegypty* JH acts on male accessory gland function by regulating l asparaginase expression and triacyglycerol. JH can increase in size of MAGs, as well as an increase in total RNA and protein content of MAG. JH can also increase the expression acp genes in the MAG. Male accessory gland proteins (Acps) act as key modulators of reproductive success in insects by influencing the female reproductive physiology and behavior. JH act as gonadotropins by regulating vitellogenesis and oogenesis in female insect.

According to Tomberlin *et al* (2002) temperature affects the BSF sex ratio because at the optimal temperature, namely 30℃, it will produce a ratio of more females than males, then at higher temperatures, the number of females will decrease and at higher temperatures above 30℃ The sex ratio between males and females is almost equal. The ideal temperature for BSF mating and oviposition is around 27 - 37℃ and humidity 40 - 60% (Amrul *et al*., 2022). BSF males will only choose active females and BSF females will only choose males that are physiologically superior to prevent the production of defective eggs (Giunti *et al*., 2018). The best sex ratio for BSF females and males for producing a large number of eggs is 60:40 (Putra & Safa'at, 2020). In silk moths (*Samiacynthia ricini*) mating and fecundity are important factors that influence survival, sex ratio, population genetics, and productivity (Uyun, 2021). Fertility and fecundity of imago may be influenced by the food consumed during the larval phase, the physiological condition of the larvae, and the quality of the food consumed during the larval phase (Taufika *et al*., 2022). The sex ratio results obtained by adding nappier grass extract are written in table 1.

The results of phytochemical analysis of nappier grass (*P. purpureum*) taken south of the UNSOED biology faculty were analyzed the presence of phytochemical compounds which include saponin, alkaloid, steroid/terpenoid, tannin and flavonoid. The results of the phytochemical analysis can be seen on Table 2.

Table 4.2. Phytochemical Test Results of Nappier Grass (*Pennisetum purpureum*)

Compound	Result
Saponin	+
Alkaloid	+
Steroid/terpenoid	+
Tanin	+
Flavonoid	+

The results obtained from the analysis of alkaloid compounds in Mayer's reagent were that a white precipitate was formed. it is known that nappier grass (*P. purpureum*) is positive for containing alkaloids. Flavonoids and alkaloids resemble juvenile hormone. Flavonoids have juvenile hormone activity which influences the development of insects in the larval phase, causing the larval phase to last longer than usual (Elimam *et al*., 2009). The mechanism of action of flavonoid compounds as growth inhibitors contained in nappier grass extract has activity as an ecdyson blocker which can inhibit the action of the ecdysone hormone so that the moulting process does not occur in the larvae. Alkaloid compounds also affect the three main hormones that influence the growth process, namely ecdysontropin, ecdysone and juvenile hormone.

CONCLUSIONS

Napier grass extract 600 ppm had the highest egg production effect which the average number of egg production was 209 mg (10.157,4 – 10.282,8 eggs). Napier grass extract 600 ppm had the highest percentage egg that hatched effect which the average of percentage egg that hatched was 88,759%. The optimum sex ratio for the cultivation was at napier grass extract 200 ppm, which is 60.42% female and 39.58% male, 600 ppm has the highest number of female (80.28%), while 0 ppm has the highest number of male (58.43%).

REFERENCES

Al-Jorany, R.S. and Al-Khazraji, H.I., 2021. Effect of Plant Extracts of *Withania somnifera* (L.) Dunal. On Some Biological Performance of Cotton Leaf Worm (*Spodoptera littoralis Boisd.*).

Amrul, N.F., Kabir Ahmad, I., Ahmad Basri, N.E., Suja, F., Abdul Jalil, N.A. and Azman, N.A., 2022. A review of organic waste treatment

using black soldier fly (*Hermetia illucens*). *Sustainability*, *14*(8), p.4565.

Auliani, R., Elsaday, B., Apsari, D.A. and Nolia, H., 2021. Kajian Pengelolaan Biokonversi Sampah Organik melalui Budidaya Maggot Black Soldier Fly (Studi Kasus: PKPS Medan). *Jurnal Serambi Engineering*, *6*(4).

Aradilla A. S., 2009, Uji efektivitas larvasida ekstrak ethanol daun mimba (*Azadirachta indica*) terhadap larva *Aedes aegypti*. Skripsi. Semarang: Universitas Diponegoro Semarang.

Bede, J. C., Goodman, Walter, G. G and Tobe S.S., 1999. Insect Juvenile Hormone III in the Sedge, *Cyperus iria* L.: Distribution and Possible Biological Significance. IUPAC

Beesigamukama D, Mochoge B, Korir NK, Fiaboe KKM, Nakimbugwe D, Khamis FM, Subramanian S, Dubois T, Musyoka MW, Ekesi S, Kelemu S, Tanga CM., 2020. Exploring black soldier fly frass as novel fertilizer for improved growth, yield, and nitrogen use efficiency of maize under field conditions. *Frontiers in Plant Science*, 11, p.574592.

Bilen, J., Atallah, J., Azanchi, R., Levine, J.D. and Riddiford, L.M., 2013. Regulation of onset of female mating and sex pheromone production by juvenile hormone in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences*, *110*(45), pp.18321-18326.

Bosch, G., Zhang, S, Dennis, GABO., and Wouter, HH., 2014. Protein quality of insects as potential ingredients for dog and cat foods. *Journal of Nutritional Science,* 3; pp. 1-4.

Campbell, N. A. 2008. *Biologi Jilid I Edisi 8*. Erlangga: Jakarta.

Cummins, V., Rawles, S. D, Thompson, K., Velasquez, A., Kobayashi, Y., Hager, J., and Webster C., 2017. Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture,* 473; pp. 337-344.

Caruso, D., Devic, E., Subamia, I.W., Talamond, P. and Baras, E., 2014. *Technical handbook of domestication and production of diptera Black Soldier Fly (BSF) Hermetia illucens, Stratiomyidae*. IRD Edition: Marseille.

Darmawan, M., Sarto, and Prasetya, A., 2017. Budidaya larva black soldier fly (*Hermetia illucens*) dengan pakan limbah dapur (daun singkong). Dalam: Simposium Nasional

Diener, S., Zurbrugg, C., and Tockner, K., 2009. Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. *Wasta Management & Research*, 27; pp. 603-610.

Gaubard, Y., 2005. Juvenile hormone binding proteins-importance on the JH action. *Lund University, Introductory paper*, (168).

Giunti, G., Campolo, O., Laudani, F. and Palmeri, V., 2018. Male courtship behaviour and potential for female mate choice in the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae). *Entomologia Generalis*, *38*(1), pp.29-46.

Gobbi P, Martínez-Sánchez A and Rojo S., 2013. The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). *European Journal of Entomology,* 110(3); p. 461.

Gujar, H. & Palli, S.R., 2016. Juvenile hormone regulation of female reproduction in the common bed bug, Cimex lectularius. *Scientific reports*, *6*(1), pp.1-10.

Harbourne, J. B., 1987, *Metode fitokimia penuntun cara modern menganalisis tumbuhan, Diterjemahkan oleh Koasasih Padmawinata dan Imam sudiro, Edisi 1*, pp. 9-10, ITB: Bandung.

Hakim, A. R., Prasetya, A. and Petrus, HTBM., 2017. Studi Laju Umpan Pada Proses Biokonversi Limbah Pengolahan Tuna Menggunakan Larva *Hermetia Illucens*. *Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan*. 12(2); pp. 179–92.

Hartfelder, K., 2000. Insect juvenile hormone: from" status quo" to high society. *Brazilian Journal of Medical and Biological Research*, *33*, pp.157-177.

Hoc, B., Noël, G., Carpentier, J., Francis, F. and Caparros Megido, R., 2019. Optimization of black soldier fly (*Hermetia illucens*) artificial reproduction. *PloS one*, *14*(4), p.e0216160.

Kinasih, I., Putra, R. E., Permana, A. D., Gusmara, F. F., Nurhadi, M. Y, Anitasari, R. A., 2018. Growth performance of black soldier fly larvae (*Hermetia illucens*) fed on some plant

based organic wastes. HAYATI *Journal of Biosciences*, 25(2); pp. 79-84.

Leyria, J., Orchard, I. and Lange, A.B., 2022. Impact of JH signaling on reproductive physiology of the classical insect model, Rhodnius prolixus. *International Journal of Molecular Sciences*, *23*(22), p.13832.

Macavei, L. I., Benassi, G., Stoian, V., and Maistrello, L., 2020. Optimization of *Hermetia illucens* (L.) egg laying under different nutrition and light condition. *PLoS One* 15(4): e0232144

Miranda, C.D., Cammack, J.A. and Tomberlin, J.K., 2019. Life-history traits of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), reared on three manure types. *Animals*, *9*(5), p.281.

Nirmala, W., Purwaningrum, P. and Indrawati, D., 2016. Pengaruh Komposisi Sampah Pasar Terhadap Kualitas Kompos Organik Dengan Metode Larva Black Soldier Fly (BSF). In *Prosiding Seminar Nasional Pakar* (pp. 1-29).

Onsongo, V. O, Osuga, I. M., Gachuiri, C. K., Wachira, A. M., Miano, D. M., Tanga, C. M., Ekesi, S., Nakimbugwe, D., and Fiaboe, KKM., 2018. Insects for income generation through animal feed: effect of dietary replacement of soybean and fish meal with black soldier fly meal on broiler growth and economic performance. *Journal of Economic Entomology*, 111; pp. 1966- 1973.

Pang, W., Hou, D., Ke, J., Chen, J., Holtzapple, M. T., Tomberlin, J. K., Chen, H., Zhang, J., and Li, Q., 2020. Production of biodiesel from CO2 and organic wastes by fermentation and black soldier fly. *Renewable Energy*, 149; pp. 1174- 1181.

Pastor, B., Velasquez, Y., Gobbi, P., and Rojo, S., 2015. Conversion of organic wastes into fly larval biomass: bottlenecks and challenges. *Journal of Insects as Food Feed*, 1(3); pp. 179–93.

Perkasa, H. D.,2019. Biokonversi Sampah Organik Menggunakan Larva Lalat Tentara Hitam. *Jurnal Biodjati*, 2(1), pp.8-13.

Putra, R.E. and Safa'at, N., 2020. Study on sex determination and impact of sex ratio to reproduction success in black soldier fly. *Jurnal Biodjati*, *5*(2), pp.191-198.

Racmawati, R., Buchori, D., Hidayat, P., Hem, S. and Fahmi, M.R., 2010. Perkembangan dan kandungan nutrisi larva *Hermetia illucens* (Linnaeus)(Diptera: Stratiomyidae) pada bungkil kelapa sawit. *Jurnal Entomologi Indonesia*, *7*(1), pp.28-28.

Rantala, M.J., Vainikka, A., and Kortet, R., 2003. the role of juvenile hormone in immune function and pheromone production trade-offs: a test of the immunocompetence handicap principle. Pro.R.Soc.Lond.B, 270; pp. 2257-2261.

Sheppard, D. C., Tomberlin, J. K., Joyce, J. A., Kiser, B. C., Sumner, S. M., 2002. Rearing methods for the black soldier fly (Diptera: Stratiomyidae). *Journal of Medical Entomology*, 39; pp. 695-698.

Taufika, R., Sumarmi, S. and Hartatie, D., 2022. Pemeliharaan ulat grayak (*Spodoptera litura Fabricius*) (Lepidoptera: Noctuidae) menggunakan pakan buatan pada skala laboratorium. *AGROMIX*, *13*(1), pp.47-54.

Testa, N.D., Ghosh, S.M. and Shingleton, A.W., 2013. Sex-specific weight loss mediates sexual size dimorphism in *Drosophila melanogaster*. *PloS one*, *8*(3), p.e58936.

Tomberlin, J. K., & D. C. Sheppard., 2002. Factors infuencing mating and oviposition of black soldier ßies (Diptera: Stratiomyidae) in a colony. *J. Entomol. Sci*. 37: 345Ð352

Uyun, M.S., 2021. Perilaku Kawin dan Fekunditas Ngengat Sutera *Samia cynthia ricini* (Biosduval) (Lepidoptera: Saturniidae).

Zheng, H., Wang, N., Yun, J., Xu, H., Yang, J. and Zhou, S., 2022. Juvenile hormone promotes paracellular transport of yolk proteins via remodeling zonula adherens at tricellular junctions in the follicular epithelium. *PLoS Genetics*, *18*(6), p.e1010292.