Jurnal Biodjati 5(2):174-181, November 2020
e-ISSN : 2541-4208
p-ISSN : 2548-1606

JURNAL BIODJATI

http://journal.uinsgd.ac.id/index.php/biodjati

INFLUENCE OF MATES VIRGINITY ON BLACK SOLDIER FLY, *Hermetia illucens* L. MATING PERFORMANCE, EGG PRODUCTION AND QUALITY

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Received : July 17, 2020
Accepted : September 08, 2020

DOI: 10.15575/biodjati.v5i2.9049

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Abstract. *Hermetia illucens (L.), the black soldier fly (BSF), has raised attention due to its potential in solving various organic waste problems and the benefits of the prepupa biomass as an alternative highly nutritious livestock feed. The availability of BSF populations strongly depends on mating success and reproduction. The mechanism of sexual selection during the mating period also determines the success rate of mating and reproduction and the survivorship of the offspring. Here, we analyzed how the influence of different mating status (virginity) of mating pairs on mating success, daily oviposition, the number of eggs and fertility of eggs. BSF reared in semi-outdoor screen cages with five replication and four treatment of mates virginity combination. An analysis of variance (ANOVA) was used to assess differences in mating and reproductive performance among treatment. Male and female BSF performed the remating activity. The virginity of males and females significantly influenced mating and oviposition frequency. Mate choice was influenced by the virginity of mates. However, virginity status of mates did not affect the number of eggs, eggs weight, and eggs fertility. Understanding of mate selection behavior in relation to virginity in BSF served as important information to obtain the sustain population in the various scale of rearing design application.*

Keywords: *black soldier fly, mating behavior, mates virginity, oviposition, sexual selection*

Citation
Permana, A. D., Fitri, L. L. & Julita, U. (2020). Influence of Mates Virginity on Black Soldier Fly, *Hermetia illucens* L. Mating Performance, Egg Production and Quality. *Jurnal Biodjati*, 5(2), 174-181.

INTRODUCTION

Black soldier fly (Diptera: Stratiomyidae) is an important insect in nature especially related to its role as a decomposer of various organic wastes (Julita et al., 2019; Permana et al., 2018; Surendra et al., 2016; Zhang et al., 2010). Several studies have shown the ability of BSF larvae to convert various organic wastes into prepupa biomass which harvested for animal feedstock (Gao et al., 2019; Kinasih et al., 2018; Manurung et al., 2016; Gobbi et al., 2013; Diener et al., 2009; Myers et al., 2008). The economic value of the black soldier fly (BSF) is well established, however, some important aspects for managing the sustained population of BSF related to its reproductive success are relatively unexplored. Reproduction is the most essential phase to efficient rearing development of BSF (Julita et al., 2020; Hoc et al., 2019) which highly related to the availability and sustainability of BSF population in both small and mass scale rearing installation.

It is well known that the quality of mating pairs influenced the reproductive performance of BSF (Nguyen, 2015). Several studies, in insects, reported some of the advantages of mating related to the virginity of mating pairs (Tanner et al., 2019; Xu & Wang, 2009; Lewis & Iannini, 1995; Arnaud & Haubruge 1999). Males and females significantly preferred virgin to non virgin mates for mating, suggesting that virginity affects reproductive fitness (Jimenez-Perez & Wang, 2004; Shelly et al., 2004; Xu, 2010). Young and virgin individuals are preferred when choosing a mating partner because they ensure higher reproductive potential (Vickers, 1997). Virgin males can produce greater spermatophores (Kaitala & Wiklund, 1995; Bissoondath & Wiklund, 1996) and virgin females have better reproductive output (Tanner et al., 2019; Vickers, 1997; Xu & Wang, 2009). This study was designed to assess the influence of male and female virginity status on mating and reproductive performance of BSF. Considering the effects of mates virginity on ovipositing behaviors and fertility of offspring, thus we measured several parameters such as total mating frequency, the number of egg-laying females, the total number of eggs, and the fertility of eggs. This study revealed the mating and reproductive behavior of BSF in relation to virginity, as important information to develop an understanding of variables which may influence the success egg production in the rearing system.

MATERIALS AND METHODS

Study Site

All research took place in an integrated garden of the Faculty of Science and Technology, UIN Sunan Gunung Djati, Bandung, Indonesia. The experiment performed inside 60 x 60 x 60 cm^3 screened breeding cages placed in a 4 x 4 x 4 m^3 semi-outdoor screen house. This research was conducted for two months, in October-November 2019.

Source of BSF

Eggs were collected from a BSF colony housed in a screen cage (3 × 3 × 3m) maintained on the roof-top of The School of Life Sciences and Technology building located at Institut Teknologi Bandung. After hatching, the larvae were fed on commercial chicken feed (brand HI-PRO-VITE) with 70% moisture in a 30 x 25 x 10 cm plastic container. In the pupal stage, all individuals were removed from the containers and collected for the experiments.

Influence of Virginity on Mating Success and Oviposition Frequency

To ensure individual virginity, the uniformly sized of BSF final stage pupae (characterized by dried, tiff and dark pigmentation) were maintained individually in a plastic cup (height 11 cm, diameter 6 cm) covered with a black sheet until they emerge to adult. Adult BSF were distinguished by sex, then each group of virgin males and females were kept in different cages before being used for experiments. BSF used was one-day-old male and female individuals. In this study, four treatments were performed based on the mating status or male and female virginity (Table 1), namely:

Table 1. Combination of virginity mating pairs of BSF

Virginity combination of mating pairs	
Male	Female
Virgin (V)	Virgin (V)
Virgin (V)	Non-virgin (NV)
Non-virgin (NV)	Virgin (V)
Non-virgin (NV)	Non-virgin (NV)

Male and female non-virgin (mated) are individuals which have completed copulation. Immediately after the mating pairs finish copulation, marked by the release of the genital contact (Julita et al., 2020), each male and female was collected in different cages, until a non-virgin group was sufficient for experimentation. A number of 30 pairs of adult BSF were used in each combination of virginity treatments. All treatments were carried out in 60 × 60 × 60 cm3 adult screen cages placed in a semi-outdoor screen house. The adult cages were provided with water (ad libitum) and decaying organic waste (a mixture of fruits wastes; papaya, pineapple and mangoes) as an egg-laying stimulant for females. The egg-laying stimulant medium was placed in a container (20 × 15 × 5 cm) at the center of the cage and with several strips wooden, act as an ovitrap, placed above the medium. Several observational parameters including total mating frequency, daily mating frequency, total oviposition frequency, daily oviposition frequency, number of eggs, egg weight and egg fertility were recorded. Mating frequency (the number of mating pairs) was initiated on the date of adult release into the treatment cages. Observations were made for 15 min periods at 1 h intervals during daylight hours each day. The observation was conducted every day for 14 days from 06.00 a.m. to 18.00 p.m. The oviposition frequency or rate of oviposition was determined by recording female oviposit and egg clutches deposited daily on the ovitrap (15× 5 cm in length). The ovitraps were replaced daily, and the numbers of egg clutches were calculated.

During the observation of all treatments, the environmental conditions were measured for 14 days. The average temperature was 27.8°C; a minimum temperature was 24.4°C and the maximum temperature was 29.3°C. Relative humidity was around 80%, minimum humidity was 73% and the maximum humidity was 86%. The light intensity ranged from 7854.17-10856.39 lux, with an average of 9556.80 lux.

Statistical Analysis

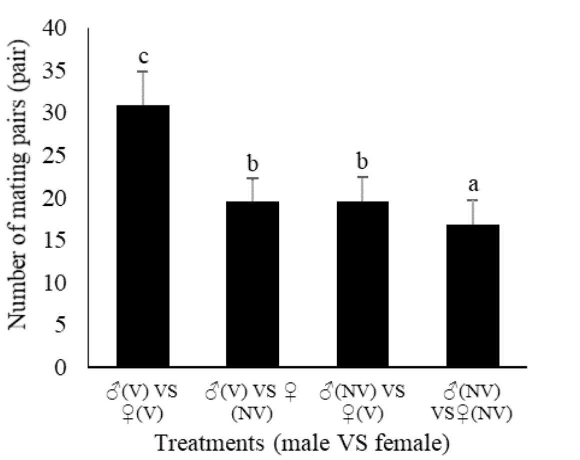
To determine differences in total mating frequency, frequency of oviposition, number of eggs, eggs weight and eggs fertility among treatments, a statistical analysis of variance (ANOVA) was performed with Duncan test as posthoc test. The confidence level applied in this study was 95%. All statistical analyses were performed using SPSS 25.0.

RESULTS AND DISCUSSION

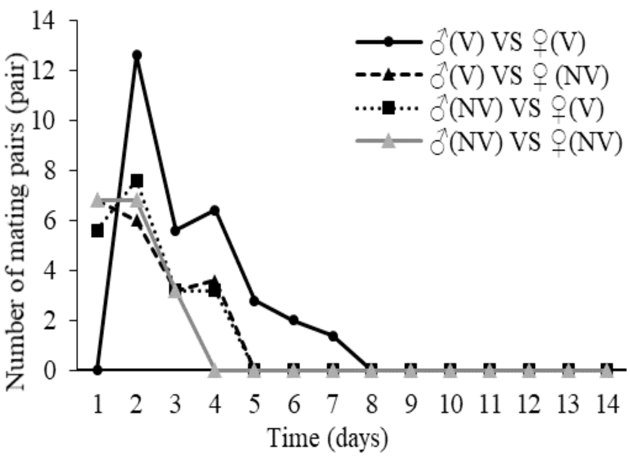
Influence of Male and Female Virginity on the Mating Frequency

Virginity status of mates in adult BSF influenced the mating frequency and oviposition frequency but did not affect egg number, egg weight, and egg fertility. Mating frequency revealed significant differences among treatment ($P < 0.05$) (Figure 1a). The total mating frequency of male and female virgin treatment was significantly higher than other combination and the lowest mating frequency was recorded on non-virgin mating pairs (Figure 1a). The mating behavior of male and female virgin pairs began at the second day of observation, while other treatments started from the first day of observation. The number of mating pairs tends to decrease from the first day of the mating period to the last day at all treatments. The mating period of virgin pairs occurred for six days and tends to be longer than other mating pairs, while non-virgin mating pairs have the shortest mating period of only three days and the frequency was decreasing with time (Figure 1b).

This study showed multiple mating activity on adult BSF was observed and the virginity status of mating pairs affected the frequency of mating, which different from Giunti et al. (2018) who used different observational method. Multiple copulations allow females to get an additional supply of sperm and obtain nutrition from males (Jimenez-Perez et al., 2003; Wang & Davis, 2006). Multiple mating behavior was found in the female of other insect such as hide beetle (*Dermestes maculates*) (McNamara, 2004; Archer & Elgar, 1999), pseudoscorpion (*Cordylochernes scorpioides*) (Newcomer et al., 1999), and *Ephestia kuehniella* (Xu, 2010). This behavior may related to the strategy of females to gain material and physiological resource that do not obtain from previous pair (Lemaitre el al., 2009) and genetic benefit of better male (Xu, 2010; McNamara, 2004; Jennions & Petrie, 2000; Archer & Elgar, 1999; Newcomer et al., 1999; Zeh et al., 1998).



(a)



(b)

Figure 1. The average of mating frequency in the BSF virginity mating pairs treatment a) Total mating frequency, b) Frequency of daily mating. Bars with different letters are significantly different (P <0.05)

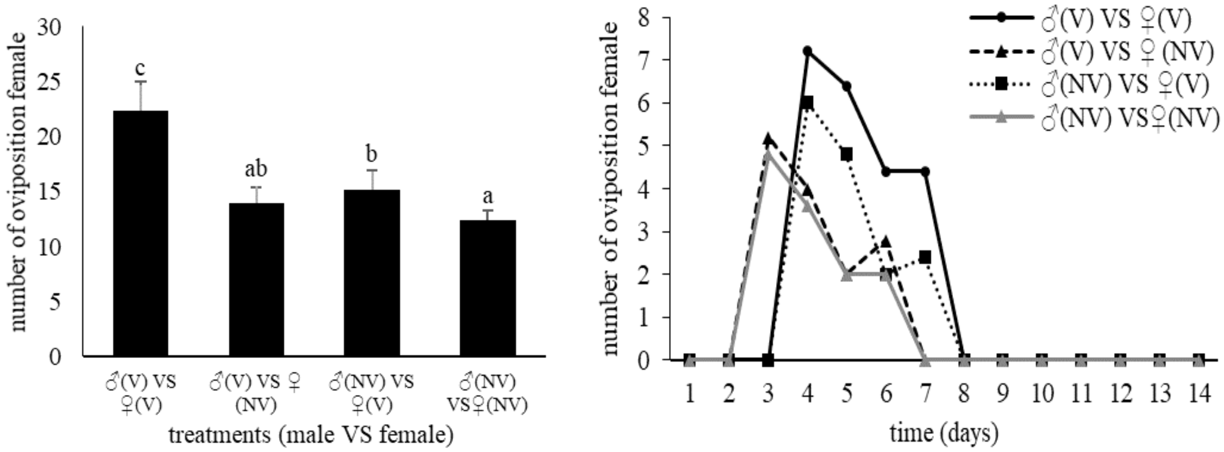
Oviposition Frequency, Egg Mass, Fecundity and Egg Fertility

There was a significant difference between treatments in the mean parameters of the number of oviposition females. Virgin female-male pairs have a significantly higher frequency of oviposition females compared to other treatments (Figure 2a). There were differences in the frequency patterns of daily oviposition females. Virgin females which mated with both types of males (virgin and non virgin) oviposited their eggs from the fourth day to the seventh day and the number of daily oviposition females was higher than non-virgin females. On the other hand, non-virgin females oviposited their eggs from the third day to the sixth day. The average frequency of daily oviposition in females tended to decrease from the first oviposition until the end of the egg-laying period and only lasted for four days in all treatment (Figure 2b).

The virginity did not significantly affect the number of eggs, egg weight, and egg fertility although male-female virgin pairs had a lower average of egg number and total egg weight (7648 eggs) compared to others (8283-8418 eggs) (Figure 3a). Egg viability produced from all combinations was relatively similar, between 76-78% (Figure 3b).

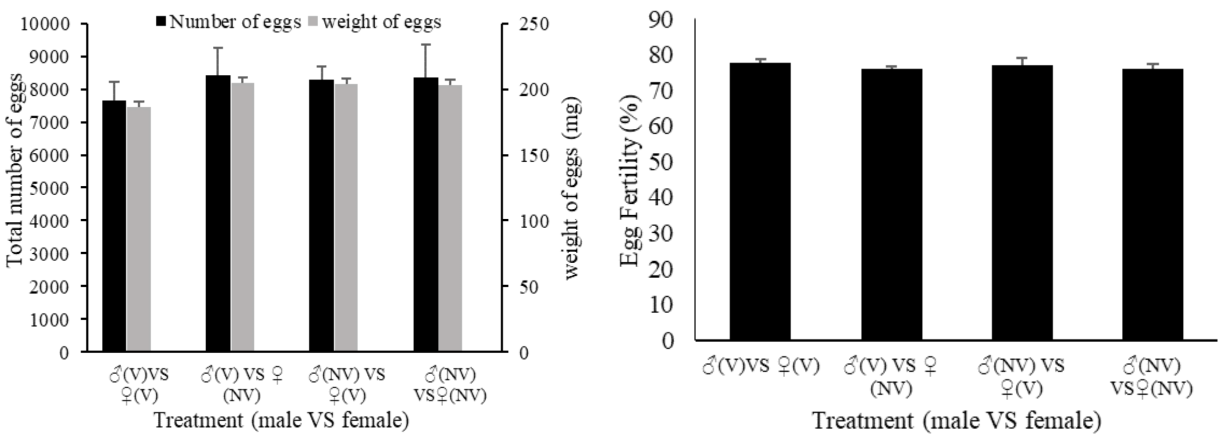
Based on this study, polyandry strategy in this species did not increase the viability of its offspring which also showed in other insects such as *Ephestia kuehniella* (Xu, 2010) and *Nysius huttoni* (Wang & Davis, 2006).

This is contrary to the 'genetic incompatibility hypothesis' (Zeh & Zeh, 2001) which assumed that the advantage of females from polyandry is to obtain higher offspring viability. However, in this research, the remating BSF females may get offspring with higher genetic diversity, since they are copulated by different males, that reducing sib competition (Robinson, 1992), disease transfer (Arnqvist & Nilsson, 2000), and inbreeding costs (Cornell & Tregenza, 2007) and in advance may provide adaptation advantages in ever-changing feed and environmental condition. Another possible explanation of this result may relate to the effect of innate factors on egg production and the length of females life span (Xu, 2010). Further studies are required to test these hypotheses on *Hermetia illucens*.



(a) (b)

Figure 2. The average of oviposition frequency in the BSF virginity mating pairs treatment a) frequency of total oviposition females, b) frequency of daily oviposition females. Bars with different letters are significantly different (P <0.05)



(a) (b)

Figure 3. The average of the total number of eggs in each virginity treatment of BSF mating pairs a) the total number and total weight of eggs, b) egg viability (average number of eggs that hatch into larvae).

The present study illustrated that virginity status of mates influenced mate choice in BSF. Although studies showed the benefit of multiple mating in term of possible nutrient transfer from male to female which increase the fecundity and egg viability (Maklakov, et al., 2005, Boggs, 1990, Arnqvist & Nilsson, 2000), this study clearly did not show it. Whether male did not inject nutrient to female, differences in egg production mechanism or the possible trade-off factors (Parker, 2006; Bonduriansky et al., 2008; Rönn et al., 2007; Green & Tregenza, 2009) which hinder the benefit of multi mating are questions to be answered by future studies.

ACKNOWLEDGEMENTS

This research was supported by the Research and Community Service Program (P3MI) funding ITB, which was granted to the first author and the Indonesia Endowment Fund for Education (LPDP scholarship), the Ministry of Finance of the Republic of Indonesia, which was granted to the third author.

REFERENCES

Archer, M. S. & Elgar, M. A. (1999). Female Preference for Multiple Partners: Sperm Competition in the Hide Beetle, *Dermestes maculatus* (DeGeer). *Animal Behaviour*, 58(3), 669-675.

Arnaud, L. & Haubruge, E. (1999). Mating Behaviour and Male Mate Choice in *Tribolium castaneum* (Coleoptera, Tenebrionidae). *Behaviour*, 136(1), 67-77.

Arnqvist, G. & Nilsson, T. (2000). The Evolution of Polyandry: Multiple Mating and Female Fitness in Insects. *Animal Behaviour*, 60(2), 145-164.

Bissoondath, C. J. & Wiklund, C. (1996). Effect of Male Mating History and Body Size on Ejaculate Size and Quality in Two Polyandrous Butterflies, *Pieris napi* and *Pieris rapae* (Lepidoptera: Pieridae). *Functional Ecology*, 10(4), 457-464.

Boggs, C. L. (1990). A General-Model of the Role of Male-Donated Nutrients in Female Insects Reproduction. *American Naturalist*, 136(5), 598-617.

Bonduriansky, R., Maklakov, A., Zajitschek, F. & Brooks, R. (2008). Sexual Selection, Sexual Conflict and the Evolution of Ageing and Life Span. *Functional Ecology*, 22(3), 443-453.

Cornell, S. J. & Tregenza, T. (2007). A New Theory for the Evolution of Polyandry as a Means of Inbreeding Avoidance. *Proceedings of the Royal Society of London Series B-Biological Sciences*, 274, 2873-2879.

Diener, S., Zurbrugg C. & Tockner K. (2009). Conversion of Organic Material by Black Soldier Fly Larvae: Establishing Optimal Feeding Rates. *Waste Management Res*, 27(6), 603–610.

Gao, Z., Wang, W., Lu, X., Zhu, F., Liu, W., Wang, X. & Lei, C. (2019). Bioconversion Performance and Life Table of Black Soldier Fly (*Hermetia illucens*) on Fermented Maize Straw. *Journal of cleaner production*, 230, 974-980.

Giunti, G., Campolo, O., Laudani, F. & 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), 29-46.

Gobbi, P., Martinez-Sanchez, A. & 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), 461–468.

Green, K. & Tregenza, T. (2009). The Influence of Male Ejaculates on Female Mate Search Behaviour, Oviposition and Longevity in Crickets. *Animal Behaviour*, 77(4), 887-892.

Hoc, B., Noel, G., Carpentier, J., Francis F. & Megido R. C. (2019). Optimization of Black Soldier Fly (*Hermetia illucens*) Artificial Reproduction. *PLoS ONE*, 14(4), 1-13.

Jennions, M. D. & Petrie, M. (2000). Why do Females Mate Multiply? A Review of the Genetic Benefits. *Biological Review,* 75(1): 21-64.

Jimenez-Perez, A., Wang, Q. & Markwick, N. (2003). Remating Behavior of *Cnephasia jactatana* Walker Females (Lepidoptera: Tortricidae). *Journal of Insect Behavior*, 16(6), 797-809.

Jimenez-Perez, A. & Wang, Q. (2004). Male Remating Behavior and its Effect on Female Reproductive Fitness in *Cnephasia jactatana* Walker (Lepidoptera: Tortricidae). *Journal of Insect Behavior*, 17(5), 685-694.

Julita, U., Fitri, L. L., Putra, R. E. & Permana A. D. (2019). Survival and Reproductive Value of *Hermetia illucens* (Diptera: Stratiomyidae) on Vegetable and Fruits Waste Rearing Substrate. *IOP Conf. Series: Journal of Physics: Conf. Series,* 1245, 012002.

Julita, U., Fitri, L. L., Putra, R. E. & Permana, A. D. (2020). Mating Success and Reproductive Behavior of Black Soldier Fly *Hermetia illucens* L. (Diptera, Stratiomyidae) in Tropics. *Journal of Entomology*, 17(3), 117-127.

Kaitala, A. & Wiklund, C. (1995). Female Mate Choice and Mating Costs in the Polyandrous Butterfly *Pieris napi* (Lepidoptera, Pieridae). *Journal of Insect Behavior*, 8(3), 355-363.

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 Bioscience*, 25(2), 79-84.

Lemaitre, J. F., Rigaud, T., Cornet, S. & Bollache, L. (2009). Sperm Depletion, Male Mating Behaviour and Reproductive 'Time-out' in *Gammarus pulex* (Crustacea, Amphipoda). *Animal Behaviour*, 77(1), 49-54.

Lewis, S. M. & Iannini, J. (1995). Fitness Consequences of Differences in Male Mating Behaviour in Relation to Female Reproductive Status in Flour Beetles. *Animal Behaviour*, 50(5), 1157-1160.

Maklakov, A. A, Kremer, N. & Arnqvist, G. (2005). Adaptive Male Effects on Female Ageing in Seed Beetles. *Proceedings of the Royal Society B-Biological Sciences*, 272(1580). 2485-2489.

Manurung, R., Supriatna, A., Esyanti, R. E. & Putra, R. E. (2016). Bioconversion of Rice Straw by Black Soldier Fly Larvae (*Hermetia illucens*): Optimal Feed Rate for Biomass Production. *Journal of Entomology and Zoology Studies*, 4(4), 1036-1041.

McNamara, K. B., Jones, T. M. & Elgar, M. A. (2004). Female Reproductive Status and Mate Choice in the Hide Beetle, Dermestes maculatus. *Journal of Insect Behavior*, 17(3), 337–352.

Myers, H. M., Tomberlin, J. K., Lambert, B. D. & Kattes D. (2008). Development of Black Soldier Fly (Diptera: Stratiomyidae) Larvae Fed Dairy Manure. *Journal of Environment Entomology*, 37(1), 11-15.

Newcomer, S. D., Zeh, J. A. & Zeh, D. W. (1999). Genetic Benefits Enhance the Reproductive Success of Polyandrous

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Females. *Proceedings of the National Academy of Sciences of the United States of America*, 96(18) 10236-10241.

Nguyen, T. T. X., Tomberlin, J. K. & Vanlaerhoven, S. (2015). Ability of Black Soldier Fly (Diptera: Stratiomyidae) Larvae to Recycle Food Waste. *Environmental Entomology*, 44(2), 406–410.

Parker, G. A. (2006). Sexual Conflict Over Mating and Fertilization: an Overview. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 361(1466), 235-259.

Permana, A. D., Ester, J. & Putra R. E. (2018). Growth of Black Soldier Fly (*Hermetia illucens*) Larvae Fed on Spent Coffee Ground. *IOP Conf. Series: Journal of Physics: Conf. Series*, 187 012070.

Robinson, G. E. (1992). Regulation of Division of Labor in Insect Societies. *Annual Review of Entomology*, 37, 637-665.

Rönn, J., Katvala, M. & Arnqvist, G. (2007). Coevolution Between Harmful Male Genitalia and Female Resistance in Seed Beetles. *Proceedings of the National Academy of Sciences of the United States of America*, 104(26): 10921–10925.

Shelly, T. E. (2004). Scent Marking by Males of the Mediterranean Fruit Fly, *Ceratitis capitata* (Diptera: Tephritidae). *Journal of Insect Behavior*, 17, 709–722.

Surendra, K. C., Olivier, R., Tomberlin, J. K., Jha, R. & Khanal, S. K. (2016). Bioconversion of Organic Wastes into Biodiesel and Animal Feed via Insect Farming. *Renewable Energy*, 98: 197–202.

Tanner, J. C., Garbe, L. M. & Zuk, M. (2019). When Virginity Matters: Age and Mating Status Affect Female Responsiveness in Crickets. *Animal Behaviour*, 147, 83-90.

Vickers, R. A. (1997). Effect of Delayed Mating on Oviposition Pattern, Fecundity and Fertility in Codling Moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *Australian Journal of Entomology*, 36(2), 179-182.

Wang, Q. & Davis, L. K. (2006). Females Remate for Sperm Replenishment in a Seed Bug: Evidence from Offspring Viability. *Journal of Insect Behavior*, 19(3), 337-346.

Xu, J. & Wang, Q. (2009). A Polyandrous Female Moth Discriminates Against Previous Mates to Gain Genetic Diversity. *Animal Behaviour*, 78(6): 1309-1315.

Xu, J. (2010). Reproductive Behaviour of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *Dissertation*. Massey University: Palmerston North, New Zealand. 1–178.

Zeh, J. A., Newcomer, S. D. & Zeh, D. W. (1998). Polyandrous Females Discriminate Against Previous Mates. *Proceedings of the National Academy of Sciences of the United States of America,* 95(23), 13732-13736.

Zeh, J. A. & Zeh, D. W. (2001). Reproductive Mode and the Genetic Benefits of Polyandry. *Animal Behaviour*, 61(6), 1051-1063

Zhang, J., Huang, L., He, J., Tomberlin, J. K., Li, J., Lei, C., Sun, M., Liu, Z. & Yu, Z. (2010). An Artificial Light Source Influences Mating and Oviposition of Black Soldier Flies, *Hermetia illucens*. Journal of Insect Science, 10, 1-7.