International Journal of Life Sciences and Biotechnology
Aydin, G. and E. Turantepe, Oviposition preferences of Black Soldier Fly (Hermetia illucens (L., 1758) (Diptera: Stratiomyidae)) on different manures. International Journal of Life Sciences and Biotechnology, 2025 8(1): p. 51-57.
https://doi.org/10.38001/ijlsb.1199393
Araştırma Makale/ Research article

Oviposition preferences of Black Soldier Fly (*Hermetia illucens* (L., 1758) (Diptera: Stratiomyidae)) on different manures

Gökhan Aydın [1], Ergin Turantepe[2]

ABSTRACT
Hermetia illucens L. (Diptera: Stratiomyidae) called as black soldier fly (BSF), is known as an efficient bio-converter of organic waste are mostly rearing for use as fish and farming feed. Studies on BSF cultivation are generally related to the discovery of the optimum organic substrates of the larval stage. The 'preference-performance principle' suggests that female adults prefer to oviposit in substrates that maximise offspring fitness. Therefore, the substrate needs to be also attractive to females for oviposition. To reveal the best substrates in which is attractive for the female to lay eggs is also extremely important for the better larval development. In this study, five different poultry manures, such as goat, peafowl, pheasant, and chicken, quail manure and a non-manure environment (control) were used in the experimental setup for BSF females to lay eggs in the study. Goat manure was least preferred by BSF adult females for laying eggs during the experiments. The most preferred manures for laying eggs by BSF were found peafowl, pheasant, and chicken, respectively. In the percentage similarity analysis, it was calculated that the number of eggs obtained from goat manure was the least and was different from all other manure tested. According to percentage similarity results the most similar group were found peafowl manure and chicken manure with 58.83%. The study also provides detailed information on the BSF breeding.

ARTICLE HISTORY
Received
27 September 2024
Accepted
16 December 2024

KEY WORDS
Insect farming,
poultry manures,
oviposition,
Hexapoda,
attractant

Introduction

Due to its geographical location, Türkiye hosts three different Hotspots (protection priority areas), namely Irano-Anatolian, Caucasus, and Mediterranean [1-3]. In parallel with the plant species richness and high endemism rate (almost 33%), insect biodiversity is also quite rich in the country [4-6]. While new insect species are being discovered every day due to the richness of plants and the favourable location of countries, some species are unfortunately included in the "critically endangered (CR)" or "extinct (EX)" category due to the negative effects of some agricultural policies [7-11]. The populations of insect species living in their natural habitats are significantly decreasing due to some negative anthropogenic and/or ecological factors, some tropical insect species, such as *Hermetia illucens* L. (Diptera: Stratiomyidae) called black soldier fly (BSF), is known as an efficient bio-converter of organic waste, are rearing for use as fish and farming feed in Türkiye [12-17].
Although not yet widely used, it is possible to use *H. illucens* for waste management other than farming feed [18-19]. While there are numerous studies on usage of BSF as farming feed, there are limited numbers of research that provide insight into the production procedures of it.
Insects play an important role in many areas around the world, including food security, environmental health, biotechnology, agriculture and medicine [20]. The production process (optimum temperature, humidity, food preferences, oviposition preference, etc.) of beneficial insects used as food, such as the *H. illucens*, needs to be improved and standardized. Assume that the Black Soldier Fly (BSF), designated for use as aquaculture feed, is subjected to testing with "Food A" as part of the production process. The larvae, which are nourished with "Food A," are subsequently desiccated and processed into fish meal, and the resultant impact on the development of Rainbow Trout is assessed. If, conversely, the BSF larvae were to be fed "Food B" instead of "Food A," it is likely that the effects on the development of Rainbow Trout would differ. Thus, the anticipated response would be "most probably NOT." It is worth noting that numerous studies have examined the implications of BSF as fish feed and/or poultry feed on the development of aquatic species or livestock, yet

1 Isparta University of Applied Sciences, Atabey Vocational School, Atabey-Isparta / Turkiye
2 Süleyman Demirel University, Faculty of Medicine, Department of Medical Biology, 32260, Isparta, Türkiye
*Corresponding Author: Gökhan Aydın, gokhanaydin@isparta.edu.tr

they often fail to disclose pertinent information regarding the production procedures employed [21-24]. Although the primary objective of our investigation is not to detail the production procedures for *H. illucens*, such information is included in the Materials and Methods section to assist researchers intending to conduct analogous studies in the future.

The aim of this study was to investigate the egg laying preferences of the BSF during the oviposition period, which is the most important of the production stages, using different types of manure.

Material and Methods

Rearing of *Hermetia illucens*

Before the experiments began, the *H. illucens* was allowed to produce one generation under laboratory conditions. To this end, eggs were began to be reared in a nutrient medium prepared by moistening with a 50:50 (%) mixture of goat manure and wheat bran, with the addition of water (50-60%) in a 5.5-liter production container, maintained at a constant temperature of 27 °C and 60% humidity. The production container was covered with a tulle large enough for the larvae to pass through, preventing the eggs from contacting water. The nutrient medium was kept moist by spraying a certain amount of water each day. After the first-stage larvae hatched from the eggs and passed through the tulle and reached the nutrient medium, organic waste supplement (homemade meals, fruit peels, etc.) was added at a rate of one-tenth of the bottom of the production container. When the larvae reached an average size of 6-7 mm, they were transferred to larger production containers and continued to be fed with organic waste at 2-day intervals depending on the number of larvae. As the larvae reached maturity, the number and size of the production containers were also increased in accordance with the growth of the larvae. The supplementation of organic waste was terminated upon the alteration of the larvae's color from white-yellowish to brown. At the onset of the prepupa stage, the larvae were separated from one another through a process of sieving, whereby they were passed through a series of progressively finer sieves in order to remove residual organic waste. Larvae in the prepupa period were placed in a different climate cabin in production containers with a wide base area and low height. The pupal period individuals were placed on shelves with a volume of 3 m^3 surrounded by tulle at 4 corners, and kept at 85% humidity and 29 ℃ until they emerged as adults. Artificial flowers with broad leaves were placed to resemble the natural environment for the adults emerging from the pupa. The environment where the adults were kept was sprayed 3 times a day. Mated females were taken to the experimental setup to lay eggs. Each time, 10 mated females were taken into the experimental setup prepared to lay eggs. This application was repeated a total of 10 times to determine the number of replications.

Preparation of the Experimental Setup

The experimental setup was designed by us to hypothesize adult female *Hermetia illucens* individuals would prefer which manure to lay eggs (Fig. 1). Five different poultry manure, goat manure and a non-manure environment (as control) were used in the experimental setup.

In the mechanism, the "release box" where adult females were placed was designed in the area where six different pipes meet. Six devices were designated at the ends of the pipes with lids where different moistened manure would be placed were designed. Goat manure, quail manure, chicken manure, pheasant manure and peafowl manure were placed in these devices. One device was left empty as a control group. A vacuum pump was used to ensure that the manure odors could reach the release box where the adult females were located. The areas where these connected to the pipes were designed to be funnel-shaped towards the inside of the chamber and are designed to be large enough for adult female individuals to pass through (Fig. 2). In addition, 1 mm diameter holes were drilled on the lidded boxes to reduce the vacuum created by the vacuum pump connected to the top cover of the release box and to allow the poultry manure odors in the lidded chambers to reach the release box.

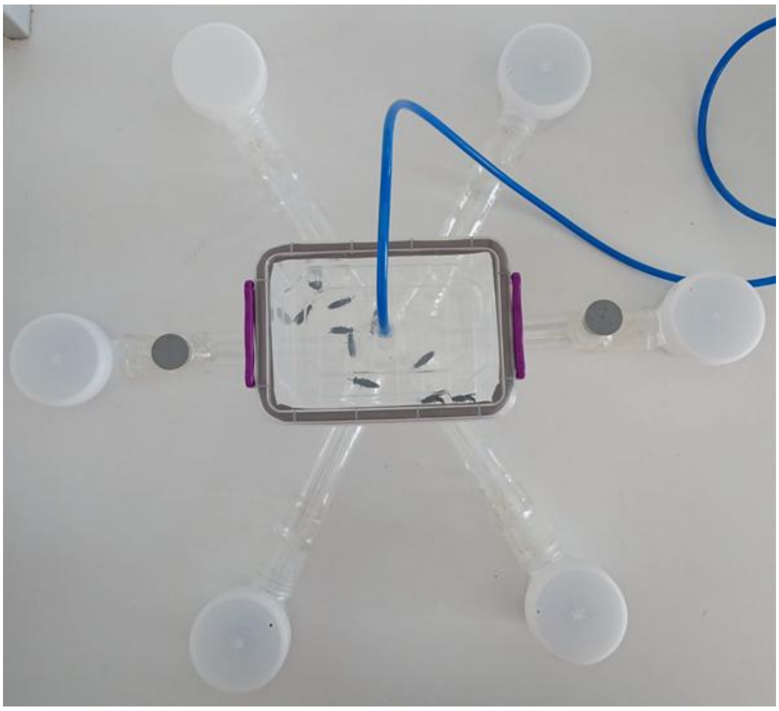

Fig 1 Experimental setup used to test which manure *Hermetia illucens* adult females would prefer to lay eggs

The location of the experimental setup was kept fixed and the lidded boxes and the experimental setup were cleaned in each new trial. In the experiment, the manures were placed in lidded boxes moistened by 95%. The vacuum pump's suction pressure was adjusted to 0.132 atm (100 mm Hg) (Preliminary studies conducted prior to the experiment concluded that this pressure was the most appropriate). After the experimental setup was completed, the captured females were transferred to the box called the release box connected to the system and the study was started. A total of 10 replications were conducted with 10 females in each experiment.

Fig 2 Lidded plastic container designed to be funnel-shaped for storing manures (left), the vacuum pump (middle), and vacuum pump's suction pressure (right)

Statistical Analysis

The means of two samples with equal variance obtained from manure X and manure Y were analysed by t - test to understand whether there was a significant difference in the effect of manure on the oviposition behaviour preferences of female adults of *H. illucens* in the experiments (P<0.01). LSD test was performed in order to difference between eggs laid of female adults of BSF on different manures.
The Multi Variate Statistical Package (MVSP) 3.11c program was used to classify the manures were taken into the experiment [25]. The classification method was chosen as the arithmetic group averages (Unweighted Pair Group Method with Arithmetic Mean - UPGMA) in the evaluation of the obtained data. Percentage similarity was used to calculate the similarities of different manures used in the experiments.

Results and Discussion

Mean number of *Hermetia illucens* eggs on manures of chicken and goat, pheasant and goat, peafowl and goat, peafowl and quail, peafowl and release box, peafowl and empty, peafowl and chicken were found statistically different by t - test while the rest combination manures in terms of average of eggs were found not significant (P<0.01) (Table 1).

Boafo et al. (2023) have been used six organic substrates for *H. illucens* production on their study and it has been evaluated for their suitability as oviposition attractants and larval development. In the oviposition tests, millet porridge mash has been found the most preferred substrate for egg laid, whereas from the other substrates (chicken manure, pig manure, fruit waste, pito mash, and waste from roots and tubes) have been recovered only a few eggs [26]. *H. illucens* larva has been found strongly preferred pig manure over the mass-rearing diet [27]. In another study, chicken, pig and cow manures have been used for evaluated to larval development of *H. illucens*. Larva on pig and cow manure have been recorded relatively greater abundance [28]. Agricultural waste, rice bran, vegetable waste, fruits waste, and household waste, have been mixed with goat manure, have been used to determine the preference to eggs laid by *H. illucens*. According to study; the highest number of eggs of BSF have been declared in household waste then vegetable waste, rice bran, and fruits waste while the lowest eggs have been found on agricultural waste [29].

Table 1 Number of eggs (Mean±SD) laid by *Hermetia illucens* adult females on different manures.

Manure	n	Mean±SD	Goat	Quail	Release Box	Empty	Chicken	Pheasant	Peafowl
Goat	10	0.2±0.17 c*	1.00000						
Quail	10	0.7±1.12 bc	0.18246	1.00000					
Release Box	10	0.9±0.54 bc	0.01792	0.63012	1.00000				
Empty	10	1.1±2.54 bc	0.10166	0.51725	0.72314	1.00000			
Chicken	10	1.6±1.82 b	**0.00578**	0.11452	0.16734	0.45905	1.00000		
Pheasant	10	2±3.33 ab	**0.00708**	0.06724	0.09427	0.25573	0.58433	1.00000	
Peafowl	10	3.5±2.27 a	**0.00000**	**0.00014**	**0.00012**	**0.00282**	**0.00825**	0.06053	1.00000

* The same letters in the column containing the mean and Standard Deviation (SD) values indicate that the means are not statistically different from each other according to LSD test (P<0.01). The P values indicated in bold were found to be statistically significant according to the t - test (P < 0.01; t = 2,10092204).

The most preferred substrate for oviposition by BSF for laid eggs were calculated peafowl manure, pheasant manure and chicken manure, respectively. Goat and quail manures proved be the least attractive manures for BSF to lay eggs. The number of eggs counted in these manures was found even lower than the non-manure environment used as control.

Percentage similarity results showed that the most similar group were found peafowl and chicken manure with 58.83%. The similarity rate of the group consisting of peafowl and chicken to pheasant was calculated as 25.27%. According to the similarity analysis results, it was noticed that the most different manure was found as goat manure and its similarity rate to all other groups was found only 11.10% (Fig 3).

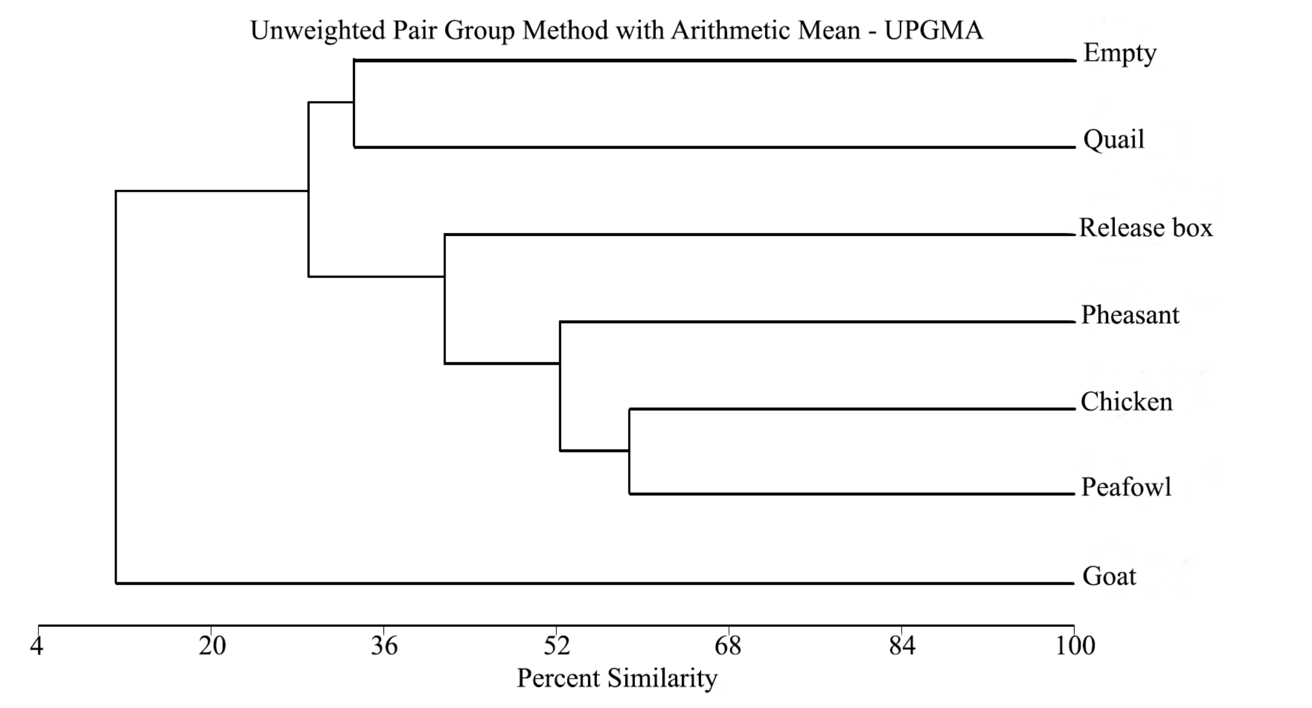

Fig 3 Percentage similarity rates of the manures used in the experiments where *Hermetia illucens* adult females laid eggs

Conclusion

Many previous studies have focused on which substrate is the most nutritious for larval development. The substrate must also be attractive to females for oviposition. The 'preference-performance principle' suggests that adult females prefer to oviposit in substrates that maximize offspring fitness [26]. To reveal the best substrates that are attractive for the females for oviposition is also extremely important for the better larval development.

According to our results; goat manure, which was found to be statistically different from the other substrates tested, was found to be the least preferred manure for BSF when compared with poultry manure. Therefore, it is recommended that goat manure should not be used as an egg-laying substrate for BSF. Adult females of BSF favoured to oviposit to peafowl, pheasant and chicken and no statistically significant difference was found between these poultry manure. Peafowl, pheasant and chicken or a mixture of these manures in certain proportions can be recommended for future studies. However, it is not easy to find peafowl and pheasant manures in Turkey. Chicken manure, which has no statistical difference between peafowl and pheasant, can be easily found.

In the calculated percentage similarity analysis showed that chicken and peafowl manures were found the most similar group with 58.82%. Therefore, chicken manure can be recommended as a suitable substrate for both the insect's egg laid preference and larval development.

Abbreviations

Standard Deviation (SD); critically endangered: CR; Extinct: EX; *H. illucens*: *Hermetia illucens*;
BSF: Black Soldier Fly; LSD: MVSP: The Multi Variate Statistical Package; UPGMA: Unweighted Pair Group Method with Arithmetic Mean; LSD: Least Significant Difference; °C: Degree (Celsius); atm: atmospheric pressure/A standard atmosphere.

Acknowledgments

We would like to thank Prof. Dr. Bülent YAŞAR for providing the opportunity for this study at Isparta University of Applied Science, Faculty of Agriculture, Department of Plant Protection, Isparta-Türkiye.

Funding

The author did not receive support from any organization for the submitted work.

Data Availability statement

The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards

Conflict of interest / Çıkar çatışması

The author declare no conflict of interest.

Ethical standards

The study is proper with ethical standards.

Authors' contributions

During the study, Ergin TURANTEPE conducted field and lab. research, Gökhan AYDIN wrote the article.

References

1. Aydın, G., Biyolojik Çeşitlilikte Bitki-Böcek Etkileşimi: Tarım Alanları, Doğal ve Yarı Doğal Habitatlar. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 2011. 15(3): p. 178-185. https://doi.org/10.19113/sdufbed.19680 (in Turkish with English abstract).
2. Aydın, G. and İ. Şen, Determination of arthropod biodiversity and some ecological parameters of Erdal Şekeroğlu (Isparta, Turkey) and Kadıini (Antalya, Turkey) cave ecosystems with evaluation of usability of insects in cave mapping. Turkish Journal of Entomology, 2020. 44 (4): p. 539-557. https://doi.org/10.16970/entoted.770018.
3. Aydın, G. and İ. Karaca, Balcalı-Adana'da Farklı Habitatlarda Çukur Tuzak Örnekleme Yöntemi Kullanılarak Hesaplanan Biyolojik Çeşitlilik Parametrelerinin Karşılaştırılması. 1.Uluslararası 5. Ulusal Meslek Yüksekokulları Sempozyumu. 27-29 Mayıs 2009, Selçuk Üniversitesi Kadınhanı Faik İçil Meslek Yüksekokulu, Konya, 2009. p. 163-177 (in Turkish with English abstract).
4. Aydın, G. and C. Kazak, Selecting Indicator Species Habitat Description and Sustainable Land Utilization: A Case Study in a Mediterranean Delta. International Journal of Agriculture & Biology, 2010. 12(6): p. 931-934.
5. Özbek, M and G. Aydın, "A new amphipod from the depths of the Morca Sinkhole (Anamur, Türkiye): *Gammarus morcae* n. sp. (Amphipoda: Gammaridae), with notes on cavernicolous amphipods of Türkiye, Turkish Journal of Zoology, 2023. 47(2): p. 81-93. https://doi.org/10.55730/1300-0179.3118 Available at: https://journals.tubitak.gov.tr/zoology/vol47/iss2/4
6. Lillig, M. and G. Aydın, Three species of Tenebronidae new to the Turkish fauna (Insecta: Coleoptera). Zoology in the Middle East, 2006. 37: p. 118-120.
7. Aydın, G., Vulnerability of *Megacephala* (*Grammognatha*) *euphratica euphratica* Latreille & Dejean, 1822 (Coleoptera: Cicindelidae) in natural and disturbed salt marsh and salt meadow habitats in Turkey. African Journal of Biotechnology, 2011. 10 (29): p. 5692-5696
8. Aydın, G. and İ. Karaca, The Effects of Pesticide Application on Biological Diversity of Ground Beetle (Coleoptera: Carabidae). Fresenius Environmental Bulletin, 2018. 27 (12A): p. 9112-9118 - WOS:000455562500050
9. Aydın, G., Distribution of the Dune Cricket *Schizodactylus inexpectatus* (Orthoptera: Schizodactylidae) in the Çukurova Delta, southern Turkey. Zoology in the Middle East, 2005. 36. p. 111-113. doi: 10.1080/09397140.2005.10638136.
10. Aydın, G and A, Khomutov, "The Biology, Nymphal Stages, and Life Habits of the Endemic Sand Dune Cricket *Schizodactylus inexpectatus* (Werner, 1901) (Orthoptera: Schizodactylidae)," Turkish Journal of Zoology, 2008. 32(4): p. 427-432. Available at: https://journals.tubitak.gov.tr/zoology/vol32/iss4/10
11. Šekeroğlu, E. and G. Aydın, Distribution and habitats of the tiger beetle *Megacephala euphratica* in the Çukurova Delta, southern Turkey (Coleoptera: Cicindelidae). Zoology in the Middle East, 2002. 27(1): p. 87-90. https://doi.org/10.1080/09397140.2002.10637943
12. Kar, S., H.E. Şamlı and L. Arın, Kara Asker Sineği *Hermetia illucens* (Linneaus, 1758): Biyoloji, Üretim ve Hayvan Beslemede Kullanımı. KSU, Tarım ve Doğa Derg., 2018. 21(2): p. 246-263. doi: 10/18016/ksudobil.323450.
13. Sevilmiş, U., S. Seydoşoğlu, T. Ayaşan, M.E. Bilgili and U. Sevilmiş, Siyah Asker Sineğinin (*Hermetia illucens* L.) Yem Kaynağı Olarak Değerlendirilmesi. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 2019. 9(4): p. 2379-2389. doi: 10.21597/jist.586778
14. Doğan, H. and F. Turan, The Usage of Black Soldier Fly (*Hermetia illucens*) Larvae Meal as Alternative Protein Source in Carp Diets (*Cyprinus carpio*). Acta Aquatica Turcica. 2021. 17(4): p. 508-514. doi: 10.22392/actaquatr.887967
15. Shah, S.R:A. and İ.S. Çetingül, Black Soldier Fly (*Hermetia illucens*) Larvae as an Ecological, Immune Booster and Economical Feedstuff for Aquaculture. Marine Science and Technology Bulletin. 2022. 11(1): p. 52-62. doi: 10.33714/masteb.1041493
16. Uslu, A.A., O.T. Özel, G.N. Örnekçi, B. Çelik, E.C. Çankırılıgil, İ. Coşkun and G. Uslu, Insect Larval Meal as A Possible Alternative to Fish Meal in Rainbow Trout (*Oncorhynchus mykiss*) Diets: Black Soldier Fly (*Hermetia illucens*), Mealworm (*Tenebrio molitor*). Journal of Limnology and Freshwater Fisheries Research, 2023. 9 (1): p. 43-52. doi: 10.17216/limnofish.1081945
17. Akdemir, F., Alabalık Yemlerine Farklı Oranlarda İlave Edilen Siyah Asker Sineği Larvası (*Hermetia illucens*)'nın Büyüme Performansı ve Bazı Kan Parametreleri Üzerine Etkileri. Dicle Üniversitesi Veteriner Fakültesi Dergisi, 2023. 16 (1): p. 27-32. doi: 10.47027/duvetfd.1271625.
18. Purkayastha, D. and S. Sarkar, Sustainable waste management using black soldier fly larva: a review. Int. J. Environ. Sci. Technol., 2022. 19: p. 12701–12726. https://doi.org/10.1007/s13762-021-03524-7
19. Ferronato, N., R. Paoli, F. Romagnoli, G. Tettamanti, D. Bruno and V. Torretto, Environmental impact scenarios of organic fraction municipal solid waste treatment with Black Soldier Fly larvae based on a life cycle assessment. Environ Sci Pollut Res., 2024. 31: p. 17651–17669. https://doi.org/10.1007/s11356-023-27140-9
20. Genc M. and B. Aydin, 7th new insect species with high potential to be used in the production of a natural red dye: *Dactylopius opuntiae* (Cockerell, 1896) (Hemiptera: Dactylopiidae). Textile Research Journal, 2024. 94(3-4): p. 477-483. doi:10.1177/00405175231215098
21. Shati, S. and M.A. Opiyo, Black soldier fly (*Hermetia illucens*) larvae meal improves growth performance, feed utilization, amino acids profile, and economic benefits of Nile tilapia (*Oreochromis niloticus*, L.). Aquatic Research, 2022. 5(3): p. 238-249. doi: 10.3153/AR22023

22. Nairuti, R.N., Utilization of Black Soldier Fly (*Hermetia illucens* Linnaeus) Larvae as a Protein Source for Fish Feed: A Review. Aquaculture Studies, 2022. 22(2): p. 697-697. doi: 10.4194/AQUAST697
23. Muslumin, B., D. Yonarta, E. Heriyati and H. Helmizuryani, Impact of black soldier fly (*Hermetia illucens*) fresh meal on the growth performance, digestive enzymes, hematology, and intestinal histology of cork fish (*Channa striata*). Turkish Journal of Veterinary and Animal Sciences, 2023. 47(4): p. 324-333. doi: 10.55730/1300-0128.4301.
24. Ellawidana, E.W.D.M., R.K. Mutucumaranad, H.A.D. Ruwandeepika and M.P.S. Magamage, Nutritional Composition and Apparent Metabolizable Energy Value of Black Soldier Fly Larvae (*Hermetia illucens* L.) Full-Fat Meal for Broiler Chickens. Türk Tarım - Gıda Bilim ve Teknoloji Dergisi, 2023. 11 (10): p. 1825-1833. doi: 10.24925/turjaf.v11i10.1825-1833.5992.
25. Kovach, W. L., A Multi variate Statistical Package. 1999, United Kingdom: Kovach Computing Services.
26. Boafo, H.A. D.S.J.C. Gbemavo, E.C. Timpong-Jones, V. Eziah, M. Billah, S.Y. Chia, O.F. Aidoo, V.A. Clottey and M. Kenis, Substrates most preferred for black soldier fly *Hermetia illucens* (L.) oviposition are not the most suitable for their larval development. Journal of Insects as Food and Feed, 2023. 9(2): p. 183-192, doi 10.3920/JIFF2022.0034.
27. Parodi A., K.V. Dijk, J.J.A. Van Loon, J.V. Schelt and H.H.E. Van Zanten, Black soldier fly larvae show a stronger preference for manure than for a mass-rearing diet. Journal of Applied Entomology, 2020. p. 144: 560–565, doi: 10.1111/jen.12768
28. Awasthi M.K., T. Liu, S. K. Awasthi, Y. Duan, A. Pandey and Z. Zhang, Manure pretreatments with black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae): A study to reduce pathogen content, Science of The Total Environment, 2020. 737: 139842, doi: https://doi.org/10.1016/j.scitotenv.2020.139842.
29. Indri, I., S. Sjam, A. Gassa and V.S. Dewi, Implication of types of feeds combined goat manure for preference black soldier fly (BSF): *Hermetia illucens* L. Earth and Environmental Science, 2021. 807: 022085. doi:10.1088/1755-1315/807/2/022085