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Developmental biology of black soldier fly (BSF) larvae (*Hermetia illucens*) influenced by various substrate types

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Abstract

The black soldier fly (*Hermetia illucens*) is increasingly recognized for converting organic waste into valuable resources, such as high-protein larvae and nutrient-rich frass. Using a two-factor experimental design, this study aimed to determine the developmental biology of BSF larvae influenced by substrate types and ratios in Camiguin Island, Philippines. Factor A was composed of substrate types (chicken, cow, goat, and pig manure), while Factor B was composed of substrate ratios (1:1, 1:2, and 2:1 manure with rice bran). Larval growth, assessed across six instars, revealed significant differences in length, width, weight, and developmental duration. At the sixth instar, pig manure (A4) produced the longest larvae (17.282 mm), with the highest length (18.620 mm) recorded in the 1:1 pig manure and rice bran ratio (A4B1). This combination also yielded the broadest larvae (4.765 mm). Chicken manure (A1) produced the heaviest larvae (0.138 g), while the 2:1 pig manure and rice bran ratio (A4B3) resulted in the heaviest larvae among all treatments (0.148 g). In terms of developmental duration, chicken manure (A1) facilitated the shortest larvae-to-pupa transition (18.42 days), while pig manure (A4) resulted in the fastest pupa-to-adult stage (13.75 days). The 1:1 ratio (B1) had the shortest pupa-to-adult transition (13.69 days) and adult emergence (7.92 days), while the 2:1 ratio (B3) exhibited the fastest overall emergence (8.25 days). These findings highlight the significant effects of substrate types and ratios on BSF larval development, demonstrating their potential for advancing sustainable agriculture, waste management, and nutrient recycling.

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Introduction

The agriculture sector has grown significantly in the last 20 years, in both land use and production, to meet the world's food demand. Climate changes pose major challenges to food security (Scala *et al.*, 2020). The black soldier fly, scientifically known as *Hermetia illucens,* is a beneficial insect belonging to the order Diptera, family Stratiomyidae, and sub-family Hermetiinae. They are black in color and medium in size similar to different species of wasps. The head region is wider and has well-developed compound eyes. The length of its antennae is longer than its head. This insect has membranous wings that are folded horizontally on the abdomen. The legs are black, and whitish tarsi are present on them. These black soldier flies are generally observed near the outdoors and livestock, in decaying organic waste matter, and animal or plant material (Meena and Meena, 2023). Human activities have a considerable impact on the environment. Organic waste management has been considered one of the most important and major environmental problems. It is now fast-growing yet difficult to solve due to the increased waste production (Scala *et al.*, 2020).

In connection with this, the extensive use of inorganic fertilizer to boost food production and meet the dietary requirements of a rapidly growing population and the management of organic waste is a challenge that is both growing and challenging to tackle because of the associated high-cost disposal of waste product (Scala *et al.*, 2020). The negative effects on ecosystems supporting agriculture become more severe. Sustainable waste management solutions are critical for achieving these agricultural outputs. In terms of waste valorization, the use of black soldier flies in trash recycling has created new opportunities for bioconversion strategies. Numerous studies have proven the black soldier fly's ability to convert kitchen waste, animal manure, agricultural waste, and other organic resources into a dependable and valuable organic fertilizer product (Manurung *et al.*, 2016). The use of black soldier fly larvae (BSFL) as an alternative for food waste and animal manure disposal offers significant environmental and economic benefits (Sivanantharaja and Gnaneswaran, 2018).

These benefits include reduced greenhouse gas emissions from landfills, decreased reliance on commercial fertilizers, and enhanced soil carbon storage. By efficiently converting organic waste into valuable resources, BSFL contributes to waste reduction, promotes sustainable agricultural practices, and supports a circular economy, all while mitigating the environmental impacts associated with traditional waste disposal and fertilizer production. The comparison of the biology of black soldier fly larvae reared in different animal manures as substrates has not yet been studied. Moreover, information on the frass nutritional content is lacking. This study aimed to determine the biology of black soldier fly larvae as influenced by various substrate types.

Materials and methods

Place and duration of the study

The study was conducted in a constructed screen house at Tangaro, Catarman, Camiguin Philippines from June to August 2024. Additionally, the farmer's field day was conducted in Tangaro, Catarman, Camiguin Philippines in October 2024.

Materials

The materials that were used in the study are the following: an analytical weight balance was used to weigh the larvae, pupae, and adult BSF, and a caliper was used to measure the length and width of the larvae and adult BSF, ocular micrometer under the stereomicroscope was also used to measure the length and diameter of the larvae, pupa and adult BSF, and plastic containers was used as rearing bin for the BSF larvae, plastic strainer was used to separate the frass and the larvae, record book for data gathering, camera for documentation.

Experimental design and treatments

This experiment was laid in a 4×3 factorial in a Completely Randomized Design, each replicated four times. Factor A was the different substrates and factor

B focused on the different ratios of the substrate waste per treatment. The treatments are as follows:

Factor A
A_1 - Chicken dung and rice bran
A_2 - Cow manure and rice bran
A_3 - Goat manure and rice bran
A_4 – Swine manure and rice bran

Factor B
B_1 – 1:1 ratio
B_2 – 1:2 ratio
B_3 – 2:1 ratio

Rearing facility and bin preparation

A total of forty-eight uniform plastic containers were utilized as rearing bins. The BSFL was reared in a rain house shelter, which provided optimal conditions. The facility was organized to facilitate a controlled and productive rearing process.

Substrate preparation

Fresh chicken dung, cow manure, carabao manure, and swine manure collected from local sources were mixed with rice bran. Each manure should be mixed with rice bran in specific ratios according to the treatment and moisture content. Thorough mixing was done to distribute nutrients evenly throughout the substrate. Water was added gradually while mixing until the desired moisture level was achieved.

Rearing of black soldier fly

A total of twenty grams of black soldier fly (BSF) eggs were purchased from Nueva Ecija, Philippines. Each treatment received 0.25 grams of BSF eggs, with four replicates per treatment. The BSF eggs were incubated in rearing bins for two to four days until they hatched. Once hatched, the larvae were fed with substrate and transferred to larger containers after five days. The substrates were provided with ad libitum, and the amount of substrate consumed by each treatment was observed weekly in kilograms. Once the substrate was fully consumed, it was collected and refilled with a new substrate. Regular monitoring of the rearing bins was conducted to ensure optimal conditions for BSF larvae growth. The larvae were expected to pupate after 18 to 22 days of feeding, depending on their diet.

Adult cage preparation

When approximately 80% of the BSF population reached the pupal stage, a fine mesh net was carefully placed over the top of each rearing bin to secure the emerging black soldier flies. This netting ensures that the adult flies are contained within the bin. Regular monitoring was done to ensure the overall mating and egg-laying performance of the adult population.

Results and discussion

In this study, black soldier fly (BSF) larvae were reared on four types of livestock manure: chicken, cow, goat, and pig. Each type of manure was provided to the larvae in three distinct ratios (1:1; 1:2 and 2:1), to identify the optimum substrate composition for BSF growth and development. Data collected included key biological parameters of BSF, such as larval, pupal, and adult length, diameter, and weight. To analyze the collected data, Analysis of Variance (ANOVA) was done to determine whether significant differences existed among the treatments in terms of BSF biology using the Statistical Tool for Agricultural Research (STAR) software version (IRRI, 2013).

Growth metrics across substrates

Length of black soldier fly larvae

Table 1 shows the mean length of black soldier fly (BSF) larvae at first instar across various substrate types. Among the treatments in factor A (substrate type), the longest larvae were observed in treatment A1 (Chicken manure) at 3.186 mm. This was followed by treatment A3 (Goat manure) with a mean of 3.142 mm. Then followed by treatment A4 (Pig manure) with a mean of 3.090 mm. The shortest length was observed in treatment A2 (Cow manure) with a mean of 3.039 mm. Statistical analysis revealed significant differences among these treatments. The aforementioned longest larval length treatment A1 (Chicken manure) was significantly different from A2 (Cow manure) and A4 (Pig manure). Similarly, treatment A3 (Goat manure) showed a significant difference from treatment A2 (Cow manure).

Table 1. Mean length of black soldier fly larval instar across various substrate types

Treatments/ Treatments combination		Mean length of black soldier fly larvae					
		1st Instar	2nd instar	3rd instar	4th instar	5th instar	6th instar
A	A1	3.186a	5.976ab	9.239	13.092	15.273	17.253a
	A2	3.039c	5.655bc	9.290	12.892	14.682	16.940a
	A3	3.142ab	6.210c	9.346	13.286	14.596	15.759b
	A4	3.090bc	6.210a	9.309	13.147	15.049	17.282a
B	B1	3.143	5.180	9.396	12.989	14.926	16.547
	B2	3.125	5.926	9.119	12.950	14.796	16.792
	B3	3.073	5.723	9.373	13.374	14.979	17.087
A × B	A1B1	3.219	6.270	9.165	12.575	14.754	16.575bcd
	A1B2	3.158	5.755	8.875	13.175	15.557	17.885ab
	A1B3	3.179	5.902	9.677	13.525	15.517	17.300abc
	A2B1	3.110	5.630	9.430	12.275	14.350	16.115bcd
	A2B2	3.042	5.667	9.267	12.725	14.845	17.800ab
	A2B3	2.965	5.667	9.172	13.677	14.852	16.905abc
	A3B1	3.154	5.430	9.657	13.175	14.730	14.877d
	A3B2	3.151	5.505	9.525	12.867	14.337	15.992bcd
	A3B3	3.119	5.377	8.855	13.815	14.720	16.407bcd
	A4B1	3.090	5.910	9.330	13.930	15.877	18.620a
	A4B2	3.151	6.755	8.810	13.032	14.442	15.490cd
	A4B3	3.029	5.945	9.787	12.480	14.827	17.737ab
cv		2.61%	9.68%	9.44%	6.95%	5.96%	7.04%

Additionally, treatment A4 (Pig manure) has a significant difference between treatment A1 (Chicken manure) and treatment A2 (Cow manure). The shortest length the treatment A2 significant difference between treatment A1 and treatment A3. In factor B (substrate ratios), the longest length was observed in treatment B1 (1:1 ratio) with a mean of 3.143 mm, followed by treatment B2 (1:2 ratio) with a mean of 3.125 mm, and the shortest length was observed in treatment B3 (2:1 ratio) with a mean of 3.073 mm. However, no significant differences were observed in substrate ratios. In treatment combination, the longest larvae were observed in A1B3 (2 chicken manure: 1 rice bran) with a mean of 3.179 mm, followed by treatment A1B2 (1 chicken manure: 2 rice bran) with a mean of 3.158 mm, and the shortest length was observed in treatment A2B3 (2 goat manure: 1 rice bran) with a mean of 2.965 mm but no significant differences were observed in treatments combinations and the results had a coefficient of variance of 2.61 percent.

At the second instar of the black soldier fly (BSF) larvae, significant differences were observed in Factor A (substrate type). The longest larvae were observed in treatment A3 (Goat manure) and A4 (Pig manure) with a mean of 6.210 mm, which significantly different from treatment A2 (Cow manure) and A1 (Chicken manure). This was followed by treatment A1 (Chicken manure) with a mean of 5.976 mm which significantly different from treatment A2 (Cow manure) and A3 (Goat manure). The shortest larvae were observed in treatment A2 (Cow manure) with a mean of 5.655 mm which significantly different from treatment A3 (Goat manure). For Factor B (substrate ratios), the longest larvae were observed in treatment B2 (1:2 ratio) with a mean of 5.926 mm, this was followed by treatment B3 (2:1 ratio) with a mean of 5.723 mm and the shortest larvae were observed in treatment B1 (1:1 ratio) with a mean of 5.180 mm. However, no significant differences were observed among the substrate ratio treatments. In terms of treatments combination, the longest larvae were observed in treatment A4B2 (1:2 pig manure and rice bran) with a mean of 6.755 mm, this was followed by treatment A1B1 (1:1 chicken manure and rice bran) with a mean of 6.270 mm while the shortest larvae were observed in treatment A3B3 (2:1 goat manure and rice bran) with a mean of 5.377 mm. No significant differences were observed among the treatment combinations and the results had a coefficient variance of 9.68 percent.

At the third instar of black soldier fly (BSF) larvae, it was observed that for Factor A (substrate types) the longest larvae were observed in treatment A3 (Goat manure) with a mean of 9.346 mm, followed by treatment A4 (Pig manure) with a mean of 9.309 mm and A2 (Cow manure) with a mean of 9.290 mm. The shortest larvae were observed in treatment A1 (Chicken manure) with a mean of 9.239 mm. However, no significant differences were observed among substrate types. For Factor B (substrate ratios), the longest larvae were observed in treatment B1 (1:1 ratio) with a mean of 9.396 mm, followed by treatment B3 (2:1 ratio) with a mean of 9.373 mm, and the shortest larvae were observed in treatment B2 (1:2 ratio) with a mean of 9.119 mm, but no significant differences were observed among the substrate ratios treatments. In terms of treatments combination, the longest larvae were observed in treatment A4B3 (2:1 pig manure and rice bran) with a mean of 9.787 mm, this was followed by treatment A1B3 (2:1 chicken manure and rice bran) with a mean of 9.677 mm and shortest larvae was observed in treatment A4B2 (1:2 pig manure and rice bran) with a mean of 8.810 mm. Similar to the previous findings, no significant differences were observed among treatment combinations and the results had a coefficient of variance of 9.44 percent.

At the fourth instar of black soldier fly (BSF) larvae, it was observed that for Factor A (substrate types), the longest larvae were observed in treatment A3 (Goat manure) with a mean of 13.287 mm, followed by treatment A4 (Pig manure) with a mean of 13.147 mm and treatment A1 with a mean of 13.092 mm. However, no significant differences were observed among substrate types. For Factor B (substrate ratios), the longest larvae were observed in treatment B3 (2:1 ratio) with a mean of 13.374 mm, followed by treatment B1 (1:1 ratio) with a mean of 12.989 mm, and the shortest larvae were observed in treatment B2 (1:2 ratio) with a mean of 12.950 mm, but no significant differences were observed among substrate ratios. In terms of treatment combination, the longest larvae were observed at treatment A1B1 (1:1 pig manure and rice bran) with a mean of 13.930 mm. This was followed by treatment A3B3 (2:1 goat manure and rice bran) with a mean of 13.815 mm. The shortest larvae were observed in treatment A2B1 (1:1 cow manure and rice bran) with a mean of 12.275 mm. Like the previous findings, no significant differences were observed among treatment combinations and the results had a coefficient variance of 6.95 percent.

At the fifth instar of black soldier fly (BSF) larvae, it was observed that for Factor A (substrate types), the longest larvae were observed in treatment A1 (Chicken manure) with a mean of 15.273 mm, followed by treatment A4 (Pig manure) with a mean of 15.049 mm and treatment A2 (Cow manure) with a mean of 14.682 mm. The shortest larvae were observed in treatment A3 (Goat manure) with a mean of 14.596 mm. However, no significant differences were observed among substrate types. For Factor B (substrate ratios), the longest larvae were observed in treatment B3 (2:1 ratio) with a mean of 14.979 mm. This was followed by treatment B1 (1:1 ratio) with a mean of 14.926 mm. The shortest larvae were observed in treatment B2 (1:2 ratio) with a mean of 14.796 mm but no significant differences were observed among substrate ratios. In terms of treatment combination, the longest larvae were observed in treatment A4B1 (1:1 pig manure and rice bran) with a mean of 15.877 mm. This was followed by treatment A1B2 (1:2 chicken manure and rice bran) with a mean of 15.557 mm. The shortest larvae were observed in treatment A3B2 (1:1 goat manure and rice bran) with a mean of 14.337 mm. Similar to the previous findings, no significant differences were observed among treatment combinations and the results had a coefficient variance of 5.96 percent.

At the sixth instar of the black soldier fly (BSF) larvae, it was observed that for Factor A (substrate types), the longest larvae were observed in treatment A4 (Pig manure) with a mean of 17.282 mm. This was followed by treatment A1 (Chicken manure) with a mean of 17.253 mm and treatment A2 (Cow manure) with a mean of 16.940 mm. The shortest larvae were observed in treatment A3 (Goat manure) with a mean of 15.759 mm. Statistical analysis revealed significant differences were observed in substrate types. The aforementioned longest larval length, treatment A4 (Pig manure) was significantly different in treatment A3 (Goat manure). While the shortest larvae, treatment A3 (Goat manure) was significantly different in treatments A1 (Chicken manure), A2 (Cow manure) and A4 (Pig manure). For Factor B (substrate ratios), the longest larvae were observed in treatment B3 (2:1 ratio) with a mean of 17.087 mm. This was followed by treatment B2 (1:2 ratio) with a mean of 16.792 mm. The shortest larvae were observed in treatment B1 (1:1 ratio) with a mean of 16.547 mm.

However, no significant differences were observed among substrate ratios. In terms of treatments combination, the longest larvae were observed in treatment A4B1 (1:1 pig manure and rice bran) with a mean of 18.620 mm. This was followed by treatment A1B2 (1:2 chicken manure and rice bran) with a mean of 17.885 mm. The shortest larvae were observed in treatment A3B1 with a mean of 14.877 mm. There is a significant difference was observed among treatments combinations. The longest larvae, treatment A4B1 (1:1 pig manure and rice bran) were significantly different among treatments A1B1 (1:1 chicken manure and rice bran), A2B1 (1:1 cow manure and rice bran), A3B1 (1:1 goat manure and rice bran), A3B2 (1:2 goat manure and rice bran), A3B3 (2:1 goat manure and rice bran), and A4B2 (1:2 pig manure and rice bran). While the shortest larvae, treatment A3B1 (1:1 goat manure and rice bran) were significantly different among treatments A1B2 (1:2 chicken manure and rice bran), A1B3 (2:1 chicken manure and rice bran), A2B2 (1:2 cow manure and rice bran), A2B3 (2:1 cow manure and rice bran), A4B1 (1:1 pig manure and rice bran) and A4B3 (2:1 pig manure and rice bran). The results had a coefficient variance of 7.04 percent.

The observed differences in larval growth could be attributed to the nutritional quality of the substrates used, as suggested by Katayane (2014), who observed that the nutritional quality of their food directly influences the growth of black soldier larvae. Similarly, Makkar *et al.* (2014) emphasized that the nutritional content within the body of black soldier fly (BSF) larvae at each instar stage was significantly affected by the quality and quantity of nutrients available in their food media. This was further supported by Cicilia and Susila (2018), who highlighted that the organic matter content in the food media directly impacts the body weight of BSFL. As summarized by Widigdyo and Normawati (2023), the quality of the substrate was important in determining the developmental success and overall biomass accumulation of BSF larvae.

Width of black soldier fly larvae

Table 2 shows the mean width of black soldier fly (BSF) larval instar across various substrate types. For Factor A (substrate types), the broadest larvae were observed in treatment A1 (Chicken manure) with a mean of 0.9170 mm. This was followed by treatment A3 (Goat manure) with a mean of 0.9141 mm and treatment A4 (Pig manure) with a mean of 0.9140 mm. The thinnest larvae were observed in treatment A2 with a mean of 0.9060 mm.

However, no significant differences were observed among substrate types. For Factor B (substrate ratios), the broadest larvae were observed in treatment B1 (1:1 ratio) with a mean of 0.9147 mm. This was followed by treatment B2 (1:2 ratio) with a mean of 0.9118 mm. The thinnest larvae were observed in treatment B3 (2:1 ratio) with a mean of 0.9117 mm, but no significant differences were observed among substrate ratios. In terms of treatments combination, the broadest larvae were observed in treatment A3B1 (1:1 goat manure and rice bran) with a mean of 0.9235 mm and this was followed by treatments A1B2 (1:2 chicken manure and rice bran) and A4B2 (1:2 pig manure and rice bran) with a mean of 0.9230 mm. The thinnest larvae were observed in treatment A2B2 (1:2 goat manure and rice bran) with a mean of 0.9000 mm but no significant differences were observed among treatments combinations and the results had a coefficient of variance of 1.82 percent.

Table 2. Mean width of black soldier Fly (BSF) larval instars across various substrate types

Treatments/ Treatments combination		Mean width of black soldier fly larvae					
		1st instar	2nd instar	3rd instar	4th instar	5th instar	6th instar
A	A1	0.9170	1.840^{ab}	2.741	3.246^{a}	3.942	4.310^{a}
	A2	0.9060	1.762^{bc}	2.707	2.797^{b}	3.818	4.207^{a}
	A3	0.9141	1.647^{c}	2.632	2.837^{b}	3.736	3.888^{b}
	A4	0.9140	1.944^{a}	2.711	3.363^{a}	3.948	4.295^{a}
B	B1	0.9147	1.761	2.718	3.095	3.896	4.131
	B2	0.9118	1.815	2.647	3.059	3.908	4.170
	B3	0.9117	1.819	2.729	3.028	3.779	4.225
A ×B	A1B1	0.9160	1.842	2.757	3.115^{abcd}	3.930	4.110^{bcd}
	A1B2	0.9230	1.805	2.607	3.445^{a}	4.012	4.435^{ab}
	A1B3	0.9120	1.875	2.857	3.177^{abc}	3.882	4.385^{abc}
	A2B1	0.9045	1.695	2.752	2.865^{cde}	3.777	4.015^{bcdc}
	A2B2	0.9000	1.790	2.727	2.887^{bcde}	3.980	4.467^{ab}
	A2B3	0.9135	1.802	2.642	2.640^{de}	3.697	4.140^{bcd}
	A3B1	0.9235	1.637	2.572	3.025^{abcd}	3.812	3.632^{e}
	A3B2	0.9015	1.650	2.790	2.457^{e}	3.812	3.942^{cde}
	A3B3	0.9175	1.655	2.535	3.027^{abcd}	3.582	4.090^{bcde}
	A4B1	0.9150	1.872	2.790	3.375^{ab}	4.062	4.765^{a}
	A4B2	0.9230	2.015	2.462	3.447^{a}	3.827	3.835^{de}
	A4B3	0.9040	1.945	2.880	3.267^{abc}	3.955	4.285^{bcd}
cv		1.82%	8.72%	11.01%	9.73%	8.69%	7.04%

At the second instar of black soldier fly (BSF) larvae, it was observed that in Factor A (Substrate types) the broadest larvae were observed in the treatment A4 (Pig manure) with a mean of 1.944 mm. This was followed by treatment A1 (Chicken manure) with a mean of 1.840 mm and treatment A2 (Cow manure) with a mean of 1.762 mm. The thinnest larvae were observed in treatment A3 (Goat manure) with a mean of 1.647 mm. Statistical analysis reveals significant differences among substrate types. The aforementioned broadest larvae, treatment A4 (Pig manure) was significantly different from treatments A2 (Cow manure) and A3 (Goat manure). While the thinnest larvae, treatment A3 (Goat manure) was significantly different from treatment A1 (Chicken manure), and A4 (Pig manure). For Factor B (Substrate ratios), the broadest larvae were observed in treatment B3 (2:1 ratio) with a mean of 1.819 mm and this was followed by treatment B2 (1:2 ratio) with a mean of 1.815 mm.

The thinnest larvae were observed in the treatment B1 (1:1 ratio) with a mean of 1.761 mm but no significant differences were observed among substrate ratios. In terms of treatments combinations, the broadest larvae were observed in treatment A4B2 (1:2 pig manure and rice bran) with a mean of 2.015 mm. This was followed by treatment A4B3 (2:1 pig manure and rice bran) with a mean of 1.945 mm. The thinnest larvae were observed in treatment A3B1 (1:1 goat manure and rice bran) with a mean of 1.637 mm. Similar to the previous findings, no significant differences were observed among treatment combinations and the results had a coefficient of variance of 8.72 percent.

At the third instar, it was observed that in Factor A (Substrate types) the broadest larvae were observed in treatment A1 (Chicken manure) with a mean of 2.741 mm. This was followed by treatment A4 (Pig manure) with a mean of 2.711 mm. The thinnest larvae were observed in treatment A3 (Goat manure) with a mean of 2.632 mm. However, no significant differences were observed among substrate types. For Factor B, the broadest larvae were observed in treatment B3 (2:1 ratio) with a mean of 2.729 mm and this was followed by treatment B1 (1:1 ratio) with a mean of 2.718 mm). The thinnest larvae were observed in treatment B2 (1:2 ratio) with a mean of 2.647 mm but no significant differences were observed among substrate ratios. In terms of treatment combinations, the broadest larvae were observed in treatment A4B3 (2:1 pig manure and rice bran) with a mean of 2.880 mm. This was followed by treatment A1B3 (2:1 chicken manure and rice bran) with a mean of 2.857 mm. The thinnest larvae were observed in treatment A4B2 (1:2 pig manure and rice bran). Similar to the previous findings, no significant differences were observed among treatment combinations and the result had a coefficient of variance of 11.01 percent.

At the fourth instar, it was observed that in Factor A (Substrate types) the broadest larvae were observed in treatment A4 (Pig manure) with a mean of 3.363 mm. This was followed by treatment A1 (Chicken manure) with a mean of 3.246 mm and treatment A3 (Goat manure) with a mean of 2.837 mm. The thinnest larvae were observed in treatment A2 (Cow manure) with a mean of 2.797 mm. Statistical analysis revealed significant differences among substrate types. The aforementioned broadest larvae, treatment A4 (Pig manure) was significantly different from treatments A2 (Cow manure) and A3 (Goat manure). The thinnest larvae, treatment A2 (Cow manure) was significantly different from treatments A1 (Chicken manure) and A4 (Pig manure). For Factor B (Substrate ratios), the broadest larvae were observed in treatment B1 (1:1 ratio) with a mean of 3.095 mm and this was followed by treatment B2 (1:2 ratio) with a mean of 3.059 mm. The thinnest larvae were observed in treatment B3 (2:1 ratio) with a mean of 3.028 mm. In terms of treatment combinations, the broadest larvae were observed in treatment A4B2 (1:2 pig manure and rice bran) with a mean of 3.447 mm and this was followed by treatment A1B2 (1:2 chicken manure and rice bran) with a mean of 3.445 mm. The thinnest larvae were observed in treatment A3B2 (1:2 goat manure and rice bran) with a mean of 2.457 mm. Statistical analysis also revealed significant differences among treatment combinations. Wherein the broadest larvae, treatment A4B2 (1:2 Pig manure and rice bran) was significantly different from treatments A2B1 (1:1 cow manure and rice bran), A2B2 (1:2 cow manure and rice bran), A2B2 (2:1 cow manure and rice bran), A3B2 (1:2 goat manure and rice bran). While the thinnest larvae treatment A3B2 (1:2 goat manure and rice bran) was significantly different from treatments A1B1 (1:1 chicken manure and rice bran), A1B2 (1:2 chicken manure and rice bran), A1B3 (2:1 chicken manure and rice bran), A3B1 (1:1 goat manure and rice bran), A3B3 (2:1 goat manure and rice bran), A4B1 (1:1 pig manure and rice bran), A4B2 (1:2 pig manure and rice bran) and A4B3 (2:1 pig manure and rice bran). The results had a coefficient of variance of 9.73 percent.

At the fifth instar, it was observed that in Factor A (Substrate types) the broadest larvae were observed in treatment A4 (Pig manure) with a mean of 3.948 mm. This was followed by treatment A1 (Chicken manure) with a mean of 3.942 mm and treatment A2 (Cow manure) with a mean of 3.818 mm. The thinnest larvae were observed in treatment A3 (Goat manure) with a mean of 3.736 mm. However, no significant differences were observed among substrate types. For Factor B (Substrate ratios), the broadest larvae were observed in treatment B2 (1:2 ratio) with a mean of 3.908 mm and this was followed by treatment B1 (1:1 ratio) with a mean of 3.896 mm. The thinnest larvae were observed in treatment B3 (2:1 ratio) with a mean of 3.779 mm but no significant differences were observed among substrate ratios. In terms of treatment combinations, the broadest larvae were observed in treatment A4B1 (1:1 pig manure and rice bran) with a mean of 4.062 mm and this was followed by treatment A1B2 (1:2 chicken manure and rice bran) with a mean of 4.012 mm. The thinnest larvae were observed in treatment A3B3 (2:1 goat manure and rice bran) with a mean of 3.582 mm. Similar to the previous findings, no significant differences were observed among treatment combinations and the results had a coefficient of variance of 8.69 percent.

At the sixth instar, it was observed that in Factor A (Substrate types) the broadest larvae were observed in treatment A1 (Chicken manure) with a mean of 4.310 mm. This was followed by treatment A4 (Pig manure) with a mean of 4.295 mm and treatment A2 (Cow manure) with a mean of 4.207 mm. The thinnest larvae were observed in treatment A3 (Goat manure) with a mean of 3.888 mm. Statistical analysis revealed significant differences among substrate types. The aforementioned broadest larvae, treatment A1 (Chicken manure) was significantly different from treatment A3 (Goat manure). While the thinnest larvae, treatment A3 (Goat manure) was significantly different from treatments A1 (Chicken manure), A2 (Cow manure), and A4 (Pig manure). For Factor B (Substrate ratios), the broadest larvae were observed in treatment B3 (2:1 ratio) with a mean of 4.225 mm and this was followed by treatment B2 with a mean of 4.170 (1:2 ratio) mm. The thinnest larvae were observed in treatment B1 (1:1 ratio) with a mean of 4.131 mm. However, no significant differences were observed among substrate types. In terms of treatment combinations, the broadest larvae were observed in treatment A4B1 with a mean of 4.765 mm and this was followed by treatment A1B2 with a mean of 4.435 mm. The thinnest larvae were observed in treatment A3B1 with a mean of 3.632 mm. Statistical analysis revealed significant differences among treatment combinations. Wherein, the broadest larvae, treatment A4B1 (1:1 pig manure and rice bran) significantly differed from treatments A1B1 (1:1 chicken manure and rice bran), A2B1 (1:1 cow manure and rice bran), A2B3 (2:1 cow manure and rice bran), A3B1 (1:1 goat manure and rice bran), A3B2 (1:2 goat manure and rice bran), A3B3 (2:1 goat manure and rice bran), A4B2 (1:2 pig manure and rice bran), and A4B3 (2:1 pig manure and rice bran). While the shortest larvae, treatment A3B1 (1:1 goat manure and rice bran) significantly differed from treatments A1B1 (1:1 chicken manure and rice bran), A1B2 (1:2 chicken manure and rice bran), A1B3 (2:1 chicken manure and rice bran), A2B1 (1:1 cow manure and rice bran), A2B2 (1:2 cow manure and rice bran), A2B3 (2:1 cow manure and rice bran), A4B1 (1:1 pig manure and rice bran) and A4B3 (2:1 pig manure and rice bran. The results had a coefficient of variance of 7.04%.

Li *et al.* (2014) as cited by Miranda *et al.* (2020), highlighted that the chemical and physical composition of animal manure can vary significantly. Chen *et al.* (2003) noted that poultry manure typically contains higher nutrients and lower fiber content compared to dairy manure.

These compositional differences may explain the significant variation in larval development observed between treatments in this study during the prepupal stage, as nutrient-rich substrates likely promote faster and larger growth in BSFL.

Weight of black soldier fly larvae

Table 3 shows the mean weight of black soldier Fly (BSF) larval instars across various substrate types. The third instar of black soldier fly (BSF) larvae shows that for Factor A (Substrate types), the heaviest larvae were observed in treatment A4 (Pig manure) with a mean of 0.011 g. This was followed by treatment A1 (Chicken manure) with a mean of 0.009 g and treatment A2 (Cow manure) with a mean of 0.008 g. The lightest larvae were observed in treatment A3 (Goat manure) with a mean of 0.006 g. Statistical analysis revealed significant differences among substrate types. Wherein, the heaviest larvae in treatment A4 (Pig manure) were significantly different from treatments A1 (Chicken manure), A2 (Cow manure), and A3 (Goat manure). The lightest larvae in treatment A3 (Goat manure) were significantly different from treatments A1 (Chicken manure), A2 (Cow manure) and A4 (Pig manure). For Factor B (Substrate ratios), the heaviest larvae were observed in treatments B1 (1:1 ratio) and B2 (1:2 ratio) with a mean of 0.009 g. The lightest larvae were observed in treatment B3 (2:1 ratio) with a mean of 0.008 g. However, no significant differences were observed among substrate ratios. In terms of treatment combinations, the heaviest larvae were observed in treatment A4B2 (1:2 pig manure and rice bran) with a mean of 0.013 g and this was followed by treatment A4B1 (1:1 pig manure and rice bran) with a mean of 0.011 g. The lightest larvae were observed in the treatments A3B2 (1:2 goat manure and rice bran) and A3B3 (2:1 goat manure and rice bran) with a mean of 0.006 g but no significant difference was observed across treatment combinations. The results had a coefficient of variance of 32.27%.

Table 3. Mean weight of black soldier fly (BSF) larval instars across various substrate types

Treatments combination		Mean weight of black soldier fly larvae			
		3rd instar	4th instar	5th instar	6th instar
A	A1	0.009[b]	0.033	0.108[a]	0.138[a]
	A2	0.008[c]	0.032	0.091[c]	0.113[ab]
	A3	0.006[d]	0.031	0.084[d]	0.103[b]
	A4	0.011[a]	0.033	0.101[b]	0.135[a]
B	B1	0.009	0.033	0.097	0.117
	B2	0.009	0.030	0.091	0.120
	B3	0.008	0.034	0.100	0.129
A × B	A1B1	0.009	0.035	0.096	0.130
	A1B2	0.009	0.027	0.116	0.141
	A1B3	0.008	0.038	0.113	0.142
	A2B1	0.008	0.032	0.085	0.101
	A2B2	0.007	0.032	0.095	0.120
	A2B3	0.008	0.031	0.092	0.117
	A3B1	0.007	0.030	0.088	0.092
	A3B2	0.006	0.033	0.072	0.106
	A3B3	0.006	0.030	0.091	0.111
	A4B1	0.011	0.033	0.117	0.145
	A4B2	0.013	0.028	0.083	0.112
	A4B3	0.009	0.037	0.104	0.148
cv		32.27%	27.85%	20.52%	19.43%

At the fourth instar, it was observed that for Factor A (Substrate types) the heaviest larvae were observed in the treatment A1 (Chicken manure) and A4 (Pig manure) with a mean of 0.033 g. This was followed by the treatment A2 (Cow manure) with a mean of 0.032 g. The lightest larvae were observed in the treatment A3 (Goat manure) with a mean of 0.031 g. However, no significant difference was observed across the substrate types. For Factor B (Substrate ratios), the heaviest larvae were observed in treatment B3 (2:1 ratio) with a mean of 0.034 g and this was followed by treatment B1 (1:1 ratio) with a mean of 0.033 g. The lightest larvae were observed in treatment B2 (1:2 ratio) with a mean of 0.030 g but no significant differences were observed across substrate ratios. In terms of treatment combinations, the heaviest larvae were observed in treatment A1B3 (2:1 chicken manure and rice bran) with a mean of 0.038 g and this was followed by treatment A4B3 (2:1 chicken manure and rice bran) with a mean of 0.037 g. The lightest larvae were observed in treatment A1B2 (1:2 chicken manure and rice bran) with a mean of 0.027 g. Similar to the previous findings, no significant differences were observed across treatment combinations and the result had a coefficient of variance of 27.85 percent.

At the fifth instar, it was observed that for Factor A (Substrate types) the heaviest larvae were observed in treatment A1 (Chicken manure) with a mean of 0.108 g. This was followed by treatment A4 (Pig manure) with a mean of 0.101 g and treatment A2 (Cow manure) with a mean of 0.091 g. The lightest larvae were observed in the treatment A3 (Goat manure) with a mean of 0.084 g. Statistical analysis revealed significant differences across substrate types.

Wherein the heaviest larvae treatment A1 (Chicken manure) was significantly different from treatments A2 (Cow manure), A3 (Goat manure), and A4 (Pig manure). While the lightest larvae in treatment A3 (Goat manure) were significantly different from treatment A1 (Chicken manure), A2 (Cow manure), and A4 (Pig manure). For Factor B, the heaviest larvae were observed in treatment B3 (2:1 ratio) with a mean of 0.100 g and this was followed by treatment B1 (1:1 ratio) with a mean of 0.097. The lightest larvae were observed in treatment B2 (1:2 ratio) with a mean of 0.091 g but no significant differences were observed across substrate ratios. In terms of treatment combinations, the heaviest larvae were observed in treatment A4B1 (1:1 pig manure and rice bran) with a mean of 0.117 g and this was followed by treatment A1B2 (1:2 chicken manure and rice bran) with a mean of 0.116 g. The lightest larvae were observed in the treatment A3B2 (1:2 goat manure and rice bran) with a mean of 0.072 g. Similar to the previous findings, no significant differences were observed across treatments and the results had a coefficient of variance of 20.52 percent.

At the sixth larval instar, it was observed that for Factor A (Substrate types) the heaviest larvae were observed in treatment A1 (Chicken manure) with a mean of 0.138 g. This was followed by treatment A4 (Pig manure) with a mean of 0.135 g and treatment A2 (Cow manure) with a mean of 0.113 g. The lightest larvae were observed in treatment A3 (Goat manure) with a mean of 0.103 g. Statistical analysis revealed significant differences across substrate types. Wherein the heaviest larvae in treatment A1 (Chicken manure) were significantly different from treatment A3 (Goat manure). While the lightest larvae in treatment A3 (Goat manure) were significantly different from treatment A1 (Chicken manure) and A4 (Pig manure). The heaviest larvae were observed in treatment B3 (2:1 ratio) with a mean of 0.129 g and this was followed by treatment B2 (1:2 ratio) with a mean of 0.120 g. The lightest larvae were observed in treatment B1 (1:1 ratio) with a mean of 0.117g.

However, no significant differences were observed across substrate ratios. In terms of treatment combinations, the heaviest larvae were observed in treatment A4B3 (2:1 pig manure and rice bran) with a mean of 0.148 g and this was followed by treatment A4B1 (1:1 pig manure and rice bran) with a mean of 0.145g. The lightest larvae were observed in treatment A3B1 (1:1 goat manure and rice bran) with a mean of 0.092 g but no significant differences were observed across treatment combinations. The result has a coefficient of variance of 19.43 percent.

As noted by Miranda *et al.* (2020), poultry manure typically has a higher nutrient content and lower fiber levels compared to dairy manure, which could explain the differences in development and survivorship observed in this study. Manure composition affects larval growth, and variations in nutrient availability, fiber content, and texture between manure types likely contribute to the differences in BSF larval weights. Optimizing manure selection and composition is therefore essential for enhancing growth rates and development in BSF production systems.

Developmental period of black soldier fly larvae

Table 4 shows the mean development period of the black soldier fly across various substrate types. In the larvae to pupa period, for Factor A (Substrate types) the shortest period was observed in treatment A1 (Chicken manure) with a mean of 18.42 days. This was followed by treatment A4 (Pig manure) with a mean of 18.67 days and treatment A3 (Goat manure) with a mean of 18.83 days. The longest period was observed in treatment A2 (Cow manure) with a mean of 18.92 days. However, no significant differences were observed across substrate types. For Factor B (Substrate ratios), the shortest period was observed in treatment B1 (1:1 ratio) with a mean of 18.62 days and it was followed by treatment B2 (1:2 ratio) with a mean of 18.69 days. The longest period was observed in treatment B3 (2:1 ratio) with a mean of 18.84 days but no significant differences were observed across substrate ratios. In terms of treatment combinations, the shortest period was observed in treatments A1B2 (1:2 chicken manure and rice bran), A2B2 (1:2 cow manure and rice bran), and A3B2 (1:2 goat manure and rice bran) with a mean of 18.25 days and this was followed by treatment A1B1 (1:1 chicken manure and rice bran) with a mean of 18.50 days. The longest duration was observed in treatments A1B3 (2:1 chicken manure and rice bran), A4B2 (1:2 pig manure and rice bran), and A4B3 (2:1 pig manure and rice bran). Similar to the previous findings, no significant differences were observed across treatment combinations and the results had a coefficient of variance of 3.78 percent.

In the pupa to adult period, for Factor A (Substrate types), for Factor A (Substrate types) the shortest period was observed in treatment A4 (Pig manure). This was followed by treatments A1 (Chicken manure) and A2 (Cow manure) with a mean of 13.92 days. The longest duration was observed in treatment A3 (Goat manure) with a mean of 14.80 days. However, no significant differences were observed across substrate types. For Factor B, the shortest period was observed in treatment B1 (1:1 ratio) with a mean of 13.69 days and this was followed by treatment B2 (1:2 ratio) with a mean of 13.75 days. The longest period was observed in treatment B3 (2:1 ratio) with a mean of 14.06 days. In terms of treatment combinations, the shortest period was observed in treatment A1B2 (1:2 chicken manure and rice bran) with a mean of 13.25 days and this was followed by treatment A2B3 (2:1 cow manure and rice bran) and A3B3 (2:1 goat manure and rice bran) with a mean of 13.50 days. The longest period was observed in treatment A4B1 (1:1 pig manure and rice bran) with a mean of 14.50 days.

Similar to the previous findings, no significant differences were observed across treatment combinations and the result had a coefficient of variance of 5.39 percent.

Table 4. Mean developmental period of the black soldier fly across various substrate types

Treatments combination		Mean developmental period of black soldier fly		
		Larvae to pupa	Pupa to adult	Adult emergence
A	A1	18.42	13.92	7.92
	A2	18.92	13.92	8.33
	A3	18.83	14.80	8.83
	A4	18.67	13.42	8.33
B	B1	18.62	13.69	8.44
	B2	18.69	13.75	8.38
	B3	18.81	14.06	8.25
A × B	A1B1	18.50	13.75	8.25
	A1B2	18.25	13.25	8.50
	A1B3	19.00	14.00	8.25
	A2B1	18.75	13.75	8.75
	A2B2	18.25	13.75	8.25
	A2B3	19.50	13.50	8.50
	A3B1	18.75	14.00	8.50
	A3B2	18.25	13.75	8.25
	A3B3	18.50	13.50	8.00
	A4B1	18.75	14.50	8.50
	A4B2	19.00	14.00	8.00
	A4B3	19.00	14.25	8.50
cv		3.78%	5.39%	9.41%

In the period to adult emergence, for Factor A (Substrate types) the shortest period was observed in treatment A1 (Chicken manure) with a mean of 7.92 days. This was followed by treatment A2 (Cow manure) and A4 (Pig manure) with a mean of 8.33 days. The longest period was observed in treatment A3 (Goat manure) with a mean of 8.83 days. However, no significant differences were observed across the substrate types. For Factor B (Substrate ratios), the shortest period was observed in treatment B3 (2:1 ratio) with a mean of 8.25 days and this was followed by treatment B2 (1:2 ratio) with a mean of 8.38 days. The longest period was observed in treatment B1 (1:1 ratio) with a mean of 8.44 days but no significant differences were observed across substrate ratios. In terms of treatment combinations, the shortest period was observed in treatments A3B3 (2:1 goat manure and rice bran) and A4B2 (1:2 pig manure and rice bran) with a mean of 8 days and this was followed by treatments A1B1 (1:1 pig manure and rice bran), A1B3 (2:1 pig manure and rice bran), A2B2 (1:2 cow manure and rice bran), and A3B2 (1:2 goat manure and rice bran) with a mean of 8.25 days.

Similar to the previous findings, no significant differences were observed across treatment combinations. An observation was reported in the study of Sivanantharaja and Gnaneswaran (2018), where the larval period ranged from a minimum of 18 days to a maximum of 24 days.

Additionally, their study noted that the pupal stage lasted between 10 and 17 days. These variations may be attributed to the differences in the substrates used for feeding the black soldier fly larvae (BSFL). In contrast, Sivanantharaja and Gnaneswaran (2018) also documented a longer adult lifespan, ranging from 10 to 12 days. The adult lifespan of black soldier flies is influenced by the energy reserves accumulated during the larval stage, as adult flies lack functional mouthparts and are unable to feed.

Conclusion

This study highlights the potential of black soldier fly (BSF) farming as a sustainable solution for waste management in agriculture, with a focus on the development of BSF larvae. The findings revealed that pig and chicken manure, particularly when combined with rice bran, produced the best results for larval growth across various developmental stages. At the sixth instar, the larvae reared on a 1:1 ratio of pig manure and rice bran exhibited the longest, broadest, and heaviest larvae. The study also showed that the duration of the larvae-to-pupa and pupa-to-adult stages varied depending on the substrate type and ratio, with chicken manure and specific substrate ratios resulting in shorter development periods. These results emphasize the importance of selecting optimum substrates and their ratios to enhance larval growth, which is crucial for improving BSF farming efficiency. By focusing on the development of BSF larvae, this study provides valuable insights for farmers looking to optimize their BSF farming practices and contribute to sustainable waste management.

Recommendation(s)

Based on the findings of this study, several recommendations can be made to optimize black soldier fly (BSF) farming practices. First, it is recommended that farmers prioritize substrates such as pig and chicken manure, particularly when combined with rice bran, as these substrates demonstrate superior larval growth. The 1:1 ratio of pig manure and rice bran was particularly effective in enhancing larval development, and further experimentation with other substrate ratios could yield additional insights into optimizing growth. Additionally, comprehensive training and educational programs should be implemented to raise awareness among farmers about the benefits of BSF farming and provide detailed guidance on substrate selection, preparation, and rearing techniques. Establishing standardized protocols for BSF farming will help ensure consistency in larval development and improve overall production efficiency. Moreover, continued research into alternative substrates and their ratios is crucial for further optimization of BSF farming systems and to assess their long-term sustainability.

Finally, fostering collaboration between agricultural institutions, government agencies, and farming communities will promote the exchange of knowledge and resources, facilitating the widespread adoption and scaling up of BSF farming as a viable solution for organic waste management and sustainable agricultural practices.

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