Germination Characteristic of Frass Derived from Black Soldier Fly – Based Bioconversion of Chicken Manure and Rice Hull Biochar

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Abstract. This study aimed to determine the effects of different types and doses of BSFL Frass on tomato seed germination. A factorial randomized design with two factors and two replications was used to evaluate germination percentage, vigor index, shoot length, root length. The result showed that frass 6 produced the highest germination percentage and vigor index while a 30% frass dose yielded the best germination performance. The 10% frass dose resulted in the longest shoot and root growth. These findings suggest that BSFL frass, particularly frass 6 at moderate application rate, can enhance tomato germination and has strong potential as a sustainable biofertilizer for improving crop establishment.

1. Introduction

Indonesia is the 5th largest rice-producing country in the world, with an estimated production of 34.1 metric tons in 2024/2025, contributing about 6% of global output [13]. Rice hull, a by-product of rice milling, accounts for about 20–34% of the grain weight [4]. It contains high levels of organic compounds, lignin, cellulose, and amorphous silica [12, 3, 14]. Through pyrolysis, rice hull can be converted into rice hull biochar (RHB) [6,11]. RHB is characterized by high yield, abundance of amorphous silica and alumina [9,2]. RHB has been widely utilized as a soil amendment [5,8] to improve soil fertility, enhance plant growth, and serve as a germination medium [10,15,1].

Chicken manure is another abundant agricultural byproduct generated from the poultry industry [7]. It is rich in essential nutrients such as nitrogen, phosphorus, and potassium, making it a valuable organic fertilizer that can reduce environmental pollution and dependence on chemical fertilizers.

Black soldier fly larvae (BSFL; *Hermetia illucens L.*) have gained considerable attention for their ability to bioconvert organic waste into valuable biomass and frass. Growing BSFL on organic waste can reduce waste amounts by 25-69 % of dry matter, with bioconversion efficiencies varying between 1.4 to 23 % of dry matter. BSFL frass can be used to a soil amendment, and enhancing crop production.

Phytotoxicity assessment is often used to evaluate the safety and suitability of organic materials for plant growth through seed germination assays. Tomato (*Solanum lycopersicum* L.) is commonly used in germination studies due to its sensitivity to nutrient balance and potential inhibitors. Despite the increasing interest in BSFL frass as an organic amendment, limited studies have compared different frass types and application doses on seed germination performance. Therefore, this study aimed to determine the effects of different types and doses of BSFL frass on tomato seed germination. The findings are expected to contribute to the optimization of frass utilization as a sustainable biofertilizer within the circular bioeconomy framework.

2. Materials and methods

This research was conducted at the Hydroponic Green House, Faculty of Agriculture, University of Alwashliyah Medan, from July to August 2024. The materials used included tomato seed (CMK TAVI, Bintang Asia), Black Soldier Fly Larvae (BSFL) frass, produced from different feeding rates of topsoil. The main tools used were seed trays (540 x 280 mm, 50 planting holes, measuring cups, a hand sprayer, scissors, digital scales, an oven, a ruler, labelling material, and a record book.

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2.1 Experimental design

The experiment employed a factorial randomized design with two factors and two replications. The first factor was the type of BSFL frass, types made up from a combination of the BSFL feeding rates and substrate compositions, combining chicken manure (CM) and rice husk biochar (RHB) as follows:
B1 = Frass 1 (200 mg/larvae/day, 90% CM + 10% RHB),
B2 = Frass 2 (100 mg/larvae/day, 90% CM + 10% RHB),
B3 = Frass 3 (200 mg/larvae/day, 70% CM + 30% RHB),
B4 = Frass 4 (100 mg/larvae/day, 70% CM + 30% RHB),
B5 = Frass 5 (200 mg/larvae/day, 50% CM + 50% RHB),
B6 = Frass 6 (100 mg/larvae/day, 50% CM + 50% RHB).

The second factor was the dosage of BSFL frass, consisting of 10% (L1), 30% (L2), and 50% (L3) of the germination medium. The frass types were selected based on differences in nutrient composition arising from variations in BSFL feeding rates and substrate mixtures, which are known to influence frass quality and its suitability as a soil amendment. Data was analyzed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at a 5% significance level.

2.2 Germination Test

Germination media were prepared by mixing topsoil with the corresponding type and dosage of BSFL frass. The mixture was thoroughly homogenized and placed in the seed trays with one tomato seed sown per hole. Tomato was chosen as a test crop because of its sensitivity to nutrient balance and growth-inhibiting compounds, making it a suitable indicator species for germination bioassays.

Germination performance was evaluated based on germination percentage, vigor index, shoot length, and root length.

Germination percentage (%) = $\frac{\text{the number of normal sprout produced}}{\text{the number of seed samples tested}} x\ 100\ \%$

Vigor Index at day 10 = Were : $\frac{G1}{D1}+\frac{G2}{D2}+\frac{G3}{D3}+\frac{G4}{D4}+\ldots.+\frac{Gn}{Dn}$

G = Number of seeds that germinate on a given day
D = The time corresponding to that amount
n = Number of days in the final calculation

3. Results and discussion

The application of BSFL frass significantly influenced germination percentage (GP), vigor index (VI), shoot length (SL), and root length (RL) of tomato seedlings (Table 1). Among the frass types, frass 6 (100 mg, 50% CM:50% RHB) consistently produced the highest germination percentage and vigor index, indicating an optimal nutrient balance and minimal phytotoxicity. The enhanced germination performance could be attributed to the balanced C/N ratio and the buffering capacity of rice hull biochar (RHB), which likely mitigated ammonia and phenolic toxicity from the manure component.

Table 1. Descriptive statistics of traits under study in the BSFL frass types.

Stats	Treat	GP day 12	VI day 10	SL day 14	RL day 14
Min.	B1 (200 mg, 90CM :10 RHB)	60.00	40.16	2.00	0.76
	B2 (100 mg, 90CM :10 RHB)	56.00	11.72	2.36	0.12
	B3 (200 mg, 70CM :30 RHB)	68.00	44.86	5.80	2.06
	B4 (100 mg, 70CM :30 RHB)	68.00	42.92	5.10	1.00
	B5 (200 mg, 50CM :50 RHB)	64.00	33.80	2.68	1.24
	B6 (100 mg, 50CM :50 RHB)	72.00	51.27	5.35	1.44
Mean	B1 (200 mg, 90CM :10 RHB)	73.33	48.82	5.42	2.01

	B2 (100 mg, 90CM :10 RHB)	70.00	42.39	7.28	1.73
	B3 (200 mg, 70CM :30 RHB)	72.67	49.06	7.39	2.50
	B4 (100 mg, 70CM :30 RHB)	78.67	52.00	7.18	2.60
	B5 (200 mg, 50CM :50 RHB)	82.00	55.02	6.39	2.20
	B6 (100 mg, 50CM :50 RHB)	84.00	55.37	6.49	2.27
	B1 (200 mg, 90CM :10 RHB)	9.35	8.30	3.11	1.43
	B2 (100 mg, 90CM :10 RHB)	12.07	17.41	3.05	1.22
Standard	B3 (200 mg, 70CM :30 RHB)	4.68	4.02	0.95	0.40
Error	B4 (100 mg, 70CM :30 RHB)	7.00	5.91	1.66	1.07
	B5 (200 mg, 50CM :50 RHB)	16.15	14.29	2.16	0.64
	B6 (100 mg, 50CM :50 RHB)	12.65	4.59	0.97	0.56
	B1 (200 mg, 90CM :10 RHB)	84.00	61.51	9.44	3.79
	B2 (100 mg, 90CM :10 RHB)	84.00	61.51	10.82	3.72
Max.	B3 (200 mg, 70CM :30 RHB)	80.00	52.72	8.65	2.96
	B4 (100 mg, 70CM :30 RHB)	84.00	61.18	9.48	3.70
	B5 (200 mg, 50CM :50 RHB)	100.00	73.22	8.22	2.90
	B6 (100 mg, 50CM :50 RHB)	100.00	62.97	7.79	2.74

GP Germination Percentage (%), VI Vigour Index, SL Shoot Length (cm), RL Root Length (cm), CM Chicken Manure, RHB = Rice Hull Biochar.

However, at higher frass dosages, a decline in shoot and root growth was observed, suggesting potential phytotoxic effects due to excessive nutrient concentration or the accumulation of toxic compounds. As reported by Núñez & Barral, substrates obtained from thermo-composting may contain organic acids, ammonia, ethylene oxide, salts, phenols, and heavy metals that can induce phytotoxicity. Similarly, Song et al found that excessive composted frass could inhibit seedling growth, whereas moderate inclusion (10%) promoted leaf development in *pakchoi* (*Brassica rapa*). This suggests that the beneficial or inhibitory effects of BSFL frass depend on its composition, maturity, and concentration within the growth medium. Macro- and micronutrients are crucial for the development and growth of plants as they are vital to plant physiology [33]. The improvement in germination at moderate doses, particularly at 30% frass application (L2) suggests that an intermediate level of frass provides an optimal balance between nutrient availability and phytotoxic compound concentration (Table 2). At this level, the microbial activity and biochemical composition within the frass likely promote early seedling vigor. Frass not only supplies essential nutrients but also contains plant growth–promoting rhizobacteria (PGPR) and phytohormones such as auxins and gibberellins [34], which stimulate cell elongation and enhance germination. In addition, the rice hull biochar (RHB) fraction contributes to improved aeration, pH buffering, and moisture retention, fostering a more favorable microenvironment for root and shoot development.

Table 2. Descriptive statistics of traits under study in the dosage of BSFL frass.

Stats	Treat	GP day 12	VI day 10	SL day 14	RL day 14
	L1 (10%)	68.00	45.38	7.40	1.74
Minimum	L2 (30%)	60.00	40.16	4.20	0.92
	L3 (50%)	56.00	11.72	2.00	0.12
	L1 (10%)	76.00	51.96	8.27	2.77
Mean	L2 (30%)	79.00	52.93	6.58	2.40
	L3 (50%)	75.33	46.44	5.23	1.51
Standard	L1 (10%)	6.38	4.17	0.80	0.78
Error	L2 (30%)	12.66	10.01	1.31	0.70
	L3 (50%)	14.35	14.64	2.64	0.88
	L1 (10%)	84.00	61.51	9.48	3.79
Minimum	L2 (30%)	100.00	73.22	8.22	3.42
	L3 (50%)	100.00	64.44	10.82	2.96

GP = Germination Percentage (%), VI= Vigour Index, SL= Shoot Length (cm), RL= Root Length (cm), CM = Chicken Manure, RHB = Rice Hull Biochar.

Conversely, higher dosages (L3, 50%) resulted in a decline in germination and seedling growth, indicating potential nutrient toxicity or osmotic stress due to excessive ammonium or soluble salts. This is consistent with findings from Núñez and Barral and Mendes et al., who reported that composts rich in organic acids, ammonia, and phenolics can exhibit phytotoxicity. The observed variation among frass types (Table 3) further supports this, where frass B6 (50% CM: 50% RHB) consistently produced the highest germination and vigor index, highlighting the synergistic effect of nutrient-rich manure and biochar's buffering capacity. Similar enhancement of plant growth using biochar-based BSFL frass has been reported in chilli and maize, primarily due to improved nutrient retention

and microbial colonization in the rhizosphere.

Although the ANOVA results (Table 3) indicate that most main effects were non-significant at $p \leq 0.005$, the significant interactions between frass type and dosage underscore the importance of composition–dose balance in determining seedling response. The interaction patterns show that frass blends containing equal parts of manure and RHB (e.g., B6L2) achieved superior germination and Vigor, likely because the biochar mitigated ammonia and phenol toxicity from the manure fraction. These findings emphasize that optimal application rates should consider both nutrient composition and the physicochemical properties of the frass to avoid inhibitory effects on germination.

Table 3. Mean Squares of the studied parameters along with DMRT based Grouped means.

SOV	df	GP	VI	SL	RL
Reps	1	4.00^{ns}	306.48*	3.91^{ns}	2.83*
B (the BSFL frass types)	5	189.24*	140.56^{ns}	3.38^{ns}	0.60^{ns}
L (the dosage of BSFL frass)	2	45.78^{ns}	147.21^{ns}	27.81*	5.41*
B x L	10	250.04*	157.34*	3.74*	1.04*
Error	17	60.47	62.82	2.60	0.23
The BSFL frass types		GP	VI	SL	RL
B1 (200 mg, 90CM :10 RHB)		73.33^{bc}	48.82	5.42	2.01
B2 (100 mg, 90CM :10 RHB)		70.00^{c}	42.39	7.28	1.73
B3 (200 mg, 70CM :30 RHB)		72.67^{bc}	49.06	7.39	2.50
B4 (100 mg, 70CM :30 RHB)		78.67^{ab}	52.00	7.18	2.58
B5 (200 mg, 50CM :50 RHB)		82.00^{a}	55.02	6.39	2.20
B6 (100 mg, 50CM :50 RHB)		84.00^{a}	55.37	6.49	2.27
The dosage of BSFL frass		GP	VI	SL	RL
L1 (10%)		76.00	51.96	8.27^{a}	2.77^{a}
L2 (30%)		79.00	52.93	6.58^{ab}	2.40^{a}
L3 (50%)		75.33	46.44	5.23^{b}	1.47^{b}
Interaction The BSFL frass types & The dosage of BSFL frass		GP	VI	SL	RL
B1L1(200 mg, 90CM :10 RHB & 10 %)		84.00^{ab}	56.16^{ab}	9.11^{a}	3.74
B1L2(200 mg, 90CM :10 RHB & 30 %)		66.00^{bc}	41.74^{b}	4.83^{bcd}	1.52
B1L3(200 mg, 90CM :10 RHB & 50 %)		70.00^{bc}	48.56^{ab}	2.33^{d}	0.77
B2L1(100 mg, 90CM :10 RHB & 10 %)		78.00^{bc}	51.59^{ab}	8.70^{ab}	2.91
B2L2(100 mg, 90CM :10 RHB & 30 %)		72.00b^{c}	51.48^{ab}	6.56^{abc}	1.79
B2L3(100 mg, 90CM :10 RHB & 50 %)		60.00^{c}	24.09^{c}	6.59^{abc}	0.48
B3L1(200 mg, 70CM :30 RHB & 10 %)		70.00^{bc}	49.05^{ab}	8.03^{ab}	2.38
B3L2(200 mg, 70CM :30 RHB & 30 %)		78.00^{bc}	48.79^{ab}	6.41^{abc}	2.62
B3L3(200 mg, 70CM :30 RHB & 50 %)		70.00^{bc}	49.35^{ab}	7.73^{ab}	2.51
B4L1(100 mg, 70CM :30 RHB & 10 %)		78.00^{bc}	53.33^{ab}	8.55^{ab}	3.00
B4L2(100 mg, 70CM :30 RHB & 30 %)		82.00^{ab}	55.76^{ab}	7.75^{ab}	3.34
B4L3(100 mg, 70CM :30 RHB & 50 %)		76.00^{bc}	46.91^{b}	5.24^{abcd}	1.42
B5L1(200 mg, 50CM :50 RHB & 10 %)		70.00^{bc}	48.19^{b}	7.54^{abc}	2.39
B5L2(200 mg, 50CM :50 RHB & 30 %)		100.00^{a}	67.76^{a}	7.85^{ab}	2.65
B5L3(200 mg, 50CM :50 RHB & 50 %)		76.00^{bc}	49.12^{ab}	3.77^{cd}	1.55
B6L1(100 mg, 50CM :50 RHB & 10 %)		76.00^{bc}	53.42^{ab}	7.68^{abc}	2.22
B6L2(100 mg, 50CM :50 RHB & 30 %)		76.00^{bc}	52.09^{ab}	6.08^{abcd}	2.50
B6L3(100 mg, 50CM :50 RHB & 50 %)		100.00^{a}	60.61^{ab}	5.72^{abcd}	2.09

Means followed by different letters in the same column differ significantly from each other at p = 0.05 SOV Source of variation, df degree of freedom, GP germination percentage, VI vigour index, SL Shoot Length, RL Root Length

The interaction between BSFL frass types and dosages had a significant effect on most germination and morphological traits, suggesting that both composition and application level jointly determine seedling performance (Tables 2 and 3). These interactive responses are further illustrated in Fig. 1. Fig. 1 illustrates the interactive effects between BSFL frass types and dosages on germination and early seedling growth attributes. A clear trend was observed where moderate frass dosages, particularly from frass types with a balanced composition of chicken manure and rice hull biochar, promoted higher germination percentage and vigor index. In contrast, treatments with higher inclusion rates or unbalanced compositions tended to suppress seedling growth, reflecting potential nutrient toxicity or osmotic stress. The pattern aligns with the findings in Tables 2 and 3, confirming that both frass composition and application rate jointly influence physiological responses during germination. This suggests that the beneficial effect of BSFL frass is dosage-dependent, with moderate application optimizing nutrient availability while minimizing phytotoxic effects from excessive ammonia or phenolic compounds.

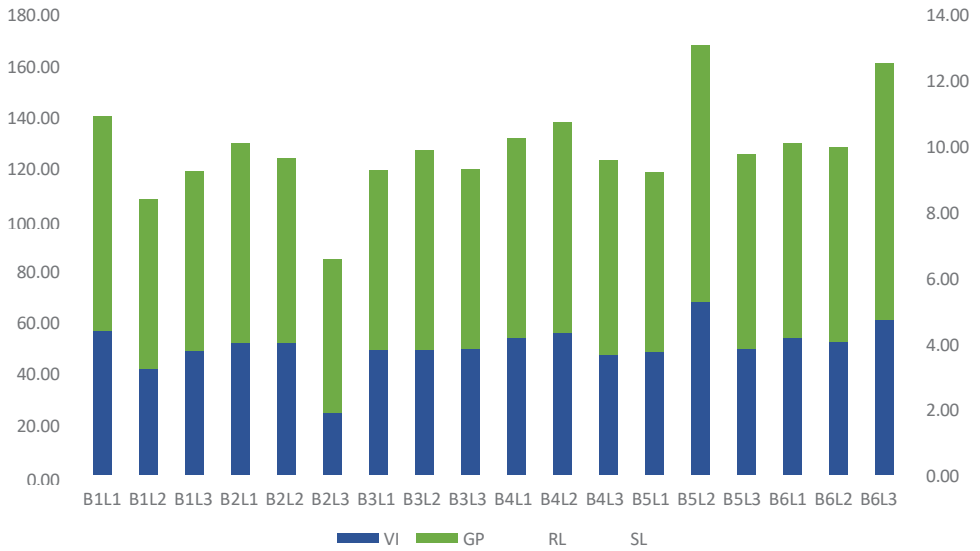

Fig. 1. Average responses of studied attributes under combination treatments.

The pattern aligns with the findings in Tables 2 and 3, confirming that both frass composition and application rate jointly influence physiological responses during germination. This suggests that the beneficial effect of BSFL frass is dosage-dependent, with moderate application optimizing nutrient availability while minimizing phytotoxic effects from excessive ammonia or phenolic compounds.

Moreover, previous studies have reported similar outcomes, indicating that soil enriched with food waste–derived BSFL frass can considerably enhance crop growth, primarily through improved nitrogen availability. The growth-promoting effects may also be attributed to the presence of essential macro- and micronutrients, as well as bioactive compounds naturally contained in the frass. Nevertheless, the optimal application rate can vary depending on the frass nutrient composition, the type of crop cultivated, existing soil fertility conditions, and environmental growing factors. To further understand the interrelationships among germination and morphological traits influenced by BSFL frass treatments, a correlation analysis was performed (Table 4). This analysis provides additional insight into how these parameters interact to reflect overall seedling performance.

Table 4 presents the Pearson correlation coefficients among germination percentage (GP), vigor index (VI), shoot length (SL), and root length (RL). A strong and significant positive correlation was observed between GP and VI (r = 0.8530*), indicating that seeds with higher germination rates tend to produce more vigorous seedlings. Similarly, GP showed a moderate but significant correlation with RL (r = 0.4871*), suggesting that early germination contributes to better root system establishment. Root length also exhibited strong positive correlations with both VI (r = 0.6458*) and SL (r = 0.7627*), highlighting the interconnected role of root development in supporting seedling growth and shoot elongation. Although SL showed a positive but non-significant association with GP and VI, this pattern implies that shoot elongation may depend more on post-germination nutrient and hormonal factors than on initial germination vigor. Overall, these correlations confirm that BSFL frass, particularly in balanced formulations such as B6L3 and B5L2— supports coordinated development between germination success, seedling vigor, and morphological growth.

Table 4. Pearson correlation between traits under study.

Variables	GP	VI	SL	RL
GP	1			
VI	**0.8530***	1		
SL	0.2081^{ns}	0.2661^{ns}	1	
RL	**0.4871***	**0.6458***	**0.7627***	1

Values in bold are different from 0 with a significance level p=0.05

These findings collectively highlight that the synergistic interaction between BSFL frass composition and application dosage plays a crucial role in enhancing seedling performance. The positive correlations among germination percentage, vigor index, and root traits indicate that balanced frass formulations, particularly those combining nutrient-rich manure with biochar can improve seed vigor and early growth by providing both chemical and biological benefits. This integrative understanding provides a foundation for determining the optimal frass composition and dosage for practical

applications in sustainable seedling production and soil fertility management.

4. Conclusion

Based on the overall findings presented above, it can be concluded that the interaction between BSFL frass composition and application rate plays a decisive role in determining tomato seed germination and seedling performance. This study demonstrated that BSFL frass significantly influenced the germination and early growth of tomato seedlings, with the most favorable results observed at a moderate application rate (30%) and in the frass composition containing 50% chicken manure and 50% rice husk biochar. The balanced nutrient content, improved aeration, and buffering properties provided by biochar effectively minimized phytotoxic effects while promoting root elongation and seedling vigor. These results suggest that properly formulated BSFL frass can serve as a sustainable biofertilizer for seedling production systems. For practical applications, optimizing frass dosage and composition is essential to ensure consistent nutrient availability without inducing toxicity. Future research should extend to field-scale validation to assess its long-term impact on soil fertility, microbial dynamics, and crop performance under diverse environmental conditions.

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