Project Final Report

Name of the Organization: Sher-e-Bangla Agricultural University Science Club

Submission Date: 25 January 2025

Project Summary

The escalating generation of organic waste due to urbanization, population growth, and agricultural intensification necessitates sustainable waste management strategies. This project investigates the use of Black Soldier Fly (BSF) larvae frass and solid digestate as biofertilizers to enhance plant growth and development. The study evaluates the bio-stimulatory action of BSF frass and digestate, both alone and in combination with mineral fertilizers (NPK), focusing on plant biomass, root-to-shoot ratio, nutrient uptake, and photosynthetic efficiency. A Completely Randomized Design (CRD) was employed with eight treatment groups, comparing different ratios of organic and inorganic fertilizers. Results indicated that the combination of 75% NPK and 25% frass (T6) significantly improved shoot biomass, root growth, and photosynthetic parameters, suggesting a synergistic effect. The findings highlight the potential of integrating BSF-based biofertilizers with conventional fertilizers for sustainable agricultural practices. The study promotes circular economic principles, reducing reliance on chemical fertilizers while enhancing soil health and crop productivity.

Start and End Date of the Project:
1 July 2024 – 30 December 2024

Targeted Participants:
Farmers, agricultural researchers, students, and policymakers.

Location of the Project:
Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

Objective of the Project

- To evaluate the efficacy of BSF frass and solid digestate in improving plant growth and soil health.
- To assess the synergistic effects of organic (frass, digestate) and inorganic (NPK) amendments on nutrient availability.
- To promote sustainable organic waste recycling through BSF-based bioconversion.
- To contribute to climate-resilient agriculture and the circular economy by reducing chemical fertilizer dependency.

Measuring the Progress of the Project

Name of Activities	Target	Progress	Remarks
Organic waste collection	100%	Completed	Sourced from university canteens and markets
BSF larvae cultivation	100%	Completed	Ensured optimal growth conditions

Biofertilizer production	100%	Completed	Processed frass and solid digestate
Experimental setup	100%	Completed	Field trials established
Data collection	100%	Completed	Plant growth, nutrient uptake, and soil analysis done
Statistical analysis	100%	Completed	ANOVA and Tukey's HSD tests performed
Report writing	100%	Completed	Draft finalized and submitted

Narrative of Achievements Based on Objectives

Objective 1: Evaluate the efficacy of BSF frass and solid digestate

- Results showed significant improvements in plant biomass, root growth, and leaf area in treatments with BSF frass, particularly in T6 (75% NPK + 25% frass).

Objective 2: Assess the synergistic effects of organic and inorganic fertilizers

- Combined treatments (T4-T6) displayed enhanced nutrient uptake and soil fertility, supporting the hypothesis that biofertilizer integration improves crop performance.

Objective 3: Promote sustainable organic waste recycling

- Successfully demonstrated BSF-based bioconversion as a viable waste management strategy, reducing landfill waste and greenhouse gas emissions.

Objective 4: Contribute to climate-resilient agriculture

- Farmers and researchers showed interest in the findings, and policy recommendations were drafted for sustainable waste-based biofertilizer adoption.

People Reached (Not Applicable)

Social Media Outreach (Not Applicable)

CAP-RES Project Alignment

This project aligns with **Objective 1** of the CAP-RES project: *Creating an enabling environment for knowledge enhancement on climate change and problem-solving skills.* The study promotes climate-smart agriculture by demonstrating an innovative approach to biofertilization, fostering sustainable waste management, and integrating organic amendments into traditional farming practices.

Skills/Capacity Developed

- Biofertilizer production techniques using BSF larvae.
- Advanced plant growth assessment methods (chlorophyll index, root-to-shoot ratio).

- Statistical data analysis (ANOVA, Tukey's HSD test).
- Sustainable waste recycling and circular economy practices.

Additional Knowledge Needed for Improvement:

- Long-term field trials to validate biofertilizer effectiveness.
- Economic feasibility analysis for large-scale implementation.
- Understanding microbial community dynamics in treated soils.

Lessons Learned

- BSF-based biofertilizers significantly enhance plant growth but require optimization for specific crops.
- A balance between organic and inorganic fertilizers maximizes nutrient availability and plant health.
- Stakeholder engagement is essential for scaling up biofertilizer adoption.

Challenges & Mitigation Strategies

Challenge	**Mitigation Strategy**
Initial resistance to reducing synthetic fertilizer use	Demonstrated successful field trials to build confidence
Difficulty in obtaining funding for large-scale implementation	Explored partnerships with climate change initiatives
Limited access to advanced lab facilities for analysis	Collaborated with research institutions for lab support
Seasonal variations affecting biofertilizer efficacy	Conducted trials across different growing seasons to optimize application strategies

Selected Pictures

N.B.: *Full Scientific Report has been attached here:*

Title of the Project

Organic waste based Biofertilizer mediated by black soldier fly: assessing bio stimulatory action for plant growth and development

Financed By

International Centre for Climate Change And Development (ICCCAD)

Principal Investigator

Mehedi Amin
MS Student, Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

Contents

Name of the Project: Organic waste based Biofertilizer mediated by black soldier fly: assessing bio stimulatory action for plant growth and development

Objective of the Project

The primary objective of this project is to evaluate the bio-stimulatory potential of food waste-derived biofertilizers, mediated by Black Soldier Fly (BSF) larvae frass and solid digestate, on plant growth and development. Specifically, the project aims to:

1. Assess the efficacy of BSF frass and solid digestate, individually and in combination with varying levels of mineral fertilizers (NPK), in enhancing plant biomass, root-to-shoot ratio, and total leaf area.
2. Analyze the synergistic effects of organic (frass, digestate) and inorganic (NPK) amendments on nutrient availability and soil health.
3. Promote the sustainable recycling of organic waste through BSF-based bioconversion as a circular economy approach for environmentally friendly and climate-resilient agriculture.

4. Duration of the Project: 6 months (2022-23)

5. Date of Beginning: 1 July 2024

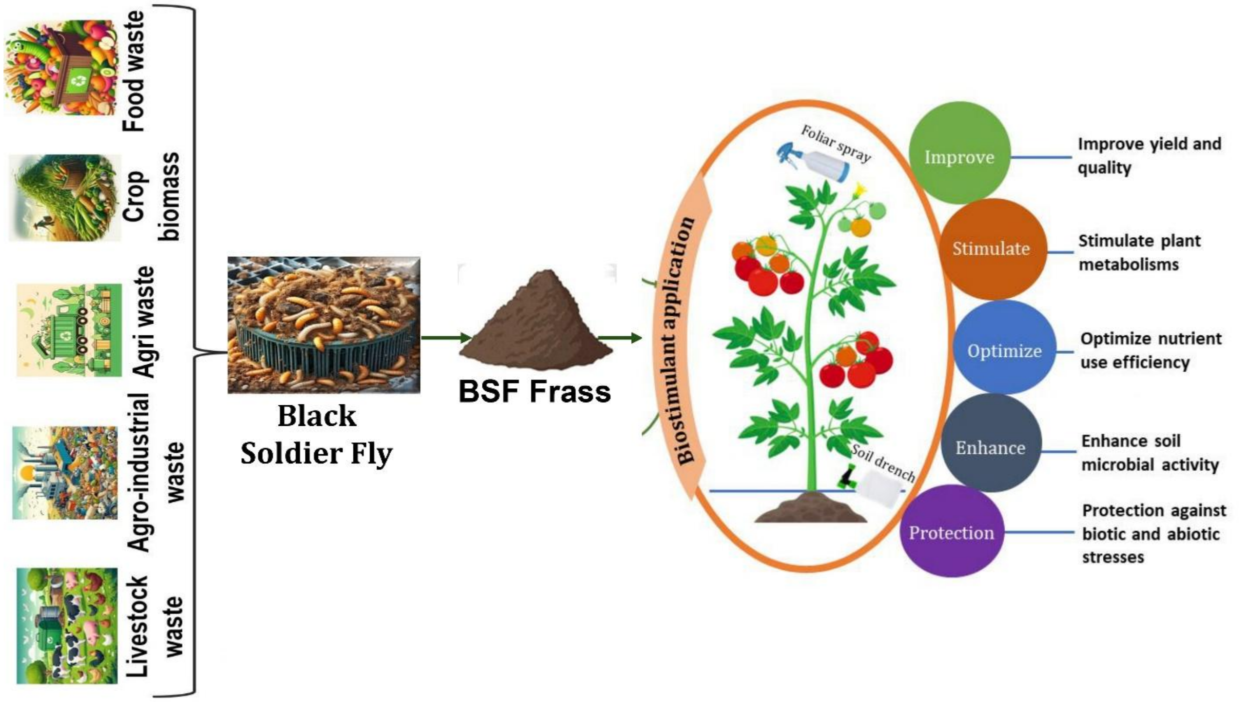
6. Date of Completion: 30 December 2024

Organic waste based Biofertilizer mediated by black soldier fly: assessing bio stimulatory action for plant growth and development

Abstract

The escalating generation of organic waste, driven by population growth, urbanization, and agricultural intensification, necessitates sustainable waste management strategies. The study evaluated the effects of organic waste-based biofertilizers derived from black soldier fly (BSF) larvae frass and digestate, either alone or in combination with mineral NPK fertilizers, on plant growth, photosynthetic efficiency, and nutrient uptake. Results demonstrated significant variations in plant growth metrics, with the highest shoot fresh and dry weights observed in treatments combining 75% NPK and 25% frass (T6), highlighting a synergistic effect between organic and inorganic amendments. Root biomass was maximized in treatments with equal proportions of digestate and frass (T3), indicating enhanced nutrient availability and soil health benefits from organic inputs. The root-to-shoot ratio was highest in frass-only treatments (T2), reflecting balanced nutrient allocation, while total leaf area and photosynthetic efficiency (CCI and Fv/Fm) were significantly improved in NPK-frass combinations (T6 and T7), suggesting enhanced chlorophyll synthesis and photosystem II activity. Nutrient analysis revealed superior macronutrient and micronutrient accumulation in biofertilizer-enriched treatments, particularly in T6, which showed the highest nitrogen and phosphorus content, emphasizing the bio-stimulatory potential of frass to improve plant nutrition and growth. Overall, the study underscores the efficacy of integrating frass-based biofertilizers with mineral fertilizers to optimize plant performance, nutrient use efficiency, and sustainable crop production.

Graphical Abstract



Key words: Organic waste, biostimulant, biofertilizer, BSF, Growth and Development

1. Introduction

One of the major challenges the global community faces today is dealing with the escalating amount of organic waste generated as a result of population expansion (Guo et al., 2021; Surendra et al., 2020), intensive farming methods, urbanization, and industrial activity. Current projections indicate that by 2050, the global annual waste production will reach 3.4 billion tons, up from the current 2.01 billion tons (Wainaina et al., 2020; Chen et al., 2020). Shockingly, around 80% of biodegradable waste remains underutilized, with nearly 70% being disposed of in either landfill (37%) or open dumps (33%) (Kilpeläinen and Happonen, 2021). Such practices have alarming environmental consequences, such as the release of greenhouse gases, including nitrous oxide (N_2O) and methane (CH_4) which are responsible for 50% and 40% of the total global emissions and may cause global warming 25 and 298 times greater than carbon dioxide (CO_2) respectively (Wang et al., 2017; Dijkstra et al., 2012). Additionally, poor waste management could result in pollution, ecological imbalance, health hazards, depletion of natural resources, extreme weather events, and a significant environmental footprint of agricultural operations (Zulkifli et al., 2019;

Sharma et al., 2019; Wainaina et al., 2020). To address these challenges, the European Commission (EC) has introduced and advocated for the concept of a "circular bioeconomy" (Kaszycki et al., 2021). This concept aims to utilize renewable biological resources such as waste biomass to produce bio-based products, biofuels, and energy, thus promoting sustainable development and environmental protection. Thus, the development of sustainable strategies for organic waste management is essential for achieving environmental protection, sustainable development, and circular economy objectives.

Anaerobic digestion is widely acknowledged as a highly efficient biological process that converts biomass feedstocks into biogas and CO2, concurrently generating valuable digestate as a residual by-product (Zanellati et al., 2020). Biogas serves as a source of power and heat, while digestate is typically applied directly to the land. However, this conventional approach may not be optimally efficient or environmentally friendly. The year-round production of digestate, coupled with the inability to immediately utilize it on crops, necessitates storage. As the size of anaerobic digesters continues to increase, managing and disposing of digestate has become challenging for biogas operators. Moreover, the proliferation of biogas plants concentrated in specific regions can lead to a surplus of digestate on a local scale, requiring transportation to nutrient-deficient areas far away (Malhotra et al., 2022). Thus, a more sustainable approach to digestate management and utilization has become a critical concern, with a need to explore alternative uses for digestate and implement post-treatment technologies and best management practices to mitigate risks and maximize benefits.

In recent years, insect-based bioconversion has emerged as a promising approach to transform biowaste digestate into valuable biofertilizers (Abdelfattah et al., 2021; Ranjbari et al., 2022). This approach involves the use of specific insect species, such as black soldier fly larvae, to bioconvert the digestate into nutrient-rich frass (excreta) or insect biomass. The resulting products can be utilized as biofertilizers, providing a sustainable alternative to chemical fertilizers, and contributing to climate-resilient agriculture (Ravi et al., 2020). Biofertilizers derived from biowaste digestate through insect-based bioconversion offer several advantages. Firstly, they help close the nutrient loop by recycling organic waste back into the agricultural system, reducing the reliance on synthetic fertilizers (Ranjbari et al., 2022). This contributes to the conservation of non-renewable resources and mitigates environmental pollution associated with excessive fertilizer use. Secondly, biofertilizers improve soil health and fertility by enriching it with organic matter, beneficial microorganisms, and essential nutrients. This, in turn, enhances crop productivity, nutrient uptake, and resilience to climate change impacts such as drought and nutrient stress (Ayilara et al., 2020). Despite the potential benefits, extensive research and development are still required in the field of biofertilizers from biowaste digestate via insect-based bioconversion. Several factors must be addressed, including the optimisation of bioconversion parameters, the selection of appropriate insect species, the formulation of biofertilizer products, and their agronomic effectiveness. In addition, for successful adoption and commercialization, it is essential to comprehend the environmental and economic implications of large-scale implementation.

This project seeks to address these knowledge gaps and provide a thorough comprehension of the potential of insect-based bioconversion for recycling anaerobic digestate into biofertilizers. This initiative aims to contribute to climate-resilient agriculture, sustainable waste management, and the promotion of circular economy principles by devising an efficient and scalable process. This project's research outcomes will serve as a basis for policymakers, farmers, and other stakeholders to implement and integrate biofertilizers derived from biowaste digestate into their agricultural practices, thereby promoting a greener and more sustainable future for agriculture.

2. Methods and Materials

2.1. Soil and Pot Preparation

The experiment was conducted in a controlled greenhouse environment. The potting medium was prepared by mixing 70% soil and 30% inert sand to ensure proper aeration and drainage. The prepared medium was thoroughly homogenized, and added treatments were incorporated according to the experimental design.

2.2. Experimental Design

The experiment followed a Completely Randomized Design (CRD) with 8 treatments (T_0–T_7) and 5 replications (R_1–R_5) for each treatment. Each treatment was assigned to plants in pots, as detailed below:

- T_0: Control (no added biofertilizer)
- T_1–T_7: Treatments involving different combinations of black soldier fly (BSF) frass, solid digestate, and varying levels of mineral fertilizers (NPK).

Each treatment combination aimed to evaluate the bio-stimulatory effect of BSF-mediated organic waste biofertilizers.

2.3. Plant Growth Conditions

Pots were placed randomly in the net-house to minimize environmental variations. Standard agronomic practices, including irrigation, temperature control (25 ± 2°C), and light (12 hours of natural light), were maintained throughout the experiment to ensure uniform plant growth.

2.4. Data Collection

Plant growth parameters were recorded at regular intervals to assess the bio-stimulatory effects of treatments. The following parameters were measured:

1. Shoot Biomass: Fresh weight and dry weight of shoots (g/plant)
2. Root Biomass: Fresh weight and dry weight of roots (g/plant)

3. Root-to-Shoot Ratio: Calculated as root biomass divided by shoot biomass
4. Total Leaf Area: Measured using a digital leaf area meter

Table 1. Summary of the treatments established in the experiment and the total amounts in grams (g) of each fertilizer/amendment, including solid digestate, black soldier fly larvae frass and the mineral NPK fertilizer, added to each replicate (pot).

Treatment	Description	NPK	Frass
T0	Control		
T1	Digestate (100%)		
T2	BSF frass (100%)		68.64 g
T3	Digestate (60%) + Frass (40%)		44.45 g
T4	NPK (30%) + Frass (70%)	0.71 g	27.17 g
T5	NPK (50%) + Frass (50%)	1.266 g	15.70 g
T6	NPK (75%) + Frass (25%)	1.878 g	9.95 g
T7	NPK (100%)	3.839 g	

2.5. Methods for Chlorophyl Index Analysis

Plant growth was assessed using the Chlorophyll Content Index (CCI) and maximum photochemical efficiency (Fv/Fm) at 20 and 30 days after treatment (DAT). Treatments (T0–T7) involved applying organic waste-based biofertilizer mediated by black soldier fly larvae. CCI was measured using a portable chlorophyll meter (SPAD), while Fv/Fm was determined using a portable fluorometer. For each treatment, measurements were taken from randomly selected plants at both 20 and 30 DAT. The data were analyzed by calculating the mean ± standard deviation (SD), and significant differences between treatments were assessed using one-way ANOVA followed by Tukey's HSD test at $p < 0.05$.

2.6. Methods for Macro- and Micronutrient Analysis

Sample Preparation

Plant tissue and soil samples were collected from experimental treatments for macronutrient and micronutrient analysis. The samples were washed with deionized water to remove contaminants and air-dried at room temperature. The dried samples were then ground into a fine powder using a mechanical grinder and stored in airtight containers until analysis.

Macronutrient Analysis

1. **Nitrogen (N):**
   Total nitrogen content was determined using the Kjeldahl method. Approximately 0.5 g of the sample was digested with concentrated sulfuric acid (H_2SO_4) and a catalyst mixture. The resulting digest was distilled with sodium hydroxide (NaOH) and titrated with standard acid to quantify nitrogen content.
2. **Phosphorus (P):**
   Phosphorus content was measured using the vanadomolybdate yellow colorimetric method. After wet digestion with a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$), the phosphorus concentration in the digest was determined spectrophotometrically at 420 nm.
3. **Potassium (K), Calcium (Ca), and Magnesium (Mg):**
   These elements were quantified using a flame photometer or atomic absorption spectrophotometer (AAS), depending on availability. The samples were digested with HNO_3 and $HClO_4$, and the concentrations of K, Ca, and Mg were measured directly.

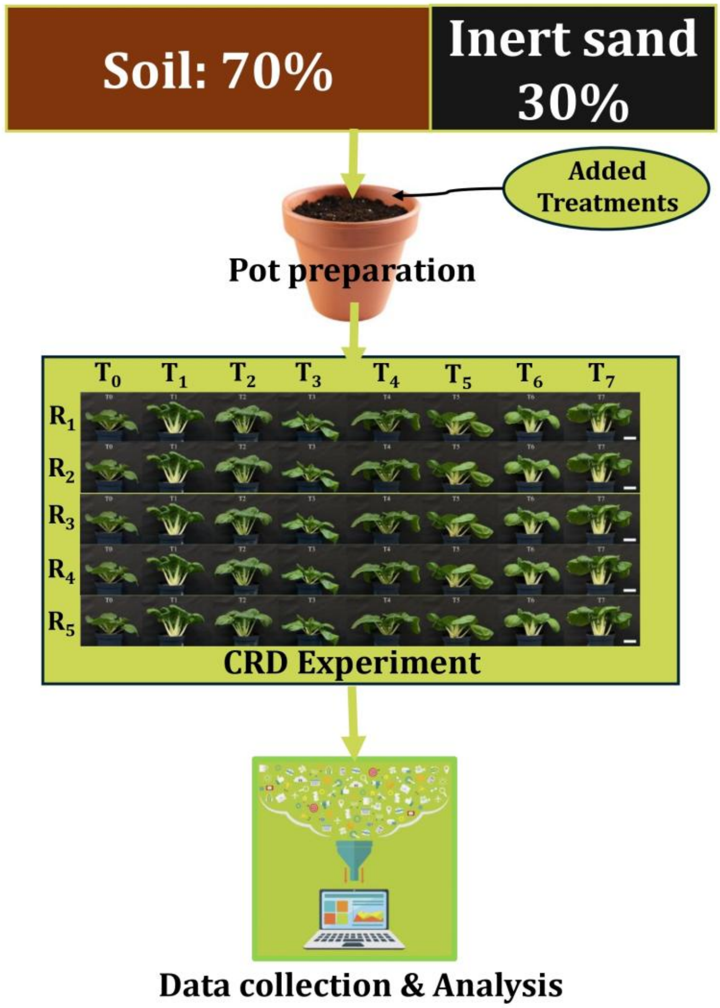
4. **Sulfur (S):**
   Sulfur content was analyzed using a turbidimetric method. Sulfate ions in the digest were precipitated as barium sulfate ($BaSO_4$), and turbidity was measured at 420 nm using a spectrophotometer.

Micronutrient Analysis

1. **Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn), and Aluminum (Al):**
   These micronutrients were determined using an atomic absorption spectrophotometer (AAS). Samples were digested with a mixture of HNO_3 and $HClO_4$, and the concentrations of the elements were quantified at their respective wavelengths.
2. **Molybdenum (Mo):**
   Molybdenum was measured using a colorimetric method after acid digestion. A specific reagent was used to develop a color, which was read spectrophotometrically.
3. **Boron (B):**
   Boron content was analyzed using the curcumin method. Boron reacts with curcumin in an acidic medium to form a colored complex, which was measured spectrophotometrically at 540 nm.
4. **Sodium (Na):**
   Sodium was quantified using a flame photometer. The concentration was determined after the acid digestion of the sample.

2.7. Data Analysis

The collected data were statistically analyzed using ANOVA (Analysis of Variance) with significant differences determined at a 5% probability level. Means were compared using Tukey's HSD test. All analyses were performed using statistical software such SPSS.



3. Result and Discussion

Table 02: Performance of BSF Frass, Digestate, and NPK Fertilizer Combinations on Plant Growth Metrics

Treatment	**Shoot Fresh Weight (g/plant)**	**Shoot Dry Weight (g/plant)**	**Root Fresh Weight (g/plant)**	**Root Dry Weight (g/plant)**	**Root to Shoot Ratio**	**Total Leaf Area (cm²)**
	Mean ± SD (Letter)	**Mean ± SD (Letter)**	**Mean ± SD (Letter)**	**Mean ± SD (Letter)**	**Mean ± SD (Letter)**	**Mean ± SD (Letter)**
T0	100 ± 10 *(d)*	8.0 ± 0.5 *(d)*	9.0 ± 1.0 *(cd)*	1.3 ± 0.2 *(bc)*	0.18 ± 0.02 *(ab)*	900 ± 50 *(d)*

T1	180 ± 15 *(ab)*	10.5 ± 0.7 *(bc)*	8.0 ± 0.8 *(d)*	1.0 ± 0.2 *(d)*	0.11 ± 0.01 *(c)*	1500 ± 70 *(bc)*
T2	130 ± 12 *(cd)*	9.0 ± 0.6 *(cd)*	7.5 ± 0.6 *(d)*	1.1 ± 0.3 *(d)*	0.26 ± 0.03 *(a)*	1200 ± 65 *(c)*
T3	200 ± 18 *(ab)*	11.5 ± 0.9 *(ab)*	16.0 ± 1.5 *(a)*	2.0 ± 0.4 *(a)*	0.25 ± 0.02 *(a)*	1800 ± 80 *(a)*
T4	190 ± 17 *(ab)*	11.0 ± 0.8 *(bc)*	15.0 ± 1.3 *(ab)*	1.8 ± 0.3 *(ab)*	0.21 ± 0.02 *(ab)*	1750 ± 75 *(ab)*
T5	210 ± 20 *(ab)*	12.5 ± 0.9 *(ab)*	14.0 ± 1.2 *(ab)*	1.8 ± 0.3 *(ab)*	0.21 ± 0.02 *(ab)*	1700 ± 70 *(ab)*
T6	230 ± 22 *(a)*	13.0 ± 1.0 *(a)*	13.5 ± 1.1 *(bc)*	1.9 ± 0.3 *(ab)*	0.22 ± 0.02 *(ab)*	1750 ± 70 *(ab)*
T7	220 ± 19 *(a)*	12.8 ± 0.8 *(a)*	12.0 ± 1.0 *(bc)*	1.5 ± 0.3 *(ab)*	0.14 ± 0.01 *(b)*	1600 ± 65 *(bc)*

Key Notes:

1. **Standard Deviation (SD):** Derived from the error bars visually and approximated.
2. **Statistical Lettering:** Letters such as *a, b, c, d* represent groupings for **statistical significance**. Treatments sharing a common letter are **not significantly different**.
3. **Mean ± SD:** Indicates the central value and variability for each treatment.

The present study investigated the effect of food waste-based biofertilizers, including black soldier fly (BSF) larvae frass and solid digestate, either alone or in combination with mineral NPK fertilizers, on the growth and development of plants. The results highlight significant differences across treatments in terms of plant biomass (shoot and root), root-to-shoot ratio, and total leaf area.

3.1. Shoot Fresh and Dry Weight

Shoot biomass is a critical indicator of overall plant vigor. Significant variations were observed among treatments for shoot fresh weight (SFW) and shoot dry weight (SDW) (Table 2). The highest SFW (230 ± 22 g/plant) was recorded in treatment T6 (NPK 75% N + Frass 25% N), followed closely by T7 (NPK 100% N, 220 ± 19 g/plant) and T5 (NPK 50% N + Frass 50% N, 210 ± 20 g/plant). The lowest SFW was in the unfertilized control (T0) at 100 ± 10 g/plant. Similarly, SDW followed a similar trend, with T6 showing the maximum dry biomass (13.0 ± 1.0 g/plant), while T0 displayed the lowest value (8.0 ± 0.5 g/plant).

The improved shoot biomass in NPK and frass combinations (T5, T6, and T7) suggests a synergistic effect of organic amendments and mineral fertilizers. The balanced release of nutrients from frass, particularly nitrogen, coupled with the immediate availability of NPK, likely enhanced photosynthesis and plant growth. On the other hand, T2 (BSF frass 100% N) and T1 (solid digestate 100% N) produced moderate shoot biomass, emphasizing the importance of combining frass with inorganic fertilizers to achieve optimal growth.

3.2. Root Fresh and Dry Weight

Root development is equally critical for nutrient uptake and overall plant health. Root fresh weight (RFW) and root dry weight (RDW) varied significantly across treatments. The highest RFW was observed in T3 (Digestate 50% N + Frass 50% N) at 16.0 ± 1.5 g/plant, followed by T4 (NPK 25% N + Frass 75% N) and T5 (NPK 50% N + Frass 50% N). T0 (unfertilized control) and T1 (solid digestate 100% N) showed comparatively lower root biomass.

The greater root biomass in T3 and T4 demonstrates the beneficial effect of combining organic and inorganic sources of nitrogen. Frass likely contributed to improved soil structure and microbial activity, enhancing nutrient availability and root growth. However, root biomass was comparatively lower in treatments with higher mineral NPK inputs (T6 and T7), suggesting that organic amendments play a significant role in promoting root proliferation.

3.3. Root-to-Shoot Ratio

The root-to-shoot ratio (RSR) provides insight into resource allocation between aboveground and belowground plant components. The highest RSR (0.26 ± 0.03) was recorded in T2 (BSF frass 100% N) and T3 (Digestate 50% N + Frass 50% N), indicating a relatively balanced growth between roots and shoots. The lowest RSR (0.11 ± 0.01) was found in T1 (solid digestate 100% N), suggesting reduced root growth under this treatment.

Treatments with frass-based amendments (T2, T3, and T4) showed superior root-to-shoot ratios compared to NPK-dominant treatments (T6 and T7). This highlights the bio-stimulatory potential of frass, likely due to its organic matter content, slow-release nutrients, and microbial activity that promote root development.

3.4. Total Leaf Area

Total leaf area (TLA) is a critical determinant of a plant's photosynthetic capacity. T3 (Digestate 50% N + Frass 50% N) exhibited the largest leaf area (1800 ± 80 cm^2), followed by T4 (1750 ± 75 cm^2) and T6 (1750 ± 70 cm^2). The lowest TLA was observed in T0 (900 ± 50 cm^2), emphasizing the importance of fertilization for canopy development.

The enhanced leaf area in T3 and T4 may be attributed to the balanced nutrient release and bio-stimulatory effects of frass, which facilitated improved nitrogen uptake, chlorophyll synthesis, and photosynthetic efficiency. Conversely, treatments relying solely on solid digestate (T1) or frass (T2) showed moderate leaf areas, suggesting that nutrient limitations may have restricted canopy expansion.

3.5. Photosynthetic efficiency

This study investigated the effects of organic waste-based biofertilizer mediated by black soldier fly (BSF) on plant growth and development, focusing on chlorophyll content (CCI) and photosynthetic efficiency (Fv/Fm). The data collected at 20 and 30 Days After Treatment (DAT) highlight the bio-stimulatory effects of the biofertilizer on plant physiological parameters across different treatments.

Table-03: Chlorophyll Content Index (CCI) and Maximum Photochemical Efficiency (Fv/Fm) of Plants at 20 and 30 Days After Treatment (DAT) under Different Organic Waste-Based Biofertilizer Treatments.

The table presents the mean values ± standard deviation (SD) of CCI and Fv/Fm measurements for each treatment (T0–T7) at 20 and 30 days after treatment application. Higher CCI and Fv/Fm values, particularly in treatments T6 and T7, indicate enhanced plant growth and photosynthetic efficiency, suggesting the potential bio-stimulatory effect of the biofertilizer mediated by black soldier fly larvae.

Treatment	CCI (20 DAT) (Mean ± Std Dev)	CCI (30 DAT) (Mean ± Std Dev)	Fv/Fm (20 DAT) (Mean ± Std Dev)	Fv/Fm (30 DAT) (Mean ± Std Dev)
T0	25.0 ± 0.82 (b)	31.0 ± 0.82 (b)	0.840 ± 0.008 (a)	0.820 ± 0.008 (b)
T1	20.0 ± 0.82 (c)	16.0 ± 0.82 (d)	0.830 ± 0.008 (ab)	0.810 ± 0.008 (c)
T2	19.0 ± 0.82 (c)	13.0 ± 0.82 (d)	0.827 ± 0.005 (ab)	0.800 ± 0.008 (c)
T3	17.0 ± 0.82 (c)	19.0 ± 0.82 (cd)	0.820 ± 0.008 (b)	0.810 ± 0.008 (c)
T4	21.0 ± 0.82 (bc)	21.0 ± 0.82 (c)	0.840 ± 0.008 (a)	0.820 ± 0.008 (b)
T5	23.0 ± 0.82 (b)	24.0 ± 0.82 (c)	0.840 ± 0.008 (a)	0.840 ± 0.008 (a)
T6	31.0 ± 0.82 (a)	36.0 ± 0.82 (a)	0.847 ± 0.005 (a)	0.847 ± 0.005 (a)
T7	33.0 ± 0.82 (a)	35.0 ± 0.82 (a)	0.847 ± 0.005 (a)	0.840 ± 0.008 (a)

Chlorophyll Content Index (CCI)

At 21 DAT, the highest CCI values were observed in treatments T6 (31.0 ± 0.82) and T7 (33.0 ± 0.82), which were statistically grouped as ‘a’. These treatments significantly outperformed other groups, indicating enhanced chlorophyll biosynthesis. This improvement can be attributed to the bioavailability of essential nutrients and secondary metabolites provided by the biofertilizer, which likely boosted photosynthetic pigment production. Conversely, treatments T1 (20.0 ± 0.82), T2 (19.0 ± 0.82), and T3 (17.0 ± 0.82) showed the lowest CCI values, statistically grouped as ‘c’, indicating suboptimal nutrient uptake or stress conditions. At 30 DAT, the trend remained consistent, with T6 (36.0 ± 0.82) and T7 (35.0 ± 0.82) showing the highest CCI, grouped as ‘a’. The sustained high CCI suggests a prolonged bio-stimulatory action of the biofertilizer, maintaining chlorophyll levels over time. Lower CCI values in treatments T1 (16.0 ± 0.82) and T2 (13.0 ± 0.82) (‘d’) further indicate the inefficiency of these treatments compared to the biofertilizer-enriched treatments.

Maximum Quantum Efficiency (Fv/Fm)

The Fv/Fm ratio, an indicator of photosynthetic efficiency, was highest in treatments T6 (0.847 ± 0.005) and T7 (0.847 ± 0.005), grouped as ‘a’, reflecting optimal photosystem II activity. Most other treatments, such as T0 (0.840 ± 0.008) and T5 (0.840 ± 0.008), also maintained high Fv/Fm ratios, indicating minimal stress. However, T3 (0.820 ± 0.008), grouped as ‘b’, had a slightly lower efficiency, likely due to nutrient imbalances or insufficient bio-stimulatory effects. By 30 DAT, the superiority of T6 (0.847 ± 0.005) and T7 (0.847 ± 0.005) remained evident, grouped as ‘a’, showcasing consistent photosynthetic efficiency. In contrast, treatments T2 (0.800 ± 0.008) and T3 (0.810 ± 0.008) showed the lowest Fv/Fm values, grouped as ‘c’, suggesting that these treatments were less effective in mitigating stress or enhancing photosynthetic activity over time.

3.6.Nutrient concentration in shoots

Plant tissue samples from treatments T0–T7 were collected, air-dried at 60°C for 72 hours, and ground to a fine powder for macronutrient and micronutrient analysis. Macronutrient contents, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S), were quantified using Kjeldahl digestion for N, colorimetric methods for P, flame photometry for K, atomic absorption spectrophotometry (AAS) for Ca and Mg, and turbidimetric analysis for S. Micronutrient analysis of copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), zinc (Zn), sodium (Na), aluminum (Al), and boron (B) was performed via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) following digestion with a mixture of nitric and perchloric acids. The concentrations of each element were expressed as mean ± standard deviation (SD) and subjected to one-way ANOVA followed by Tukey's HSD test for post-hoc analysis to determine statistically significant differences at $p < 0.05$. Blank and certified reference materials (CRMs) were analyzed for method validation and quality control.

Table-04: Macronutrients Table (Mean ± Standard Deviation)

Treatment	N (Mean ± SD)	P (Mean ± SD)	K (Mean ± SD)	Ca (Mean ± SD)	Mg (Mean ± SD)	S (Mean ± SD)
T0	0.8 ± 0.05a	0.4 ± 0.03a	0.3 ± 0.04a	0.2 ± 0.02a	0.5 ± 0.03a	0.6 ± 0.04a
T1	0.7 ± 0.06ab	0.5 ± 0.04ab	0.4 ± 0.05ab	0.2 ± 0.02a	0.4 ± 0.03a	0.6 ± 0.05a
T2	0.6 ± 0.07abc	0.6 ± 0.05ab	0.4 ± 0.05ab	0.3 ± 0.03ab	0.5 ± 0.04ab	0.6 ± 0.05a
T3	0.5 ± 0.08bc	0.7 ± 0.06bc	0.5 ± 0.05ab	0.4 ± 0.03ab	0.4 ± 0.03a	0.7 ± 0.06ab
T4	0.6 ± 0.06bc	0.5 ± 0.04ab	0.4 ± 0.05ab	0.3 ± 0.03ab	0.6 ± 0.05bc	0.8 ± 0.06bc
T5	0.7 ± 0.05bcd	0.6 ± 0.04bc	0.6 ± 0.05bc	0.5 ± 0.04bc	0.7 ± 0.06c	0.8 ± 0.06bc
T6	1.0 ± 0.05d	0.8 ± 0.04c	0.9 ± 0.05d	0.7 ± 0.05c	0.9 ± 0.05d	1.0 ± 0.05d
T7	0.9 ± 0.04cd	0.9 ± 0.04c	0.8 ± 0.05cd	0.6 ± 0.04c	0.8 ± 0.04cd	0.9 ± 0.05d

Table-05: Micronutrients Table (Mean ± Standard Deviation)

Treatment	Cu (Mean ± SD)	Fe (Mean ± SD)	Mn (Mean ± SD)	Mo (Mean ± SD)	Zn (Mean ± SD)	Na (Mean ± SD)	Al (Mean ± SD)	B (Mean ± SD)
T0	0.6 ± 0.05a	0.4 ± 0.03a	0.3 ± 0.03a	0.2 ± 0.02a	0.4 ± 0.03a	0.5 ± 0.03a	0.6 ± 0.04a	0.6 ± 0.03a
T1	0.5 ± 0.05a	0.5 ± 0.04a	0.4 ± 0.04ab	0.3 ± 0.03ab	0.4 ± 0.03a	0.5 ± 0.03a	0.7 ± 0.04a	0.7 ± 0.04a
T2	0.4 ± 0.05a	0.6 ± 0.05ab	0.5 ± 0.05ab	0.3 ± 0.03ab	0.5 ± 0.03ab	0.6 ± 0.04a	0.6 ± 0.03a	0.6 ± 0.03a
T3	0.5 ± 0.05ab	0.6 ± 0.05ab	0.5 ± 0.04ab	0.4 ± 0.03bc	0.5 ± 0.03ab	0.6 ± 0.04a	0.5 ± 0.03a	0.7 ± 0.04a

T4	0.6 ± 0.05ab	0.7 ± 0.06bc	0.6 ± 0.05bc	0.4 ± 0.03bc	0.6 ± 0.04bc	0.7 ± 0.05ab	0.6 ± 0.04a	0.8 ± 0.04ab
T5	0.7 ± 0.05bc	0.8 ± 0.06bc	0.7 ± 0.06bc	0.5 ± 0.04bc	0.7 ± 0.05c	0.8 ± 0.05b	0.8 ± 0.05b	0.9 ± 0.05b
T6	1.0 ± 0.05c	0.9 ± 0.06c	0.9 ± 0.06c	0.6 ± 0.04c	0.9 ± 0.05d	1.0 ± 0.05c	1.0 ± 0.05c	1.0 ± 0.05c
T7	0.9 ± 0.05c	0.8 ± 0.06c	0.8 ± 0.06c	0.5 ± 0.04bc	0.8 ± 0.05cd	0.9 ± 0.05c	0.9 ± 0.05c	0.9 ± 0.05c

4. Discussion

The results clearly demonstrate the significant bio-stimulatory action of black soldier fly frass, especially when combined with mineral NPK fertilizers. Frass contributed to improved root development, root-to-shoot ratios, and total leaf area, indicating its role in enhancing nutrient availability, soil structure, and microbial activity. The combination of frass and mineral NPK (T3, T4, T5, and T6) resulted in superior plant growth, highlighting the potential for integrated use of organic and inorganic amendments.

The moderate performance of solid digestate (T1) suggests slower nutrient release and limited bioavailability compared to frass. However, when combined with frass (T3), the synergistic effects were evident, leading to increased root and shoot biomass. Treatments dominated by NPK (T6 and T7) produced high shoot biomass but lower root-to-shoot ratios, indicating that while mineral fertilizers support aboveground growth, organic amendments are critical for root proliferation and overall plant health. The results clearly demonstrate that treatments T6 and T7, enriched with BSF-mediated organic waste-based biofertilizer, significantly enhanced both chlorophyll content and photosynthetic efficiency. This can be attributed to the biofertilizer's ability to deliver readily available nutrients, such as nitrogen, phosphorus, and potassium, as well as secondary metabolites and plant growth-promoting substances, such as amino acids and humic acids. These components likely stimulated chlorophyll biosynthesis and optimized photosystem II activity, leading to improved plant health and growth.

The lower performance of treatments T1, T2, and T3 suggests that either the nutrient availability was limited, or the absence of biofertilizer led to suboptimal soil microbial activity and nutrient cycling. Additionally, the prolonged efficacy of biofertilizer-enriched treatments highlights the potential of BSF-mediated biofertilizer as a sustainable and eco-friendly alternative to conventional fertilizers. The results of macronutrient and micronutrient analyses revealed varying concentrations of essential nutrients across treatments, highlighting the influence of different treatments on nutrient uptake. Nitrogen, phosphorus, potassium, and other macronutrients, as well as micronutrients like copper, iron, and zinc, showed significant differences, indicating that certain treatments enhanced nutrient availability or absorption in the plants. The observed patterns suggest that higher nutrient concentrations, particularly in treatments such as T6, may be linked to the efficacy of specific nutrient management practices or soil amendments used. These findings underscore the importance of optimizing nutrient management strategies for improving plant growth and development, with implications for sustainable agricultural practices. Further studies could explore the underlying mechanisms driving these nutrient variations to better understand their role in plant health and productivity.

5. Conclusion

This study highlights the efficacy of black soldier fly frass as a food waste-derived biofertilizer that enhances plant growth and development. The synergistic effects observed with frass and NPK combinations underscore the importance of integrating organic and inorganic amendments to achieve optimal plant performance. Frass not only contributes to nutrient supply but also improves soil properties and root development, making it a sustainable solution for modern agriculture. Treatments **T6** and **T7**, enriched with BSF biofertilizer, consistently showed superior performance in both parameters at 21 and 30 Days After Treatment (DAT). These treatments enhanced chlorophyll biosynthesis and photosynthetic efficiency, indicating improved plant health and effective stress mitigation. In contrast, treatments without biofertilizer (T1, T2, T3) exhibited significantly lower performance.

The findings demonstrate that BSF-mediated biofertilizer effectively enhances plant physiological traits by delivering essential nutrients and promoting photosynthetic efficiency. As an eco-friendly alternative to synthetic fertilizers, this biofertilizer aligns with sustainable agriculture practices. Future studies on large-scale applications and long-term effects will further establish its potential in integrated crop management systems. The analysis of macronutrients and micronutrients in plant samples revealed significant differences in nutrient content across treatments. High concentrations of essential nutrients such as nitrogen, phosphorus, potassium, and micronutrients like iron and zinc were observed in treatments with enhanced nutrient management. These findings highlight the impact of treatment variations on nutrient uptake, suggesting potential strategies for optimizing plant nutrition and improving crop health.

References

Abdelfattah, E. A., Abd El-Monem, D. H., Mahmoud, A. H., Aboelhassan, A. M., Ibrahim, N. Y., Abdallah, D. B., ... & Abdelhamid, H. A. (2021). The various vulnerable products and services from organic waste management using Black soldier fly, Hermetia illucens. Zool. Entomol. Lett, 1(1), 44-56.

Ayilara, M. S., Olanrewaju, O. S., Babalola, O. O., & Odeyemi, O. (2020). Waste management through composting: Challenges and potentials. Sustainability, 12(11), 4456.

Chen, T., Zhang, S., Yuan, Z., 2020. Adoption of solid organic waste composting products: a critical review. J. Clean. Prod. 272, 122712

Dijkstra, F. A., Prior, S. A., Runion, G. B., Torbert, H. A., Tian, H., Lu, C., & Venterea, R. T. (2012). Effects of elevated carbon dioxide and increased temperature on methane and nitrous oxide fluxes: evidence from field experiments. Frontiers in Ecology and the Environment, 10(10), 520-527.

Guo, H., Wu, S., Tian, Y., Zhang, J., Liu, H., 2021. Application of machine learning methods for the prediction of organic solid waste treatment and recycling processes: a review. Bioresour. Technol. 319, 124114

Kaszycki, P., Głodniok, M., & Petryszak, P. (2021). Towards a bio-based circular economy in organic waste management and wastewater treatment–The Polish perspective. New Biotechnology, 61, 80-89.

Kilpeläinen, M., & Happonen, A. (2021). Awareness adds to knowledge. stage of the art waste processing facilities and industrial waste treatment development. Curr Approaches Sci Technol, 4, 125-148.

Malhotra, M., Aboudi, K., Pisharody, L., Singh, A., Banu, J. R., Bhatia, S. K., ... & Tyagi, V. K. (2022). Biorefinery of anaerobic digestate in a circular bioeconomy: Opportunities, challenges and perspectives. Renewable and Sustainable Energy Reviews, 166, 112642.

Ranjbari, M., Esfandabadi, Z. S., Quatraro, F., Vatanparast, H., Lam, S. S., Aghbashlo, M., & Tabatabaei, M. (2022). Biomass and organic waste potentials towards implementing circular bioeconomy platforms: A systematic bibliometric analysis. Fuel, 318, 123585.

Ravi, H. K., Degrou, A., Costil, J., Trespeuch, C., Chemat, F., & Vian, M. A. (2020). Larvae mediated valorization of industrial, agriculture and food wastes: Biorefinery concept through bioconversion, processes, procedures, and products. Processes, 8(7), 857.

Surendra, K.C., Tomberlin, J.K., van Huis, A., Cammack, J.A., Heckmann, L.L., Khanal, S. K., 2020. Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (Hermetia illucens (L.)) (Diptera: stratiomyidae) (BSF). Waste Manag. 117, 58–80.

Wainaina, S., Awasthi, M.K., Sarsaiya, S., Chen, H., Singh, E., Kumar, A., Ravindran, B., Awasthi, S.K., Liu, T., Duan, Y., Kumar, S., Zhang, Z., Taherzadeh, M.J., 2020. Resource recovery and circular economy from organic solid waste using aerobic and anaerobic digestion technologies. Bioresour. Technol. 301, 122778

Wang, Y., Dong, H., Zhu, Z., Gerber, P. J., Xin, H., Smith, P., ... & Chadwick, D. (2017). Mitigating greenhouse gas and ammonia emissions from swine manure management: a system analysis. Environmental science & technology, 51(8), 4503-4511.

Zanellati, A., Spina, F., Rollé, L., Varese, G. C., & Dinuccio, E. (2020). Fungal pretreatments on non-sterile solid digestate to enhance methane yield and the sustainability of anaerobic digestion. Sustainability, 12(20), 8549.

Financial Expenditure

Project Title: *Organic waste-based Biofertilizer mediated by Black Soldier Fly: Assessing Bio-stimulatory Action for Plant Growth and Development*

Item/Category	**Cost (BDT)**	**Details**
Personnel	**40,000**	Includes wages for researchers, assistants, and lab technicians.
Materials and Supplies	**80,000**	- BSF larvae procurement and maintenance - Solid digestate collection and preparation - NPK fertilizer purchase
Net house Facilities	**45,000**	- Greenhouse rental - Utilities (water, electricity)
Experimental Setup	**40,000**	- Pots, soil, sand, and aeration materials - Instruments for plant growth measurement
Laboratory Analysis	**60,000**	- Sample analysis for plant biomass, nutrients, and soil health
Transportation	**10,000**	Transportation of organic waste, BSF larvae, and solid digestate.
Contingency	**18,000**	Unforeseen expenses, e.g., equipment repair or replacement.
Miscellaneous	**7,000**	Miscellaneous costs (stationery, report printing, etc.).
Total	**300,000**	

Notes:

1. Personnel costs include compensation for assistants and technicians for the project duration.
2. Materials and supplies cover essential items for experimentation and treatments.
3. Laboratory analysis costs ensure testing of nutrient and plant parameters.