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Effects of Black Soldier Fly Frass on *Brassica campestris* Growth, Biochemical Composition and Soil Quality

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Abstract

Excessive use of chemical fertilizers can lead to environmental pollution and declining soil health. Black Soldier Fly (BSF) frass is a potential organic alternative due to its nutrient content and soil-enriching properties. Due to limited information, this study was conducted to evaluate the effects of different amounts of BSF frass on *B. campestris* morphological and biochemical composition, as well as the soil properties. Six treatments were applied, including the control (T1); NPK fertilizer (T2); 2g of BSF frass (T3); 5g of BSF frass (T4); 10g of BSF frass (T5); and 5g of BSF frass + 5g of NPK fertilizer (T6) per polybag in the greenhouse. The results showed that T5 significantly enhanced plant height (25 ± 1.79cm), leaf number (9.6 ± 1.03), and fresh weight (15.47 ± 2.67g). For biochemical composition, T3 gave the highest chlorophyll content (35.18 ± 1.18), while T6 led to the highest Total Phenolic Content (2.04 ± 0.15 mg GAE/g DW) and protein concentration in leaves (23.61 ± 1.2 mg BSAE/g FW). Our findings suggest that the addition of BSF frass to the soil can effectively improve the growth and quality of *B. campestris*, supporting its potential use as a sustainable organic fertilizer in agriculture.

Keywords: Organic farming; Black Soldier Fly Frass; Plant Growth; Biochemical Properties; Soil Characteristics

Introduction

According to Kopittke et al. (2019), the amount and quality of food and crops produced have significantly decreased due to the degradation of nearly 33% of the world's soils. This degradation is caused by unsustainable agricultural practices, including the overuse of chemical fertilizers, which often result in soil nutrient depletion, reduced fertility, and long-term environmental damage.

To mitigate the negative impacts of chemical fertilizers on soil health and the broader environment, there is a growing interest in sustainable and eco-friendly agricultural practices, such as organic amendments. Among these, Black Soldier Fly (BSF) frass is becoming one of the most efficient organic fertilizers. Studies conducted by Gärttling and Schulz (2022) highlight that BSF frass is an excellent source of essential nutrients, including nitrogen, phosphorus, and potassium, as well as organic matter that supports soil health and plant growth.

This study aimed to evaluate the potential of BSF frass as a sustainable alternative for enhancing plant morphological properties and improving plant biochemical composition. At the same time, the effectiveness of BSF frass in improving soil health was determined by monitoring soil pH and electrical conductivity. In this research, we investigated the response of the rapidly growing plant Brassica campestris (*B. campestris*) to varying quantities of BSF frass. This is a commonly cultivated vegetable in Malaysia with various nutritional values and health benefits.

This research offers valuable insights into sustainable agricultural practices, particularly by promoting the use of insect-derived frass as an alternative to synthetic fertilizers. In the long term, this approach could help address critical challenges related to soil degradation, chemical dependency, and environmental sustainability.

Materials and methods

Plant Growth Experiment

B. campestris seeds were purchased from a local store and sown in germination trays filled with peatmoss. After two weeks, the seedlings were transplanted into polybags (12 × 12 inches) filled with a total weight of 3 kg of soil. Plants were arranged in a Completely Randomized Design (CRD) and grown in the greenhouse at the Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, Skudai, Johor. The BSF frass used in this study was purchased from Perkhidmatan Agro Pintar Sdn. Bhd., and the commercial NPK (15:15:15) fertilizer from YARA was used as a control chemical fertilizer treatment. The experiment consists of six treatments as described in Table 1:

Table 1 Fertilizer amount applied to *Brassica campestris*

Treatment	Medium composition	BSF frass amount
T1	No fertilizer	0
T2	NPK fertilizer (5g)	0
T3	BSF frass	2g
T4	BSF frass	5g
T5	BSF frass	10g
T6	BSF frass + NPK	BSF 5g + NPK 5g

During growth, the height of the plants was measured from the soil level to the topmost leaf's apex using a meter ruler, and the number of leaves was determined. Once harvested, the plant's fresh weight was determined immediately using an analytical balance to prevent water loss (Agustiyani et al., 2021). At the same time, root length was measured using a ruler.

Soil Analysis

The soil pH and EC value were taken and recorded every 7 days using a pH meter (Takemura DM-15, Japan) and an EC meter (Hanna Instruments® GrolineLine Soil Test™, USA), respectively.

Total Protein Extraction and Estimation of Protein Content in *B. campestris*

Total protein extraction was conducted using a modified method (Sarkar et al., 2020). Briefly, 1g of finely chopped fresh leaves and roots was placed in a mortar and pestle. The mixture was ground using liquid nitrogen until a thick paste was produced. The paste was collected in a 2 ml microcentrifuge tube. Next, 1 mL of protein extraction buffer containing 10 mM Tris-HCl (pH 8.1), 10 mM EDTA (pH 8.0), 5 mM β-mercaptoethanol, and 0.1 mg/mL of PMSF was added to the paste. Then the solutions were centrifuged at 12000 rpm for 20 min at 4°C. The final supernatants were collected into another 1.5ml microcentrifuge tube and stored at -20°C prior to use. Five µL of protein extract were mixed with 250µL of Bradford reagent, and the samples were incubated at room temperature for 5 minutes. Subsequently, the absorbance was determined using SPECTROstar Nano at 595 nm. A standard calibration curve using a standard solution of Bovine Serum Albumin (BSA), ranging from 20 to 100 µL of BSA (2 mg/mL), was used for protein estimation.

Chlorophyll Content in *B. campestris* Leaves

A Konica Minolta SPAD meter was used to measure the amount of chlorophyll in the leaves, and it was calibrated before use. Measurements were taken from the first, second and third leaves by placing the SPAD meter probe on the leaf surface, ensuring proper contact and avoiding areas with damage, major veins, or dirt.

Preparation of Aqueous Extract and Estimation of Total Phenolic Content (TPC)

Fresh *B. campestris* samples were collected, thoroughly rinsed with distilled water to remove surface impurities and oven-dried for 24 hours at 60°C (Chai and Wong, 2012). The dried leaves were crushed into a fine powder using a mortar and pestle and stored in a zip-lock bag at 4°C until further analysis.

The dried powder was extracted with deionized water at a 1:10 (dry weight: volume) ratio at 80°C for 4 hours. The heat-incubated homogenate was syringe-filtered, and the filtrate was centrifuged at 9000 rpm and 4°C for 15 min. The resulting supernatant was immediately collected and stored at -20°C for subsequent analysis.

0.2 mL of the sample extract, 0.8 mL of distilled water, and 0.1 mL of Folin-Ciocalteu reagent were added, and the mixture was incubated at room temperature for 3 min. Next, 0.3 mL of 20% sodium carbonate (Na_2CO_3) was added, and the mixture was incubated in the dark for 120 min. The absorbance of the mixture was read at 765 nm using a SPECTROstar Nano. TPC was determined using a standard calibration curve prepared from gallic acid (GA) solutions ranging from 0 to 100 mg/L and expressed as mg GAE/g of dried sample.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) for Analysis of Variance (ANOVA), followed by the Post Hoc Duncan Multiple Range Test (DMRT) at a 0.05 probability level. The results were expressed as mean ± standard error.

Results and discussion

Plant Growth Parameters

B. campestris supplied with fertilizer were significantly taller than the control throughout the experiment (Figure 1). At the fifth week, *B. campestris* in T5 (10g BSF frass) exhibited the greatest height with 25 ± 1.79cm, followed by T3 (24.4 ± 1.5cm) and T4 (24 ± 1.48cm), surpassing T2 treatment (23.9 ± 1.5cm), while T1 treatment (19 ± 0.45cm) resulted in the shortest plants. With a notable increase of 18 cm in plant height, T5 showed the most significant improvement, while T1 had the least amount of increase in plant height. Besides, there was no significant difference ($p > 0.05$) between all treatments during the first week after transplanting. However, two weeks after the fertilizer's application, there was a significantly different height ($p < 0.05$) between T2, T3, and T5, with T4 and T6, or T1, remaining unchanged until the end of the planting. While in week 3, T1 was significantly different from other treatments until the last week of the planting season. In this study, when treated plants were treated with a high amount of BSF frass, which is 10g in T5, it increased the plant height.

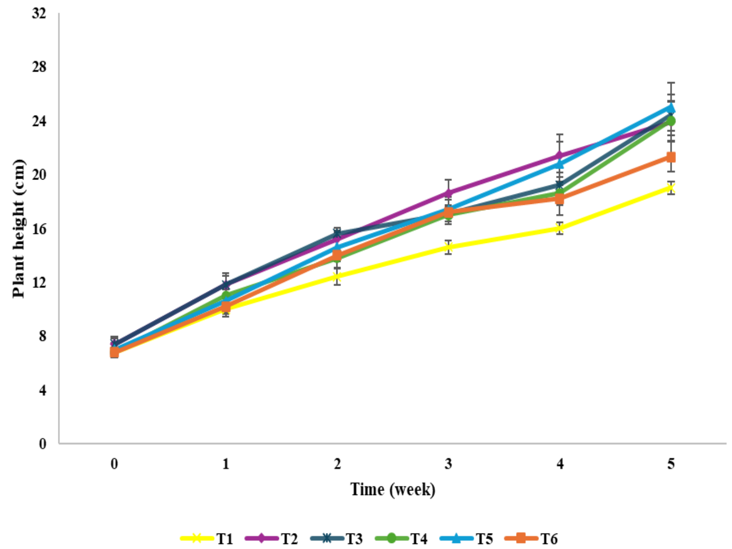

Figure 1 Plant height of *B. campestris* after treatment with different treatments. T1 = control; T2 = NPK fertilizer; T3 = 2g BSF frass; T4 = 5g BSF frass; T5 = 10g BSF frass; T6 = 5g BSF frass and 5g NPK fertilizer. Data was expressed as mean ± standard error (SE), n = 5

Figure 2 shows the number of leaves of *B. campestris* that have significantly increased in all fertilizer treatments after two weeks of transplanting. Plants treated with fertilizer treatments (T2, T3, T4, T5 and T6) produced more leaves than the control (T1). They are continuously increased better than control (T1) until week 5, except for T3 (9.2 ± 0.37). At week 5, plants treated with 5g BSF frass (T5) had a similar number of leaves as the NPK control (T2), with 10 leaves. This suggests that a moderate amount of BSF frass gave a similar response to the growth of leaves. Despite showing an increasing trend in the number of leaves, the treatments did not result in statistically significant differences among the groups throughout the study ($p > 0.05$).

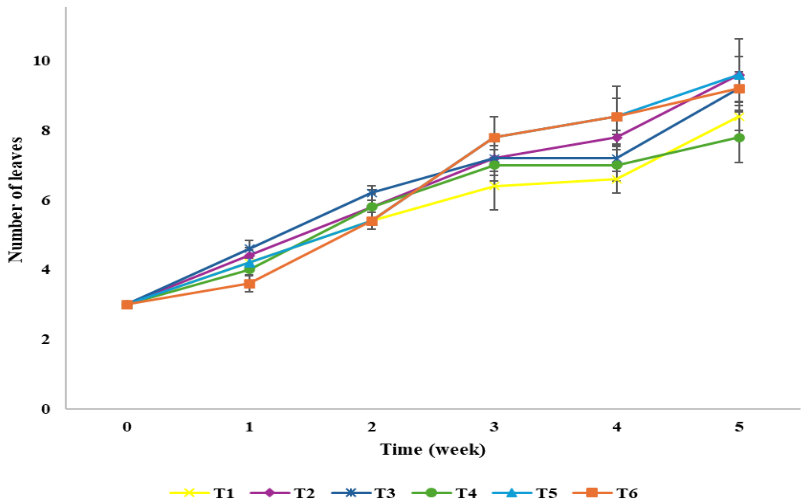

Figure 2 Number of *B. campestris* leaves for 5 weeks after transplanting in each treatment. T1 = control; T2 = NPK fertilizer; T3 = 2g BSF frass; T4 =5g BSF frass; T5 = 10g BSF frass; T6 = 5g BSF frass and 5g NPK fertilizer. Data were expressed as mean ± standard error (SE), n = 5.

The effects of different fertilizer treatments on the root length and fresh weight of B. campestris are shown in Table 2. Among all treatments, plants treated with NPK fertilizer (T2) had the shortest root length (7.8 ± 0.58cm), which is significantly lower ($p < 0.05$) compared to other treatments. In contrast, T6 exhibits the highest root length, at 12.1 ± 1.42 cm, followed by T3 (11 ± 1.23cm) and T5 (10.6 ± 0.62 cm). Nevertheless, there is no statistically significant difference in root length between the three treatments. Based on the result, the NPK fertilizer treatment (T2) resulted in significantly shorter roots, potentially due to nutrient sufficiency that reduces the need for extensive root elongation. In contrast, BSF frass treatment (T3, T5) and the combination treatment (T6) showed root lengths statistically similar but different from the control group (T1). This suggests that BSF frass is effective in promoting root elongation, likely due to its high organic matter content, slow-release nutrients, and ability to enhance soil structure. The combination treatment (T6) yielded the highest root length, suggesting a possible synergistic effect between organic and inorganic inputs (Anyega et al., 2021).

In addition, the highest fresh weights were recorded in plants subjected to T5, with values of 15.47 ± 2.67g. This was followed by plants under T6, which exhibited greater fresh weights for the whole plant (15.34 ± 1.33g) compared to T2, which recorded 13.12 ± 1.55g. However, leaf fresh weight under T4 and T3 was slightly lower than that of T2, with 9.4 ± 1.79g and 9.4 ± 1.15g, respectively. The lowest fresh weights were observed in plants treated with T1, with the whole plant weighing 5.61 ± 0.74g. Statistical analysis revealed that the effect of fertilizer treatment on the fresh weight of the whole plant was significant ($p < 0.05$). Notably, the fresh weights of T1 differed significantly from other treatments. However, no statistically significant differences ($p > 0.05$) were observed between T5 and T6 for the fresh weights of the whole plant; however, a significant difference was observed between T3 and T4 ($p < 0.05$). The highest fresh weights of the whole plants of *B. campestris* were observed in plants treated with 10g BSF frass and a combination of BSF frass and NPK fertilizer, with a minor difference. This is likely attributed to additive or synergistic effects arising from the integrated use of organic and chemical fertilizers (Anyega et al., 2021). These findings are consistent with previous studies, which report that the combined application of BSF frass or animal manure with chemical fertilizers yields the highest crop performance compared to the application of either fertilizer alone (Agustiyani et al., 2021; Anyega et al., 2021). This is also linked to enhanced soil physical properties, which increase the soil's capacity to supply both macro- and micronutrients, promoting better root development and efficient nutrient absorption, and contributing to increased biomass accumulation (Fageria & Moreira, 2011). In addition, the observed increases in plant height and root length, and leaf area may have promoted greater photosynthetic efficiency and assimilate production, leading to enhanced fresh weight in *B. Campestris*.

Table 2: Effects of different fertilizer treatments on root length and fresh weight of B. campestris after harvest in mean ± S.E. (n=5).

Plant Growth	T1	T2	T3	T4	T5	T6
Root Length (cm)	10.4±0.24ab	7.8±0.58b	11±1.23a	10.4±0.4ab	10.6±0.62a	12.1±1.42a
Fresh Weight (g)	5.61±0.74c	13.12±1.55ab	9.4±1.15bc	9.4±1.79bc	15.47±2.67a	15.34±1.33a

Note: T1 = Control; T2 = NPK control; T3 = BSF frass (2g); T4 = BSF frass (5g); T5 = BSF frass (10g); T6 = BSF frass (5g) + NPK (5g). Means followed by the different letters in column, according to Duncan's multiple range test, are significantly different ($p < 0.05$).

Soil Properties

Soil pH and EC were moderately influenced by the different treatment applications (Figures 3 and 4). From the first week to second week, soil pH was significantly increased across all treatment, however after the third week of transplanting, *B. campestris* treated with NPK fertilizer (T2) at 6.28 ± 0.16, 2g of BSF frass (T3) at 6.36 ± 0.13 and 10g BSF frass (T5) at 6.12 ± 0.21 exhibited a decrease in soil pH while increasing in control (T1) with 6.76 ± 0.07, 5g of BSF frass (T4) at 6.72 ± 0.08 and mixed fertilizers (T6) with pH number of 5.72 ± 0.31. T6 was continuously increased but fluctuated at week 5, whereas the other fertilizer treatments fluctuated. Statistical analysis showed that the pH value was only significant ($p < 0.05$) during weeks 2 and 3 after fertilizer application. Based on the results, soil pH reduction in BSF frass is primarily caused by the production of organic acids in soils, such as glycine, humic acid, and amino acids, during decomposition. The results are similar to the study published by Ullah et al. (2008). The release of these organic acids into the soils increases the hydrogen ion concentration, decreasing the soil pH (Angelova et al., 2013). The significant differences in soil pH during the first two weeks after fertilizer application are likely due to the immediate chemical interactions between the fertilizers and soil. During this early stage, nutrients are rapidly released and react with soil components, causing noticeable changes in pH. However, by the third and fourth weeks, the soil pH begins to stabilize. This stabilization may be attributed to the soil's natural buffering capacity, which helps maintain pH within a certain range despite external inputs (Jeong et al., 2016). Moreover, as plants absorb ammonium and nitrate over time, their influence on soil acidity diminishes (Hirzel et al., 2018; Vašák et al., 2015). The gradual breakdown of organic fertilizers, such as BSF frass, also results in slower nutrient release, contributing to a more moderate and steady effect on pH over time.

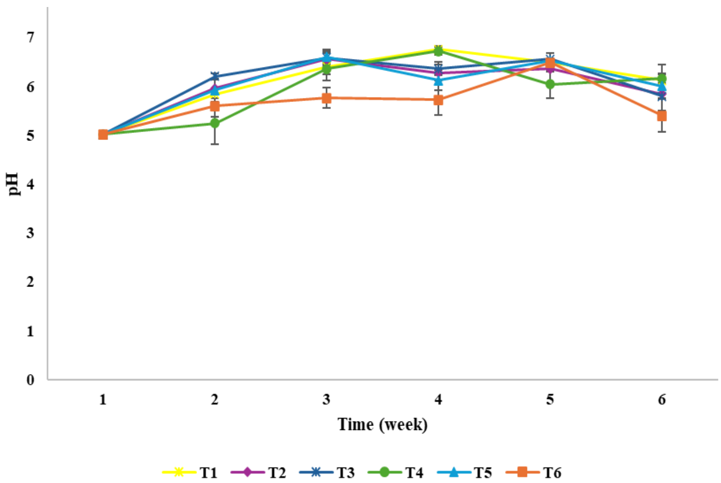

Figure 3 The effect of different treatments on soil pH value after the fifth week. T1 control; T2 NPK fertilizer; T3 = 2g BSF frass; T4 = 5g BSF frass; T5 = 10g BSF frass; T6 = 5g BSF frass and 5g NPK fertilizer. Data were expressed as mean ± standard error (SE), n = 5.

In contrast, the electrical conductivity (EC) of the soils decreased in all treatments throughout the study period (Figure 4). However, during week 3, T1 at 0.8 ± 0.06, T2 (0.83 ± 0.1), T3 (0.82 ± 0.07), and T4 with 0.83 ± 0.02 were increased, then fluctuated back by week 4 and remained stable until the end of the experiment. There was no significant difference ($p > 0.05$) observed between treatments until week 2; however, significant differences were observed at $p < 0.05$ from week 3 until the end of the experiment. Moreover, the electrical conductivity (EC) values showed significant differences among fertilizer treatments starting from week 3 to week 5, despite a general decreasing trend over time. This pattern is likely due to the delayed release and accumulation of soluble salts from both chemical and organic fertilizers. In the early weeks, EC values were relatively low and similar across treatments as the nutrients had not yet fully dissolved or mineralized (Saragih et al.). By week 3, as nutrient release intensified and ions accumulated in the soil solution, EC levels increased, leading to noticeable differences among treatments. However, according to Vašák et al. (2015), plants absorb these nutrients for growth, and some ions may have leached into deeper soil layers over time due to watering. Additionally, microbial activity and nutrient stabilization further reduced EC levels. Thus, although EC decreased as the experiment progressed, the variations remained statistically significant due to differences in fertilizer composition, nutrient release rates, and plant uptake (Angelova et al., 2013).

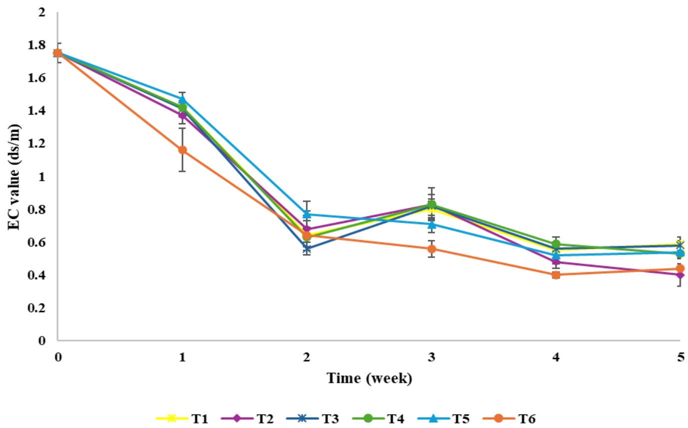

Figure 4 The effect of different treatments on soil EC value after fifth week. T1 = control; T2 = NPK fertilizer; T3 = 2g BSF frass; T4 = 5g BSF frass; T5 = 10g BSF frass; T6 = 5g BSF frass and 5g NPK fertilizer. Data was expressed as mean ± standard error (SE), n = 5

Plant Biochemical Properties

Different fertilizer treatments affected the leaf chlorophyll content of B. campestris (Figure 5). There were significant variations in the chlorophyll content over the course of five weeks between the six treatments (T1–T6), especially in the early phases. In the first week, no significant difference was observed between treatments ($p > 0.05$). As time progressed, the differences in chlorophyll content among treatments became less pronounced, with several treatments sharing the same statistical grouping, indicating no significant difference between them. This implies that the influence tended to level off over time, even though some treatments had a more immediate impact on chlorophyll production. In most weeks, treatments. like T2, T3, T5, and T6 continuously maintained higher chlorophyll content, suggesting that they promoted long-term chlorophyll accumulation. These are the same findings from Reswita et al. (2022) that use a combination treatment (30% BSF frass and 25% NPK), which produces the highest chlorophyll content in upland rice. This is due to the high nitrogen content when combining both fertilizers. On the other hand, T1 typically had lower chlorophyll levels, which suggests that it was less effective.

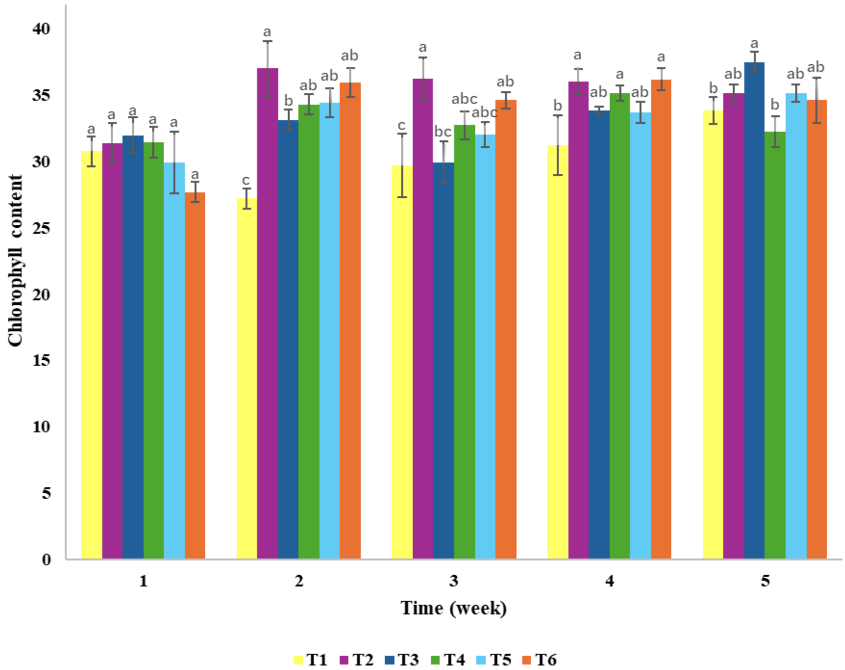

Figure 5 The effect of different treatments on chlorophyll content (SPAD value) after fifth week. Data was expressed as mean ± standard error (SE), n = 5; T1 = control; T2 = NPK fertilizer; T3 = 2g BSF frass; T4 = 5g BSF frass; T5 = 10g BSF frass; T6 = 5g BSF frass and 5g NPK fertilizer; different alphabet indicated significantly different at $P < 0.05$ using Post Hoc Duncan Multiple Range Test (DMRT).

The protein content in leaves and roots of *B. campestris* were shown in Table 3. Results revealed that T6 had the highest protein content at 23.61 ± 1.2mg BSAE/g FW, significantly higher than all other treatments. T3 (12.92 ± 1.15mg BSAE/g FW) and T4 (14.3 ± 1.06mg BSAE/g FW) had moderately high values, with no significant difference between them. T1 also showed moderate protein content with 9.76 ± 1.04 mg BSAE/g FW. T2 and T5 had the lowest protein levels, especially T2 at 4.07 ± 2.23mg BSAE/g FW, showing it is significantly lower than the others. Meanwhile, for protein content in roots showed that T3 had the highest protein content at 9.01 ± 1.96mg BSAE/g FW, followed by T2 at 5.28 ± 0.2mg BSAE/g FW. Therefore, T1, T4, T5, and T6 had similarly low values showing no significant difference between them ($p > 0.05$). The protein content in leaves is generally higher than in roots due to the distinct physiological roles each organ plays in the plant. Leaves are the primary sites of photosynthesis, a process that requires a high concentration of enzymes and proteins, particularly Rubisco, which is one of the most abundant proteins in plants. In addition, leaves contain chloroplasts, which are rich in proteins involved in capturing light energy and converting it into chemical energy. The high metabolic activity in leaves, including processes such as gas exchange, transpiration, and the synthesis of sugars and amino acids, contributes to their elevated protein content (Li et al., 2019). In contrast, roots primarily function in the absorption of water and nutrients, anchorage, and storage. These functions demand less enzymatic activity and, therefore, lower protein levels. As a result, the protein concentration in roots is generally lower than in leaves. The high protein content observed in roots under low BSF frass application (T3) is likely due to mild nutrient stress, which triggers adaptive responses in the plant. This stress condition stimulates the roots to enhance nutrient acquisition mechanisms and increase the synthesis of stress-related proteins, thereby improving their tolerance and survival (Ghosh & Xu, 2014).

The total phenolic content of *B. campestris* in response to different fertilizer treatments is shown in Table 3. Application of fertilizer improved the TPC of plants grown under T4, which displayed the least TPC while T6 resulted in maximum phenolic content with 2.04 ± 0.15 mg GAE/g DW in *B. campestris* compared to the other fertilization treatments. The TPC of *B. campestris* under different fertilizer treatments was presented in the sequence T6 > T3 > T1 > T5 > T2 > T4. Generally, there were no significant differences ($p > 0.05$) in TPC observed in *B. campestris* treated with all fertilizer treatments. Based on the TPC result, the use of BSF frass as a sole organic fertilizer resulted in a higher TPC value in *B. campestris* compared to plants grown with NPK fertilizer. This finding is consistent with previous studies on organically fertilized crops such as cassava and spinach, which demonstrated elevated phenolic compound levels (Fioroni et al., 2023). Due to its high solubility, NPK fertilizer releases nitrogen rapidly into the soil, enabling efficient nitrogen uptake by plants (Sharma & Chetani, 2017). Consequently, *B. campestris* cultivated in NPK-amended soil likely had greater access to readily available nitrogen than those grown in soil amended with BSF frass, where nitrogen is primarily in organic forms that mineralize more gradually. This slower nitrogen release from BSF frass may reduce nitrogen-induced suppression of phenolic synthesis, thereby resulting in higher TPC in plants fertilized with BSF frass. Moreover, the combined application of BSF frass and NPK fertilizer enhanced the production of TPC in *B. campestris*. This result aligns with prior research indicating that the integration of organic and inorganic fertilizers can promote higher levels of TPC in various plant species. The elevated phenolic levels observed in *B. campestris* under the combined fertilization regime may be attributed to the complementary nutrient profiles provided by BSF frass and NPK fertilizer (Anyega et al., 2021). BSF frass supplies essential nutrients, including iron, manganese, zinc, and copper, which are crucial for plant metabolic processes (Gärttling & Schulz, 2022). The frass contains chitin derived from the exoskeletons of BSF larvae, which serves as a rich nitrogen source (Basri et al., 2022; Bortolini et al., 2020). Thus, the incorporation of BSF frass may improve nutrient availability, particularly carbon and nitrogen, encouraging *B. campestris* to allocate more resources toward the biosynthesis of phenolic compounds.

Table 3 Effect of different fertilizer treatments on total protein and phenolic content in leaves and roots in mean ± S.E (n=3).

	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Protein (Leaves)	9.76 ± 1.04bc	4.07 ± 2.32d	12.29 ± 1.15bc	14.3 ± 1.06b	7.69 ± 2.24d	23.61 ± 1.2a
Protein (Root)	1.54 ± 0.64c	5.28 ± 0.2b	9.01 ± 1.96a	1.94 ± 0.66c	2.23 ± 0.81c	1.66 ± 0.26c
TPC (Leaves)	1.47 ± 0.96a	1.37 ± 0.27a	1.65 ± 0.08a	1.27 ± 0.35a	1.45 ± 0.25a	2.04 ± 0.15a

Note: T1 = Control; T2 = NPK control; T3 = BSF frass (2g); T4 = BSF frass (5g); T5 = BSF frass (10g); T6 = BSF frass (5g) + NPK (5g). Means followed by the different letters in column-according to Duncan's multiple range test, are significantly different ($p < 0.05$)

Conclusion

The application of BSF frass demonstrated notable improvements in plant growth, soil properties and biochemical properties compared to the control group. The application of 10g BSF frass (T5) resulted in the best overall plant growth performance across all four growth parameters (including plant height, number of leaves, fresh weight, and leaf number), indicating its effectiveness in promoting vegetative development. However, soil properties showed no significant differences across the treatments. In terms of biochemical properties, the combination of NPK and BSF frass yielded the highest total protein content in leaves and total phenolic content, highlighting its potential in enhancing the nutritional and antioxidant quality of the plant. These findings demonstrate that while T5 is optimal for growth, the combination treatment may be more beneficial for improving plant's biochemical profile.

Acknowledgement

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