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RESEARCH ARTICLE

Assessment of black soldier fly derived inputs and conventional fertilisers on the growth of *Amaranthus aritis* in a pot culture experiment

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Abstract

An *in vitro* study was conducted to evaluate the effects of various soil amendments on the growth performance and nutrient uptake of *Amaranthus aritis*. Five treatment conditions were evaluated, including control (Soil), soil + compost, soil + NPK, soil + compost + Black Soldier Fly (BSF) vermiwash and soil + BSF frass (15 %). Growth parameters, including crop weight, root weight, number of leaves, plant height and chlorophyll content were recorded, alongside post-harvest nitrogen, phosphorus and potassium content in both leaf and soil samples. The results revealed a significant improvement in all growth metrics with organic and integrated nutrient management treatments compared to the control. Notably, the soil + BSF frass (15 %) treatment produced the highest crop weight (16.32 g/plant), root weight (2.94 g/plant), leaf number (21/plant), plant height (54.7 cm) and chlorophyll content (4.3 mg/g FW). This treatment also yielded the highest post-harvest leaf nitrogen (4.25 %), phosphorus (0.935 %) and potassium (2.986 %) levels. These findings highlight the potential of BSF-derived amendments, particularly BSF frass, in enhancing plant growth and nutrient content, thereby offering a sustainable alternative to conventional fertilisers for leafy vegetable cultivation.

Keywords: *Amaranthus aritis*; BSF frass; nutrient uptake; soil amendment; sustainable agriculture; vermiwash

Introduction

Organic fertilisers represent a promising approach for sustainably enhancing soil health and crop productivity in modern agriculture (1, 2). They contribute to the formation of stable soil aggregates and are recognised for their role in improving soil pH, increasing soil organic matter and enriching the soil with secondary and micronutrients (3-6). Additionally, they enhance nutrient availability, uptake and efficiency of use by crops (7, 8). However, the use of organic manures remains limited, primarily due to their competing demands on farms such as being used for animal feed or as fuel for household purposes (9-11). These alternative uses often result in minimal or no organic materials being available for crop cultivation. Consequently, enhancing soil fertility through organic inputs necessitates the exploration of alternative sources of organic fertilisers. The growing demand for animal feed through large-scale insect rearing on organic materials offers a promising approach to convert organic waste into nutrient-rich fertilisers for enhancing soil fertility (12, 13).

Compost, derived from decomposed organic matter, enhances soil structure, water retention and nutrient availability while supporting beneficial microbial populations (14). When applied to soil, compost has been shown to improve plant growth parameters and soil biochemical properties (15). In addition to compost, nutrient-rich organic fertilisers derived from insects are emerging as a promising alternative to traditional organic fertilisers. The rearing of BSF larvae on various organic wastes produces a nutrient-rich frass (16-18). This frass is sufficient to suppress pathogens present in the waste and enhances waste degradation efficiency (66 %-99 %). Thus, BSF larvae serve as efficient organic cyclers and can also contribute to improving soil fertility.

Transforming BSF frass into organic fertiliser offers a rapid and effective method for recycling the nutrients contained in organic waste, thereby enhancing soil fertility. They are the potential to convert organic waste into valuable by-products such as BSF frass and vermiwash. BSF frass, the residual by-product of larval digestion, is rich in nitrogen, phosphorus, potassium and beneficial microbes, making it an effective biofertilizer (19). Similarly, BSF vermiwash, a liquid extract from BSF castings and microbial activity, contains phytohormones, micronutrients and enzymes that can promote plant growth and resistance to stress (20).

Integrating BSF-based products with conventional organic and inorganic inputs may synergistically enhance plant nutrient uptake and improve soil health. However, comparative studies assessing the efficacy of such integrated treatments, particularly BSF frass at optimal concentrations, are still limited. Therefore, the present study was conducted to evaluate the effects of different soil amendment treatments, including compost, NPK, BSF vermiwash and 15 % BSF frass, on plant nutrient content, soil fertility and overall crop performance.

Materials and Methods

Application of fertiliser for the experiment setup

The research experiment was conducted in the Agricultural College and Research Institute, Madurai. The application of various soil conditioner formulations on (*Amaranthus aritis*) grown in a pot culture mixture was conducted under controlled conditions. The compost was collected from the compost unit and mixed with a 2:1 ratio with the pot culture mixture. The NPK application was based on the fertiliser recommendation of the *Amaranthus aritis.* The BSF were reared on food, fruit and vegetable waste collected from the hostel using a plastic drum setup. The vermiwash and frass fertiliser was collected from the BSF rearing unit for the treatments (Fig. 1). A pot experiment was arranged using a completely randomised design (CRD) and the pots measured 30 × 25 cm and were filled with sandy loam soil. The temperature of the pot culture unit ranged from 28 °C-32 °C, with a relative humidity of 70 %-80 %. The experiment comprised five treatments. Seeds were sown in pots containing 10 kg of specifically formulated growth media. Five different types of growth media were prepared and used as treatment variants. The treatments and doses were fixed based on a slight modification of Agustiyani (20).

T_1 – Soil (Control)

T_2 – Soil and compost (2:1)

T_3 – Soil and chemical fertiliser NPK (equal to 10 kg/ha)

T_4 – Soil and Compost (2:1) + BSF vermiwash (100 mL/L)

T_5 – BSF Frass 15 % (300 g)

The NPK fertiliser treatment was expressed on a field-equivalent basis (10 kg/ha) in accordance with standard agronomic recommendations, while BSF frass was incorporated at 15 % (300 g pot^{-1}) on a weight basis. This distinction was made because chemical fertilisers are applied as concentrated nutrient inputs, whereas frass functions both as a nutrient source and soil conditioner; thus, a percentage incorporation better reflects its practical use and ensures uniform mixing in pot culture. The baseline nutrient composition of soil, compost, BSF frass and vermiwash is given in Table 1.

Growth parameters of *Amaranthus aritis*

The growth performance of the *Amaranthus aritis* was evaluated using a set of key parameters, which included measurements of plant height and the number of leaves per plant. After a growth period of 30-35 days from the date of sowing, the plants were carefully harvested to assess various biomass components. Specifically, the fresh weights of the plant, root system and the entire plant (total biomass) were recorded (21-23). In addition to biomass assessment, the chlorophyll content of leaves was also determined to evaluate the photosynthetic potential and physiological status of the plants.

Estimation of chlorophyll content

The chlorophyll pigments were extracted with 80 % (v/v) acetone according to the previously known procedure (24). The absorbance of the resulting extract was recorded at 663 nm and 647 nm using a spectrophotometer. Chlorophyll a and chlorophyll b concentrations were then determined individually using the corresponding calculation formulas.

$$\text{Chlorophyll a (mg/L)} = 12.25 \times A_{663} - 2.79 \times A_{647} \qquad \text{(Eqn. 1)}$$

$$\text{Chlorophyll b (mg/L)} = 21.50 \times A_{647} - 5.10 \times A_{663} \qquad \text{(Eqn. 2)}$$

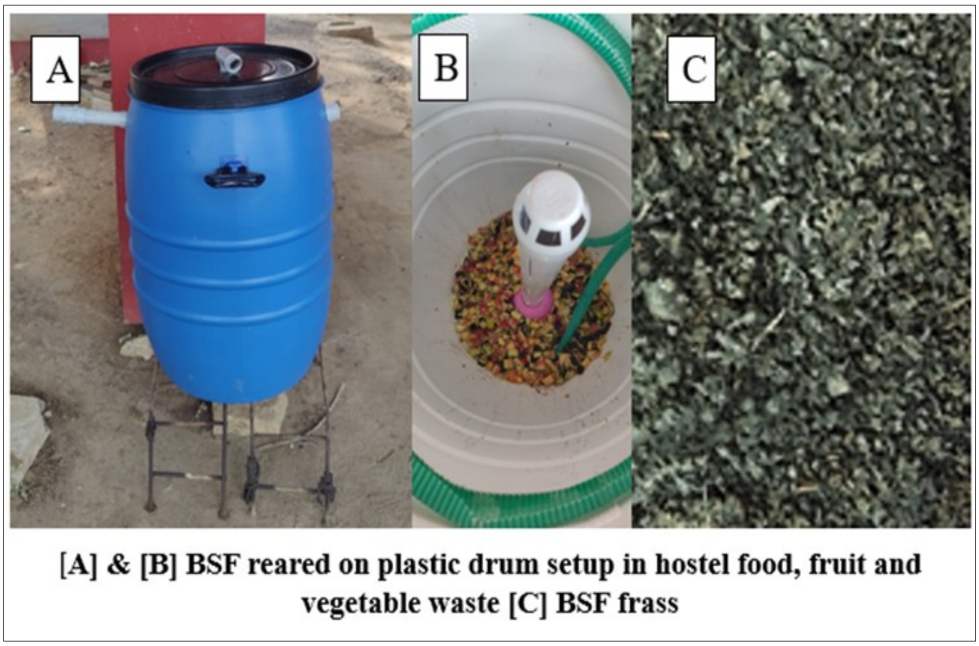

Fig. 1. Collection of BSF frass fertiliser and vermiwash from the rearing setup. A, B- BSF reared on plastic drum setup in hostel food, fruit and vegetable waste, C- BSF frass.

Table 1. Baseline nutrient composition of the soil, compost BSF frass and BSF vermiwash

S. No	Parameter	Soil	Compost	BSF frass	BSF vermiwash
1	**pH**	6.59	6.84	7.39	7.10
2	**Organic carbon (%)**	0.46	23.25	31.64	1.43
3	**Nitrogen (%)**	0.031	1.84	2.76	0.13
4	**Phosphorus (%)**	0.0005	0.83	1.63	0.04
5	**Potassium (%)**	0.011	1.24	1.73	0.18

The total chlorophyll content was then obtained as the sum of chlorophyll a and chlorophyll b:

Total chlorophyll (mg/L) = Chl a + Chl b (Eqn. 3)

Results were expressed on a fresh weight basis (mg of chlorophyll per g of leaf fresh weight).

Soil biochemical properties

Estimation of Nitrogen (N), Phosphorus (P) and Potash (K) content in soil and plants

Total nitrogen content in soil and leaf samples was estimated using the Kjeldahl digestion and distillation method (25, 26). Phosphorus (P) and potassium (K) content in leaves and soil were measured after harvest. The K and P elements were extracted by wet ashing using a mixture of concentrated acids, HNO_3 and $HClO_4$. Macro levels of K in the extract were measured using AAS (Shimadzu), while the level of P was measured using a spectrophotometer UV-Vis, Uvmini-1240 Shimadzu (27).

Statistical analysis

The experimental data, comprising five treatments with three replications each (n = 15), were analysed using AGRES software. Analysis of variance (ANOVA) was performed to determine the significance of treatment effects. Means were compared using Duncan's Multiple Range Test (DMRT) at a 5 % significance level. The critical difference (CD) values presented indicate the minimum difference required between treatment means to be considered statistically significant.

Results and Discussion

Growth performance of the *Amaranthus*

The growth performance of the test crop under different soil amendment treatments showed considerable variation in morphological parameters such as crop weight, root weight, number of leaves and plant height (Table 2, Fig. 2).

The highest crop weight (16.32 g) was recorded in the treatment receiving soil + BSF frass 15 %, followed by soil + compost + BSF vermiwash (13.78 g). The control exhibited the lowest crop biomass of 4.56 g/plant. Similarly, root weight increased significantly with organic inputs, with BSF frass 15 % again leading at 2.94 g, indicating a robust root system favourable for better nutrient uptake. A progressive increase was also observed in leaf number and plant height, with a maximum of 21 leaves/plant and 54.7 cm plant height in BSF frass 15 % treatment, followed by 18 leaves and 46.4 cm height in BSF vermiwash amended compost treatment. The control registered only 8 leaves and 8.9 cm plant height, highlighting the poor growth under nutrient-deficient conditions.

These results clearly indicate the potential of BSF frass as an effective organic fertiliser. The presence of rich organic matter, macro- and micronutrients, beneficial microbes and growth-promoting compounds such as chitin in BSF frass significantly enhances plant growth and root development (28, 29). The improved performance in the BSF vermiwash treatment further supports the synergistic effect of liquid biofertilizers when combined with compost, which improves nutrient solubilization and microbial activity in the rhizosphere (30).

Table 2. Growth parameters of the *Amaranthus aritis*

S. No	Treatments	Crop weight (Grams/plant)	Root weight (Grams/plant)	Number of leaf /plants	Plant height (cm)
1	Control (Soil)	4.56 ± 0.03	0.29 ± 0.00	8 ± 0.11	8.9 ± 0.11
2	Soil + compost	9.52 ± 0.02	1.23 ± 0.01	11 ± 0.11	16.5 ± 0.17
3	Soil + NPK	12.45 ± 0.029	1.57 ± 0.01	14 ± 0.14	21.9 ± 0.14
4	Soil + Compost + BSF vermiwash	13.78 ± 0.03	2.06 ± 0.01	18 ± 0.24	46.4 ± 0.20
5	Soil + BSF frass 15 %	16.32 ± 0.02	2.94 ± 0.02	21 ± 0.23	54.7 ± 0.23
	S.Ed	0.041	0.022	0.252	0.242
	CD (0.05)	0.091	0.069	0.561	0.539

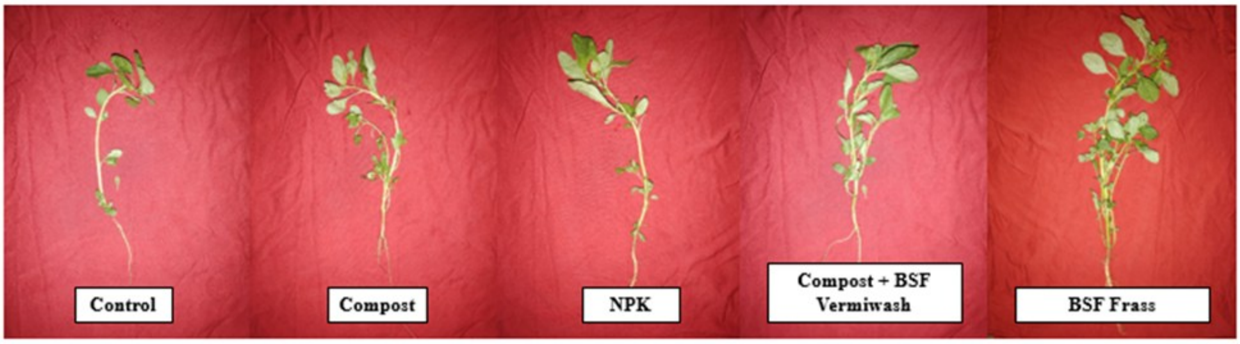

Fig. 2. Effect of *Amaranthus aritis* on black soldier fly derived inputs and conventional fertilisers.

Moreover, the application of insect-based organic fertilisers is shown to improve soil structure, nutrient retention and enzymatic activity, thereby creating a favourable environment for plant growth (31). Compared to synthetic NPK fertilisers, BSF-derived amendments not only sustained higher yields but also contributed to long-term soil fertility and sustainability. These findings reinforce the use of BSF frass at 15 % as an excellent organic amendment, potentially replacing or supplementing conventional chemical fertilisers for sustainably improving crop productivity (Fig. 3).

The chlorophyll content in *Amaranthus aritis* leaves increased with different treatments. The control had the lowest chlorophyll level at 1.8 mg/g fresh weight, showing poor nutrient availability. When compost was added, chlorophyll rose to 2.3 mg/g, showing that organic matter helped improve plant nutrition. The use of NPK fertiliser further increased it to 2.8 mg/g, since nitrogen plays a key role in chlorophyll formation (32). A bigger increase was seen with the combination of compost and BSF vermiwash (3.6 mg/g), likely due to the presence of nutrients and beneficial microbes that help plants absorb nutrients better. The highest chlorophyll content (4.3 mg/g) was recorded in the treatment with 15 % BSF frass. This shows that BSF frass, which contains nitrogen, phosphorus, potassium and helpful microbes, is highly effective in improving chlorophyll and plant growth (28, 29). These results match with earlier studies showing that using organic fertilisers, especially insect-based ones like BSF frass, can significantly increase chlorophyll levels and support better plant health (33) (Table 3, Fig. 4).

Effect of different soil amendments on the macro nutrients in *Amaranthus* leaves and soil

The study evaluates the impact of various soil amendments on the macronutrient content, specifically nitrogen (N), phosphorus (P) and potassium (K) in both the leaves and soil of *Amaranthus aritis* was shown in Table 4.

Nitrogen content

The lowest nitrogen content in leaves was recorded in the control (soil only) treatment (2.53 %), which represents the baseline nitrogen availability from unamended soil. The addition of compost (Treatment 2) marginally increased the leaf nitrogen to 2.74 %, likely due to the slow mineralisation of organic nitrogen compounds present in compost (34) (Table 4).

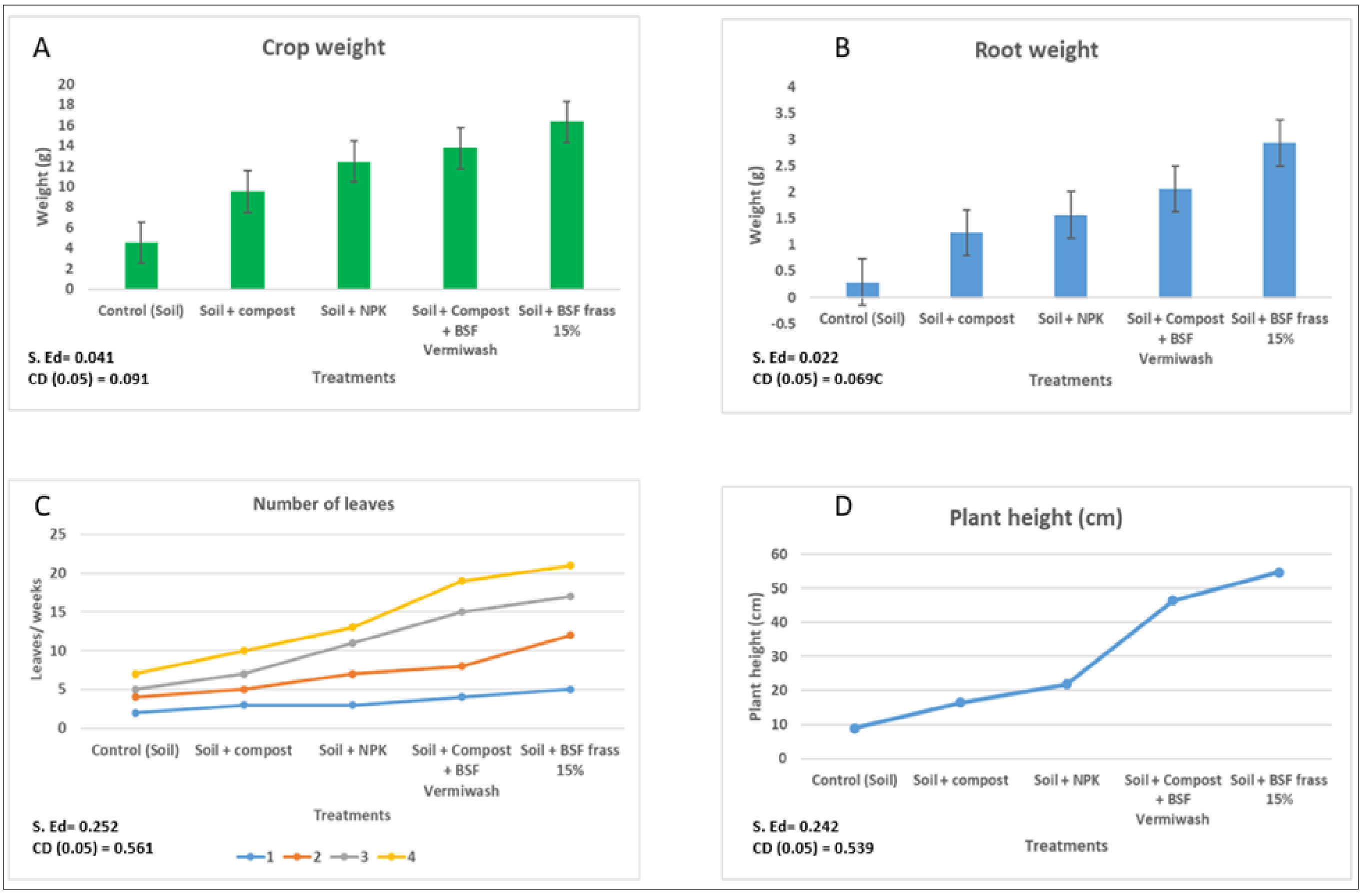

Fig. 3. Growth parameters of the *Amaranthus aritis.* A. Crop weight, B. Root weight, C. Number of leaves, D. Plant height.

Table 3. Estimation of chlorophyll content in *Amaranthus aritis*

S. No	Treatments	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total Chlorophyll content (mg/g FW)
1	Control (Soil)	1.29	0.51	1.8
2	Soil + compost	1.64	0.66	2.3
3	Soil + NPK	2.00	0.80	2.8
4	Soil + compost + BSF vermiwash	2.57	1.03	3.6
5	Soil + BSF frass 15 %	3.07	1.23	4.3
	S.Ed	0.162	0.059	0.133
	CD (0.05)	0.361	0.131	0.296

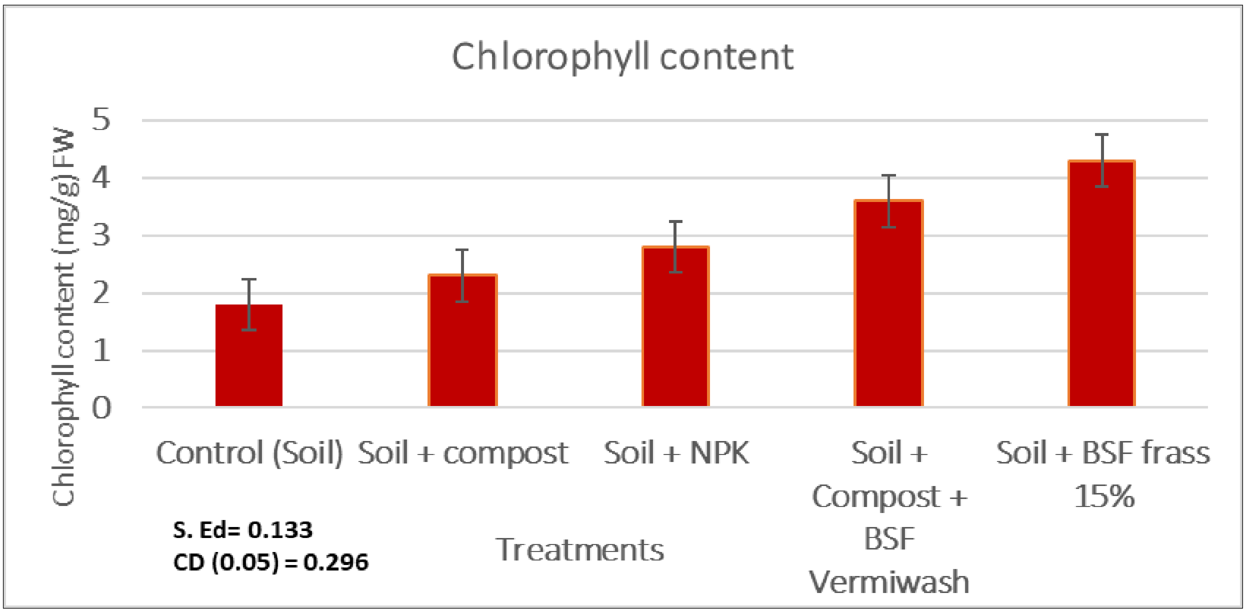

Fig. 4. Chlorophyll content of 4th week after sowing.

Table 4. Nitrogen, phosphorus and potassium content at leaf and soil on each treatment after harvesting

S. No	Treatments	Nitrogen		Phosphorus		Potash	
		Leaf	Soil	Leaf	Soil	Leaf	Soil
1	Control (Soil)	2.53	1.98	0.254	0.049	1.230	0.071
2	Soil + compost	2.74	2.10	0.812	0.074	2.453	0.294
3	Soil + NPK	3.39	2.75	0.716	0.048	1.723	0.156
4	Soil + Compost + BSF Vermiwash	3.74	3.23	0.874	0.056	1.954	0.233
5	Soil + BSF frass 15 %	4.25	3.50	0.935	0.069	2.986	0.267
	S. Ed	0.129	1.219	0.003	0.001	0.009	0.002
	CD (0.05)	0.289	2.716	0.007	0.003	0.020	0.004

Application of inorganic NPK fertilisers substantially improved leaf nitrogen content to 3.39 %, indicating enhanced nitrogen availability and plant uptake. However, even higher leaf nitrogen was observed in the treatment combining compost and vermiwash (3.74 %), showing the synergistic effect of organic matter and bioactive compounds present in BSF vermiwash (35, 36) (Fig. 5). The highest nitrogen concentration in leaves (4.25 %) was found in Treatment 5 (soil + BSF frass 15 %). BSF frass is rich in readily available nitrogenous compounds, microbial biomass and plant growth-promoting factors, which contribute to efficient nitrogen uptake by the plant (29, 37). Soil nitrogen also followed a similar trend. Control plots had the lowest soil nitrogen (1.98 %), whereas soil amended with compost alone showed a slight increase (2.10 %). The NPK-treated plots recorded a further increase (2.75 %) due to the direct supply of mineral nitrogen. Significantly higher nitrogen levels were observed in treatments receiving organic amendments in combination with BSF inputs. Soil + compost + BSF vermiwash showed 3.23 % soil nitrogen, while soil + BSF frass 15 % recorded the highest at 3.50 %. These values emphasise the role of BSF-derived inputs in improving soil nitrogen through both direct nutrient enrichment and enhancement of microbial activity, which contributes to nitrogen cycling and mineralisation (31) (Fig. 6).

Phosphorus content

The phosphorus concentration in *Amaranthus aritis* leaves varied significantly across the treatments. The highest leaf phosphorus content was observed in soil + BSF frass 15 % (0.935 %), followed by soil + compost + BSF vermiwash (0.874 %) and soil + compost (0.812 %) (Table 4). The control treatment (soil alone) recorded the lowest phosphorus content in leaves at 0.254 %. This enhanced leaf phosphorus concentration in treatments involving organic amendments can be attributed to the slow-release nature of nutrients and increased microbial activity associated with organic inputs like BSF frass and compost. The BSF frass tends to give higher leaf phosphorus uptake than compost. This is because BSF frass contains nutrients, including phosphorus, in forms that are more readily available or soluble to plants. Frass also has beneficial microbes such as nitrifying and nitrogen-fixing bacteria, which improve nutrient mobilisation and uptake in plants. The presence of chitin and antimicrobial peptides in frass can also stimulate plant growth and nutrient assimilation, enhancing nutrient uptake efficiency (38, 39). These amendments improve soil structure, microbial diversity and phosphorus bioavailability, which in turn enhances phosphorus uptake by the plants (29, 40) (Fig. 5). The superior phosphorus uptake in the BSF frass treatment is likely due to the presence of bioavailable phosphorus, organic matter and microbial stimulants in frass, which promote root proliferation and nutrient absorption (41, 42). Interestingly, while soil + NPK (0.716 %) did improve phosphorus content over the control, it was still lower than the organically enriched treatments, indicating the possible short-term nutrient availability and potential leaching associated with chemical fertilisers (43).

Regarding available phosphorus in the soil, the soil + compost treatment exhibited the highest value (0.074 %), followed closely by soil + BSF frass 15 % (0.069 %) and soil + compost + BSF vermiwash (0.056 %). Both control and NPK treatment had the lowest values, at 0.048 % and 0.048 % respectively. The increase in soil phosphorus under compost and

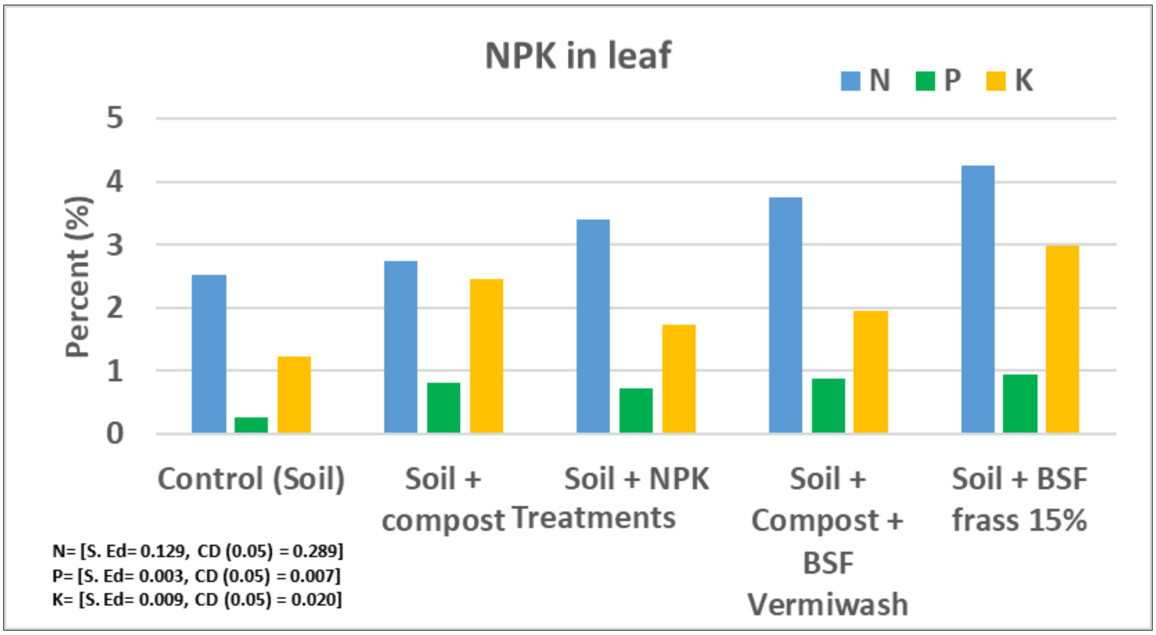

Fig. 5. NPK content in leaf after harvest.

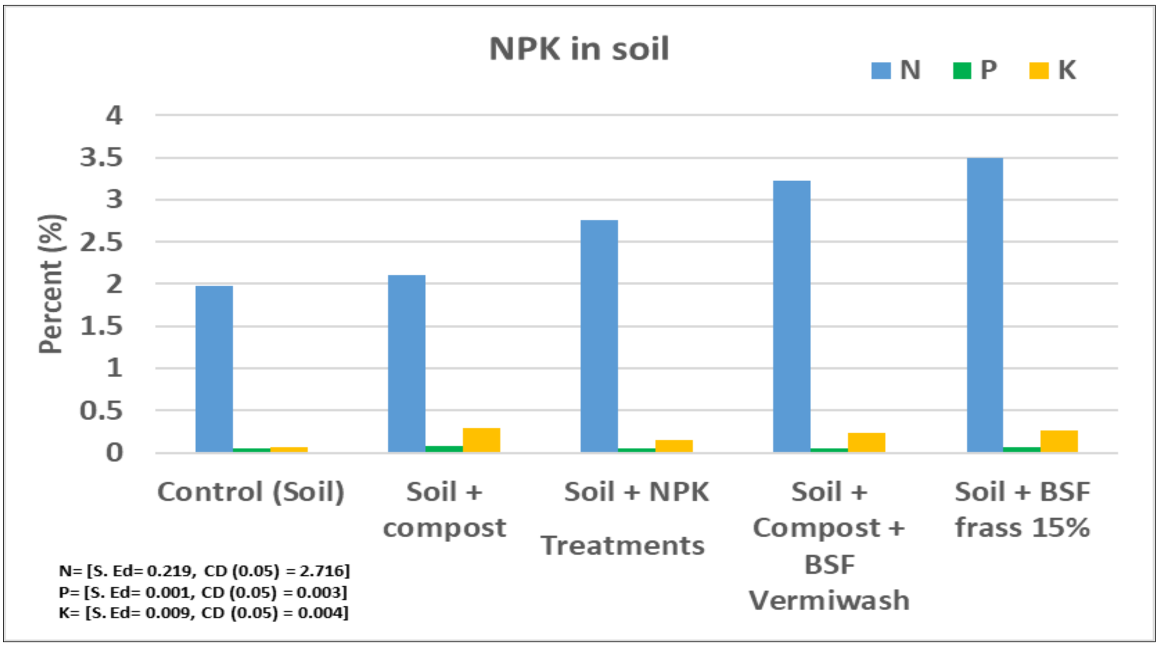

Fig. 6. NPK content in soil after harvest.

frass treatments suggests improved phosphorus retention and mobilisation, likely due to the presence of organic acids and microbial metabolites that help in solubilising bound phosphorus (40). Compost enhances soil aggregation and water-holding capacity, contributing to higher nutrient retention (43) (Fig. 6). In contrast, the lower soil phosphorus in NPK-treated soil reflects the rapid uptake and possible losses due to leaching, especially in sandy or light-textured soils. This underlines the importance of combining mineral fertilisers with organic amendments to maintain sustainable phosphorus levels in the soil (29).

Potassium content

The potassium (K) content in *Amaranthus* leaf and soil exhibited notable variation across different treatments (Table 4). Among all treatments, the highest leaf potassium content was recorded in plants grown with soil + BSF frass 15 % (2.986 %), followed by soil + compost (2.453 %). In contrast, the control (Soil alone) showed the lowest K content in leaf tissues (1.230 %) (Table 4). A similar trend was observed in soil potassium levels. The highest soil K was found in the soil + compost treatment (0.294 %), followed closely by soil + BSF frass 15 % (0.267 %) and soil + compost + BSF vermiwash (0.233 %). The control plot had the lowest soil potassium (0.071 %).

The significant increase in potassium content in both leaf and soil with BSF frass and compost treatments could be attributed to the high organic potassium present in these inputs, which are readily available to plants. BSF frass is a nutrient-rich organic byproduct containing considerable levels of macro and micronutrients including potassium and its application improves nutrient cycling and soil fertility (29, 31) (Fig. 5). Moreover, compost contributes to improved soil cation exchange capacity (CEC) and microbial activity, which enhances potassium retention and uptake by plants (44). The combined application of compost with BSF vermiwash further enhanced potassium availability, likely due to increased microbial solubilization and improved root absorption. Interestingly, Soil + NPK treatment showed moderate potassium content in leaf (1.723 %) and soil (0.156 %), highlighting that while chemical fertilizers supply essential nutrients, their immediate leaching or fixation in soil may reduce long-term availability, unlike organic inputs which release nutrients more gradually and improve soil structure and microbial health (45) (Fig. 6). These results reinforce the potential of BSF frass and compost as effective organic potassium sources, improving both crop nutritional status and soil health sustainability.

BSF frass treatment (soil + BSF frass 15 %) shows the highest nutrient contents in both leaf and soil for Nitrogen (Leaf: 4.25 %, Soil: 3.50 %), Phosphorus (Leaf: 0.935 %, Soil: 0.069 %) and Potash (Leaf: 2.986 %, Soil: 0.267 %) compared to others including control, soil + compost, soil + NPK and soil + compost + vermiwash. This indicates BSF frass substantially improves nutrient uptake and availability in leafy vegetables. The increase in nutrient content with BSF frass can be attributed to its rich organic matter content, nutrient-rich composition and its ability to improve soil health and microbial activity, leading to enhanced mineralisation and nutrient availability. Research shows composted BSF frass increases vegetable growth, yield and nutritional quality, likely due to high mineralisation rate and nutrient release synchrony with plant demand (46). BSF frass as an organic fertiliser has been reported to have low phytotoxicity, high maturity (low C/N ratio) and stability, which favour nutrient uptake and plant growth. Its integration with NPK fertiliser often results in synergistic effects, improving growth and nutrient content further. Studies indicate that BSF frass application leads to higher nitrogen uptake by plants, resulting in better leaf chlorophyll content, growth rate and yield. For example, maize and vegetable crops treated with BSF frass showed significant increases in nitrogen content in leaves and soil compared to equivalent commercial fertilisers. The use of BSF frass also aligns with sustainable agriculture practices by reducing the need for chemical fertilisers and enhancing soil fertility and crop nutritional quality (47).

Conclusion

This study demonstrates that BSF frass, particularly at a 15 % rate, significantly improves growth, biomass and nutrient uptake in *Amaranthus* compared to conventional treatments and controls. Soil + BSF frass (15 %) gave the highest crop weight (16.32 g/plant), root biomass (2.94 g/plant), leaf number (21 per plant) and plant height (54.7 cm), owing to its rich nutrient profile, organic matter and microbial activity. Soil + compost + BSF vermiwash also enhanced growth, reflecting the synergistic role of compost and bioactive compounds in nutrient solubilization. In contrast, the control recorded the lowest values, highlighting the role of organic amendments in soil fertility.

Nutrient analysis confirmed the superior fertilizing potential of BSF frass, with maximum nitrogen (4.25 % leaf, 3.50 % soil), phosphorus (0.935 % leaf, 0.069 % soil) and potassium (2.986 % leaf, 0.267 % soil). Its slow-release properties and microbial stimulation likely sustain nutrient availability. Overall, BSF frass is a sustainable biofertilizer that recycles waste, improves soil health and enhances crop productivity. In essence, BSF frass represents a sustainable, eco-friendly biofertilizer that recycles organic waste into valuable nutrients while enhancing soil health and plant productivity. Its integration into nutrient management systems aligns with circular, low-input agriculture. Combining frass with compost and vermiwash may yield synergistic formulations. Future work should focus on field trials, rate optimization and economic feasibility for wider adoption.

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Authors' contributions

AA conceptualised the study, carried out the literature search and data collection, prepared visualisations and drafted the original manuscript. SG conducted the literature review, performed data analysis and contributed to reviewing and editing the manuscript. SM assisted in data analysis and contributed to reviewing and refining the manuscript. MM supported the literature review and participated in manuscript editing. AR provided methodological support, supervision and critical revisions of the manuscript. SS managed visualisation, reference organisation and formatting of the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declared that they have no known competing financial interests or personal relationships that would have appeared to influence the work reported in this paper.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Grammarly in order to improve the language and readability. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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