Synergistic Impact of Vermicompost, Biochar and Jaggery on Antioxidants, Phenols and Flavonoids in Guava cv. L-49

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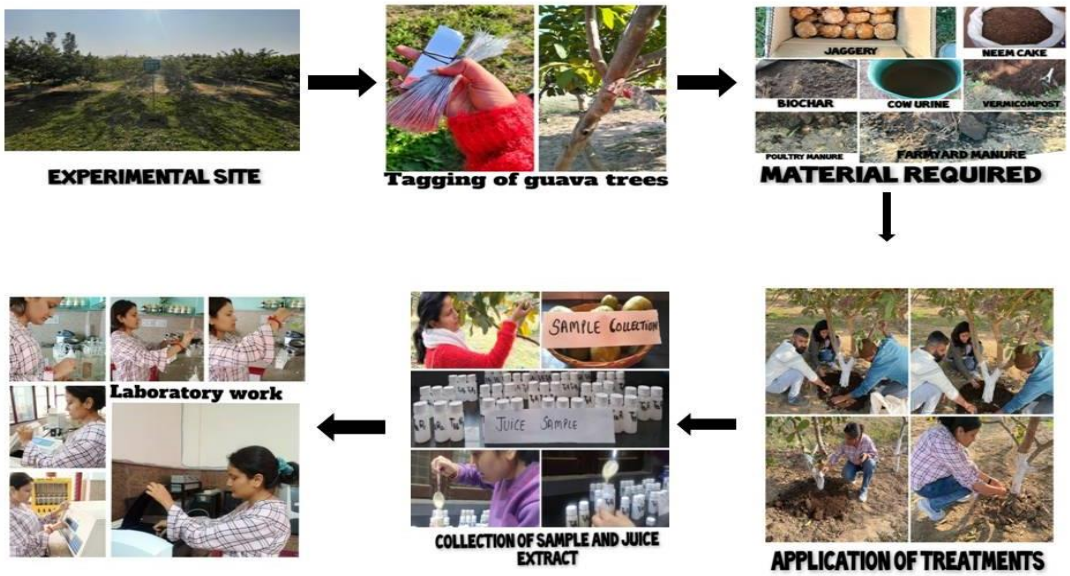
GRAPHICAL ABSTRACT



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This study was conducted over two growing seasons (2022-2023 and 2023-2024). Using a randomised block design, 16 treatments consisted of combinations of vermicompost, biochar, jaggery, poultry manure, farmyard manure, cow urine, and neem cake, and three replications were used in the study. The objective was to assess how these organic amendments affected the antioxidant, phenolic and flavonoid contents in guava fruit. The treatment T_6(Vermicompost 5 kg/tree + Biochar 7.5 kg/tree + Jaggery 1.25 kg/tree) produced the highest levels of antioxidant, phenolic and flavonoid, according to the results. T_6 in particular showed an increase in antioxidant activity from 46.48% to 48.14%, phenolic content from 29.72 mg TA/g to 30.93 mg TA/g and flavonoid content from 23.88 mg/g FW to 25.14 mg/g FW. This study provides important information for sustainable horticultural practices by highlighting the potential of organic amendments to enhance the nutritional qualities of guava cv. L-49.

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Keywords: Vermicompost; Biochar; Jaggery; Psidium guajava; Nutritional quality

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INTRODUCTION

Climate change, environmental deterioration, and the pressures of population increase present new challenges for modern agriculture. The increasing demand for agricultural products puts additional strain on crop productivity and makes it necessary for horticultural practices to decrease their adverse impacts on the environment. Thus, increasing sustainable production is essential to satisfying the world food needs in the future while having the fewest negative effects on the environment (Rojas-Downing *et al*. 2017; Abd-Elmabod *et al*. 2020). The growing global demand for organic fruit can be attributed to rising consumer awareness of health and diet. Because people believe that organic foods are better than conventional in terms of flavour, nutrition, health benefits, cleanliness, safety, and environmentally friendly manufacturing, consumers are willing to pay premium prices for them (Kamboj *et al*. 2023). To promote sustainable agriculture, it is essential to emphasize organic farming practices, particularly the use of organic fertilizers. This paper highlights the impact of various organic materials on enhancing soil health, improving crop yields, and fostering environmental sustainability. Organic fertilizers, sourced from compost, manure and green manure, enrich the soil with vital nutrients and improve its structure. Trupiano *et al*. (2017) found that organic amendments enhance microbial activity and increase soil organic matter, which improves nutrient availability for crops. Additionally, organic fertilizers reduce dependence on chemical inputs, promoting a balanced ecosystem.

Ghosh *et al*. (2014) demonstrated that these practices lead to higher soil fertility, better water retention, and increased resilience against pests and diseases. Emphasizing the integration of organic fertilizers in farming can facilitate a shift towards sustainable agricultural methods, mitigating the negative impacts of conventional practices while ensuring long-term productivity and soil health. Thus, this paper underscores the importance of organic fertilizers in advancing sustainable agriculture. Many researchers have examined the physico-chemical and organoleptic characteristics of conventional and organic fruits and vegetables; however, because cultivation management, soil type, and production size vary, results have occasionally been conflicting (Gamage *et al*. 2023).

Guava (*Psidium guajava* L.), which is extensively grown in tropical as well as subtropical areas, is known for its nutritional value. Its abundance of antioxidants, phenols, and flavonoids supports its therapeutic properties, which include anti-inflammatory and anti-cancer effects (Naseer *et al*. 2018; Shanthirasekaram *et al*. 2021). It is essential to improve these bioactive chemicals using sustainable farming methods. Because of their potential to strengthen crop quality and promote soil health, biochar and vermicompost have attracted attention (Anand *et al*. 2020). Plant secondary metabolite improves the soil characteristics and nutrient availability brought about by biochar made from pyrolysed organic sources (Santos *et al*. 2017).

Earthworms break down organic waste to produce vermicompost, which enriches the soil with vital nutrients, growth hormones, and helpful bacteria that encourage plants to produce more bioactive substances (Mohite *et al*. 2024). Research has indicated that the utilisation of vermicompost and biochar can considerably raise the antioxidant, phenolic and flavonoid content of guava fruits. For example, when treated with these amendments, organic apples and strawberries have demonstrated increased antioxidant levels, better textural qualities, and greater resilience to deterioration (Hargreaves *et al*. 2008). In a similar vein, consumers preferred organic tomatoes more because of their superior flavour, texture and rich colour, which is thought to be attributable to increased concentrations of antioxidants like lycopene and anthocyanin (Dumas *et al*. 2003). Higher concentrations of vitamins and antioxidants are a result of biochar and vermicompost improved soil structure and increased nutrient availability, which are linked to these increases in fruit quality. Because of their distinct ecological advantages, vermicompost and biochar are great soil conditioners. In addition to improving water retention and reducing heavy metal contamination, biochar raises the amount of organic matter in soil (Ennis *et al*. 2012). Rich in microbes and nutrients, vermicompost enhances soil structure and encourages plant growth. Combining these amendments improves soil health and lowers the demand for synthetic fertilisers, which promotes sustainable agriculture methods while also increasing the nutritional value of guava fruits (Ceritoğlu *et al*. 2018).

The processed form of sugarcane juice known as jaggery is rich in carbohydrates (sucrose: 72–78 g/100g), (Calcium 40–100 mg; Magnesium 70–90 mg; Phosphorous 20–90 mg; Sodium 19–30 mg; Iron 10–13 mg; Manganese 0.2–0.4 mg; Zinc 0.2–0.4 mg; Chlorine 5.3–0.0 mg; 0.1–0.9 mg) in significant amount (Sharifi-Rad *et al*. 2023).

Increased sucrose uptake by the roots enhances the retention of photosynthetic carbon in the plant's aerial parts, which results in greater biomass accumulation and a reduced root-to-shoot ratio. By transporting sugar molecules, such as sucrose, from the rhizosphere into the root cells, there is less photosynthetic carbon allocated to root growth and development. Consequently, more of the assimilated sugar is distributed to other parts of the plant, such as the fruit (Kazachkova 2023).

This study assessed how biochar and vermicompost affect the concentrations of flavonoids, phenols, and antioxidants in guava fruits. The aim was to provide more information on sustainable methods that improve the nutritional content of guava, to raise consumer awareness, and to support sustainable agricultural practices in guava cultivation by researching how these organic amendments affect the quality of guava fruit.

EXPERIMENTAL

The experiment was formulated to appraise the repercussions of various organic amendments on antioxidants, phenols and flavonoids of guava fruit. The experiment was orchestrated in a randomized block design with sixteen treatments and three replications. Each replication encompassed three plants, totaling 48 guava trees. The experiment was undertaken over two years, 2022 and 2023.

Before administering the treatments, it was essential to analyze the initial status of the orchard soil. The orchard soil utilized for the experiment was characterized as sandy clay loam, with the following nutrient composition: 0.42% organic carbon, 203.24 kg ha^{-1} of available nitrogen (N), 12.53 kg ha^{-1} of available phosphorus (P), and 138.65 kg ha^{-1} of available potassium (K). In Table 1, the experimental treatments for the study feature a range of organic amendments to assess their influence on guava quality. The following treatments were applied: Vermicompost (15 kg/tree) was used for 100% nitrogen replacement (T_1), while other vermicompost treatments included combinations with cow urine and neem cake: 10 kg/tree + 0.5 liter cow urine + 1.0 kg neem cake (T_2), 10 kg/tree + 1.0 liter cow urine + 1.5 kg neem cake (T_3), and 10 kg/tree + 1.5 liter cow urine + 2.0 kg neem cake (T_4). Additionally, vermicompost (5 kg/tree) was combined with biochar and jaggery: 5.0 kg biochar + 1.0 kg jaggery (T_5), 7.5 kg biochar + 1.25 kg jaggery (T_6), and 10 kg biochar + 1.50 kg jaggery (T_7). Poultry manure (5 kg/tree) also served as a 100% nitrogen replacement (T_8), with further combinations including 3 kg/tree + 4.0 kg biochar + 0.5 kg jaggery (T_9), 3 kg/tree + 5.0 kg biochar + 0.75 kg jaggery (T_{10}), and 3 kg/tree + 6.0 kg biochar + 1.0 kg jaggery (T_{11}). Farmyard manure (15 kg/tree) was another 100% nitrogen replacement (T_{12}), combined with cow urine and neem cake: 10 kg/tree + 0.75 liter cow urine + 1.5 kg neem cake (T_{13}), 10 kg/tree + 1.25 liter cow urine + 1.75 kg neem cake (T_{14}), and 10 kg/tree + 1.75 liter cow urine + 2.0 kg neem cake (T_{15}).

The control (T_{16}) treatment in this context refers to following the recommended package of practices without any additional or experimental treatments. This involves strictly adhering to the specified quantities of Urea (700 g), Di-Ammonium Phosphate (275 g), and Muriate of Potash (135 g) according to the tree's age, along with the recommended timing of application. This standard practice serves as a baseline against which other treatments can be compared. The treatments were applied to evaluate their impact on guava fruit quality and productivity throughout the experimental period.

The incorporation of organic amendments was slated for November. Each treatment was carefully infused into the soil encircling the base of the guava trees to ensure the highest efficacy. Vermicompost, poultry manure, and farmyard manure were uniformly scattered around each tree and lightly plowed into the soil to facilitate proper integration. Biochar was mixed directly into the soil to increase its physical and chemical properties. Jaggery was mixed in water until it was fully dissolved and applied as a liquid fertilizer to increase the capacity of absorption and effectiveness. Cow urine combined with vermicompost and neem cake in specific treatments and was infused directly to the soil to amplify nutrient availability and microbial activity. This thoroughgoing application method was designed to align with the guava growth cycle and maximize nutrient uptake during the winter months.

Table 1. Treatment Combinations of Organic Amendments for Guava Cultivation

Treatment	Description
T_1	Vermicompost (15 kg/tree) - 100% Nitrogen Replacement
T_2	Vermicompost (10 kg/tree) + Cow Urine (0.5 liter/tree) + Neem Cake (1.0 kg/tree)
T_3	Vermicompost (10 kg/tree) + Cow Urine (1.0 liter/tree) + Neem Cake (1.5 kg/tree)
T_4	Vermicompost (10 kg/tree) + Cow Urine (1.5 liter/tree) + Neem Cake (2.0 kg/tree)
T_5	Vermicompost (5 kg/tree) + Biochar (5.0 kg/tree) + Jaggery (1.0 kg/tree)
T_6	Vermicompost (5 kg/tree) + Biochar (7.5 kg/tree) + Jaggery (1.25 kg/tree)
T_7	Vermicompost (5 kg/tree) + Biochar (10 kg/tree) + Jaggery (1.50 kg/tree)
T_8	Poultry Manure (5 kg/tree) - 100% Nitrogen Replacement
T_9	Poultry Manure (3 kg/tree) + Biochar (4.0 kg/tree) + Jaggery (0.5 kg/tree)
T_{10}	Poultry Manure (3 kg/tree) + Biochar (5.0 kg/tree) + Jaggery (0.75 kg/tree)
T_{11}	Poultry Manure (3 kg/tree) + Biochar (6.0 kg/tree) + Jaggery (1.0 kg/tree)
T_{12}	Farmyard Manure (15 kg/tree) - 100% Nitrogen Replacement
T_{13}	Farmyard Manure (10 kg/tree) + Cow Urine (0.75 liter/tree) + Neem Cake (1.5 kg/tree)
T_{14}	Farmyard Manure (10 kg/tree) + Cow Urine (1.25 liter/tree) + Neem Cake (1.75 kg/tree)
T_{15}	Farmyard Manure (10 kg/tree) + Cow Urine (1.75 liter/tree) + Neem Cake (2.0 kg/tree)
T_{16}	Control - Recommended package of practices for guava cultivation

Table 2. Chemical Composition of Various Organic Materials Utilized in the Treatments

Material	N (%)	P (%)	K (%)	Other Nutrients	Source
Vermicompost	1.6 - 2.0	0.5 - 1.0	0.5 - 0.9	Calcium (Ca): 1.0-2.0%, Magnesium (Mg): 0.5-1.0%	Edwards and Arancon (2004)
Cow Urine	0.5 - 1.0	0.02 - 0.10	0.15 - 0.30	Sulfur (S): 0.03-0.05%, Urea content	Pathak and Singh (2009)
Neem Cake	2.0 - 5.0	0.5 - 1.0	1.0 - 1.5	Azadirachtin: 0.2-0.5%, Organic Carbon: 15-20%	Subapriya and Nagini (2005)
Biochar	0.5 - 1.0	0.1 - 0.5	1.0 - 2.0	Carbon (C): 70-80%, pH: 7.5-8.5	Lehmann and Joseph (2015)

Material	N (%)	P (%)	K (%)	Other Nutrients	Source
Jaggery	-	-	-	Rich in Carbohydrates (Sucrose), Minor minerals: Fe, Ca	Sharma and Singh (2008)
Poultry Manure	1.5 - 2.5	1.0 - 1.5	0.5 - 1.0	Organic Matter: 30-40%, Ca, Mg, S	Nahm (2003)
Farmyard Manure	0.5 - 1.5	0.2 - 0.5	0.5 - 1.5	Organic Carbon: 15-25%, Calcium (Ca), Magnesium (Mg)	Parthasarathy *et al*. (2008)

Sample Preparation

The seeds were removed from ripe guava fruits, which were then chopped into small pieces. The chopped guava pieces were blended with ethanol to make a consistent extract and to ensure that the target chemicals were completely dissolved in the solvent. Once the mixture was homogenised, the extract was strained using filter paper to exclude bigger particles. Alternatively, the mixture was centrifuged for 10 min at 4000 rpm to achieve a clean supernatant. The experiments that follow assessed the guava fruit antioxidants, phenols and flavonoids using this clear supernatant.

Determination of Antioxidant

According to Andrews *et al*. (2000), free radical scavenging activity was gauged by reduction in the absorbance of the 2,2 diphenyl-1-picrylhydrazyl (DPPH) methanol solution. A0.1 mM DPPH solution was prepared in methanol, and 1 mL of the guava extract was mixed with 3mL of DPPH solution. The mixture was incubated for 30 min at room temperature in the dark. Methanol was used in the control reaction. After incubation, absorbance was determined at 517 nm. DPPH scavenging activity was calculated by Eq. 1,

$$\%\ \text{Antioxidant activity} = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100 \tag{1}$$

where the control absorbance is 0.329. This formula allows for the determination of the percentage of DPPH radical scavenging activity by the guava extract (Blois 1958).

Determination of Phenolic Content

The phenolic components of guava extract were determined using the Folin-Ciocalteu technique. A 10-fold dilution of the Folin-Ciocalteu reagent (2.5 mL) was combined with 0.5 mL of the guava extract, and 2.0 mL of a 7.5% sodium carbonate solution was added to the mixture. To enable the development of colour, the reaction was incubated for 40 min at 45 °C. The absorbance at 765 nm were determined. The standard curve was prepared using tannic acid as standard (µg/mL), and data was expressed as mg/g dry weight (Agbor *et al*. 2014).

Determination of Flavonoid Content

With catechin serving as a standard, the total flavonoid content was calculated using the chromogen reagent and the method described by Delcour and Devarebeke (1985). The results were represented in mg of catechin equivalents (CE) 100/g FW, and the absorbance was measured at 640 nm. Total phenols were estimated using the Swain and Hills (1959) approach. In a test tube, 1 mL of the extract was combined with 7.5 ml of distilled water. After thoroughly mixing the material, 0.5 ml of diluted Folin-Ciocalteu reagent was added. Following a 3-min vortex, 1 mL of saturated sodium carbonate and 500 µL of water were added to the samples to make a volume of 10 mL with distilled water. After 1 h of incubation, samples were tested for absorbance at 725 nm, taking distilled water as blank. The standard curve was prepared using tannic acid as standard (µg/mL), and data was expressed as mg/g dry weight. Data were subjected to statistical analysis using SPSS software. A randomized block design was used, and critical difference (CD) was calculated.

RESULTS AND DISCUSSION

As outlined in Table 3, the study evaluated the synergistic impact of various organic amendments on the total antioxidant activity of guava cv. L-49 over two growing seasons (2022-2023 and 2023-2024). Table 3 highlights the substantial variations across treatments.

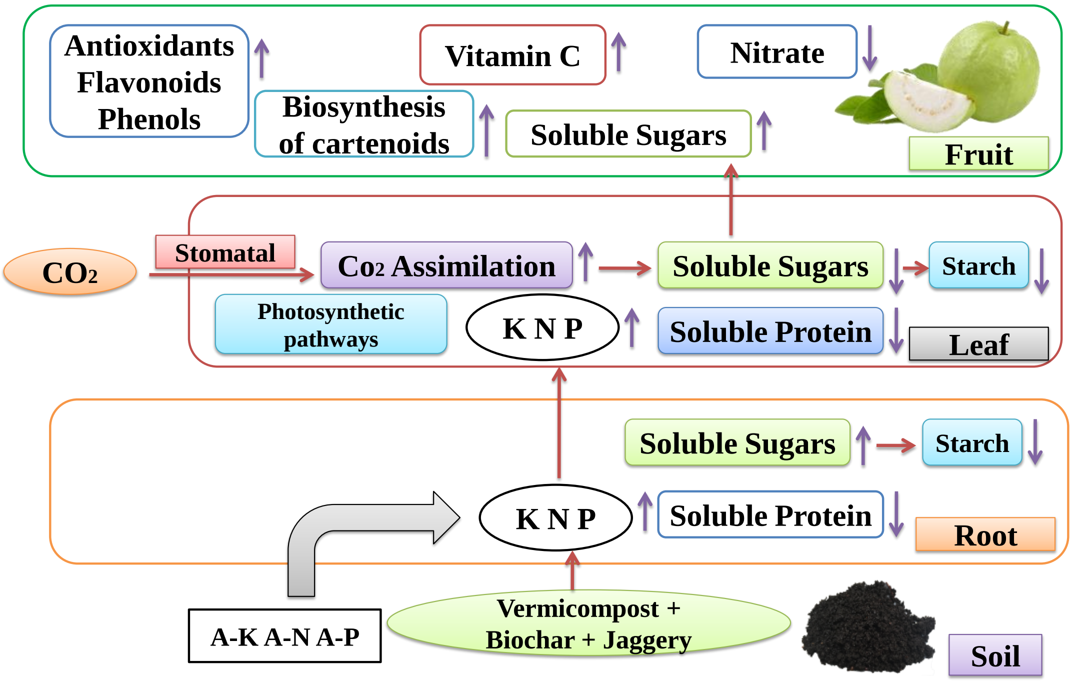

Fig. 1. An illustration of the pathway of CO_2 entering through stomata, enhancing photosynthetic pathways, and leading to increased production of soluble sugars and soluble proteins in leaves, roots, and fruits. The application of KNP (potassium, nitrogen, and phosphorus) further boosts these soluble components, while starch levels decrease. In fruits, this process enhances antioxidants, flavonoids, phenols, Vitamin C, nitrate levels, and carotenoid biosynthesis. Vermicompost combined with biochar and jaggery, as well as A-K-A-N-A-P (additional nutrients), support this nutrient flow, promoting overall plant health and fruit quality.

Table 3. Total Antioxidant Activity (%) of Guava over Two Years (2022-2023 and 2023-2024)

Treatments	Total Antioxidant Activity (%) 2022-2023	Total Antioxidant Activity (%) 2023-2024
T_1	34.81	35.54
T_2	36.73	37.55
T_3	37.19	38.09
T_4	37.28	38.71
T_5	42.02	43.85
T_6	46.48	48.14
T_7	43.88	45.59
T_8	33.79	34.65
T_9	38.12	39.02
T_{10}	38.60	39.38

T_{11}	38.94	39.66
T_{12}	34.22	35.05
T_{13}	34.90	35.72
T_{14}	35.20	36.04
T_{15}	36.14	37.05
T_{16}	27.90	27.99
Mean	37.26	38.25
S. Em. ±	0.01	0.00
C.D. (5 %)	0.02	0.01
C.D. (1 %)	0.02	0.02
C.V. (%)	0.03	0.02

Note: The treatments were as follows: Vermicompost (15 kg/tree) was used for 100% nitrogen replacement (T_1), while other vermicompost treatments included combinations with cow urine and neem cake: 10 kg/tree + 0.5 liter cow urine + 1.0 kg neem cake (T_2), 10 kg/tree + 1.0 liter cow urine + 1.5 kg neem cake (T_3), and 10 kg/tree + 1.5 liter cow urine + 2.0 kg neem cake (T_4). Additionally, vermicompost (5 kg/tree) was combined with biochar and jaggery: 5.0 kg biochar + 1.0 kg jaggery (T_5), 7.5 kg biochar + 1.25 kg jaggery (T_6), and 10 kg biochar + 1.50 kg jaggery (T_7). Poultry manure (5 kg/tree) also served as a 100% nitrogen replacement (T_8), with further combinations including 3 kg/tree + 4.0 kg biochar + 0.5 kg jaggery (T_9), 3 kg/tree + 5.0 kg biochar + 0.75 kg jaggery (T_{10}), and 3 kg/tree + 6.0 kg biochar + 1.0 kg jaggery (T_{11}). Farmyard manure (15 kg/tree) was another 100% nitrogen replacement (T_{12}), combined with cow urine and neem cake: 10 kg/tree + 0.75 liter cow urine + 1.5 kg neem cake (T_{13}), 10 kg/tree + 1.25 liter cow urine + 1.75 kg neem cake (T_{14}), and 10 kg/tree + 1.75 liter cow urine + 2.0 kg neem cake (T_{15}). The control (T_{16}) followed the recommended package of practices

Among the 16 treatments, T_6 including vermicompost (5 kg/tree) + biochar (7.5 kg/tree) + jaggery (1.25 kg/tree) demonstrated the maximum antioxidant activity. This was immediately preceded by T_5 [vermicompost (5 kg/tree) + biochar (5.0 kg/tree) + jaggery (1.0 kg/tree)]. T_7[Vermicompost (5 kg/tree) + Biochar (10 kg/tree) + Jaggery (1.50 kg/tree)] also showed similar results in respect of antioxidant activity. In contrast, T_{16} (control, recommended package of practices) had the minimum antioxidant activity. Statistical analysis indicated a very low standard error of mean (S. Em. ± 0.01 to 0.00) and coefficients of variation (C.V. %) ranging from 0.02 to 0.03%, reflecting strong precision and consistency in the results.

Table 4. Total Phenolic Content (mgTA/g) of Guava over Two Years (2022-2023 and 2023-2024)

Treatments	Total Phenolic Content (mgTA/g) 2022-2023	Total Phenolic Content (mgTA/g) 2023-2024
T_1	17.58	18.23
T_2	20.09	20.41
T_3	20.20	21.46
T_4	20.28	21.53
T_5	24.63	26.74
T_6	29.72	30.93
T_7	25.68	28.81
T_8	16.30	17.54
T_9	21.36	23.59
T_{10}	21.42	23.67
T_{11}	23.56	24.71
T_{12}	17.11	17.68
T_{13}	17.76	19.19
T_{14}	18.83	19.28
T_{15}	18.02	19.37
T_{16}	14.31	14.64
Mean	20.43	21.74

S. Em. ±	0.01	0.00
C.D. (5 %)	0.02	0.01
C.D. (1 %)	0.03	0.01
C.V. (%)	0.06	0.02

See notes following Table 4.

Table 5. Total Flavonoid Content (mg/g FW) of Guava over Two Years (2022-2023 and 2023-2024)

Treatments	**Total Flavonoid Content (mg/g FW) 2022-2023**	**Total Flavonoid Content (mg/g FW) 2023-2024**
T_1	20.03	22.93
T_2	20.51	23.61
T_3	20.75	23.82
T_4	20.98	24.05
T_5	22.79	24.96
T_6	23.88	25.14
T_7	23.58	25.05
T_8	19.18	22.47
T_9	21.41	24.21
T_{10}	21.64	24.42
T_{11}	21.83	24.76
T_{12}	19.36	22.70
T_{13}	20.08	23.17
T_{14}	20.17	23.29
T_{15}	20.32	23.52
T_{16}	16.05	16.21
Mean	20.79	23.40
S. Em. ±	0.01	0.01
C.D. (5 %)	0.02	0.02
C.D. (1 %)	0.02	0.03
C.V. (%)	0.05	0.05

See notes following Table 4.

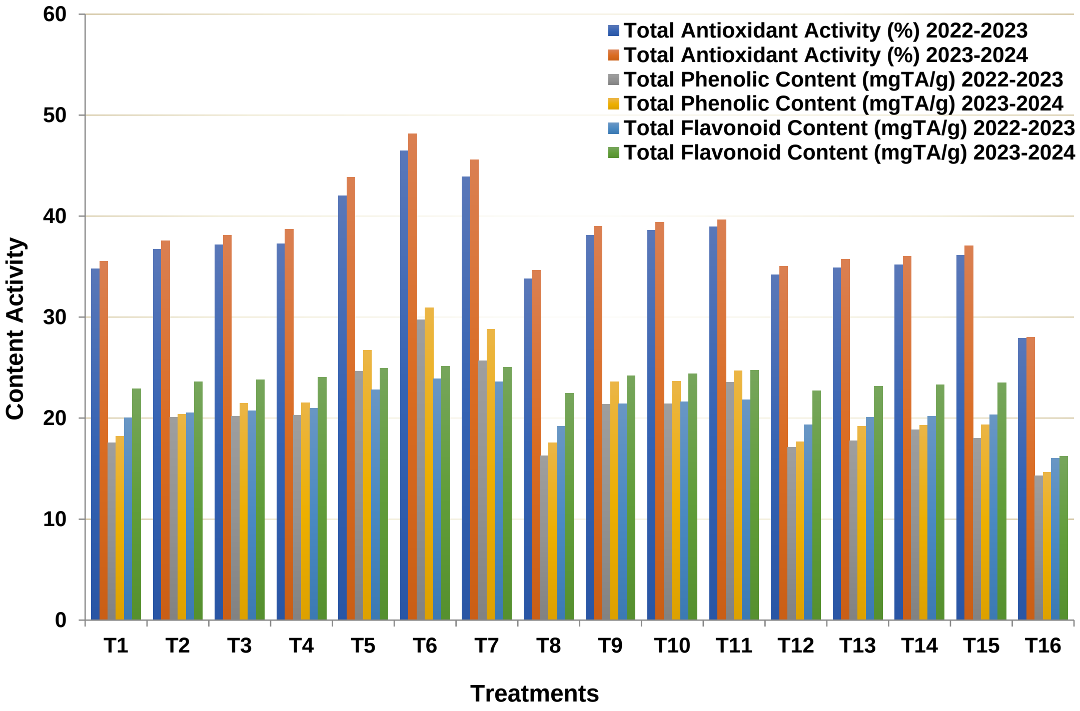

Fig 2. The bar chart compares the total antioxidant activity, phenolic content, and flavonoid content of guava across two years (2022-2023 and 2023-2024) under 16 different treatments.

The significance of the observed differences across treatments was further validated by the critical difference (C.D.) values at the 5% and 1% levels. These results demonstrate how particular organic amendments can improve the antioxidant capacity of guava, with T_6 proving to be the most effective treatment. The antioxidant activity of guava fruits is significantly enhanced by the application of vermicompost, biochar and jaggery as compared to control (T_{16}).

Table 4 presents significant differences between treatments from synergistic impact of various organic amendments on the total phenolic content of guava cv. L-49 over two growing seasons (2022-2023 and 2023-2024). T_6 [vermicompost (5 kg/tree) + biochar (7.5 kg/tree) + jaggery (1.25 kg/tree)] showed the greatest phenolic content. T_5 [vermicompost (5 kg/tree) + biochar (5.0 kg/tree) + jaggery (1.0 kg/tree)] and T_7 [vermicompost (5 kg/tree) + biochar (10 kg/tree) + jaggery (1.50 kg/tree)] also demonstrated a significant improvement. On the other hand, T_{16} (control, recommended package of practices) exhibited the least amount of phenolic content. The findings of the statistical analysis showed a very low coefficient of variation (C.V.%) ranging from 0.02 to 0.06% and a very low standard error of mean (S. Em. ± 0.01 to 0.00), indicating great precision and reliability. The significance of the differences between the treatments was further confirmed by critical difference (C.D.) values at the 5% and 1% levels. These findings highlight the ability of particular organic amendments to increase guava phenolic content; treatment T_6 was the most successful. According to Table 5, the study examining the synergistic impact of various organic amendments on the total flavonoid content of guava cv. L-49 over two growing seasons (2022-2023 and 2023-2024) demonstrated significant variations across treatments. T_6[vermicompost (5 kg/tree) + biochar (5.0 kg/tree) + jaggery (1.0 kg/tree)]had the highest total flavonoid content among the 16 treatments. T_5 [vermicompost (5 kg/tree) + biochar (5.0 kg/tree) + jaggery (1.0 kg/tree)] and T_7 [vermicompost (5 kg/tree) + biochar (10 kg/tree) + jaggery (1.50 kg/tree)] showed a significant increase in flavonoid content as well. On the other hand, T_{16} (control, recommended package of practices) had the lowest level of flavonoid content. The findings of the statistical analysis demonstrated good precision and consistency, with a low standard error of mean (S. Em. ± 0.01) and coefficients of variation (C.V. %) of 0.05 for both years. The significance of the observed differences across treatments was further corroborated by the critical difference (C.D.) values at the 5% and 1% levels. These results demonstrate how adding particular organic amendments can increase the amount of flavonoids in guava, with T_6 being the most successful treatment.

As illustrated in Fig2, the overall antioxidant activity was higher in 2022-2023 for most treatments, with some decline or stability in 2023-2024. Phenolic content generally increased slightly from 2022-2023 to 2023-2024, showing greater stability. Flavonoid content exhibited mixed trends, with some treatments improving and others declining in 2023-2024. Notably, treatments T_5 and T_6 showed significant increases in both antioxidant activity and phenolic content in 2023-2024, while treatments like T_{14} to T_{16} demonstrated less pronounced changes

The enhanced antioxidant activity, phenols and flavonoids in guava fruits due to the application of vermicompost, biochar, and jaggery, as well as the corresponding increases in carotenoids, phenolics, and flavonoids, is supported by several studies. A possible explanation is that the biochar, increasing the pH, CEC (Cation exchange capacity), N_{tot} (Total soil nitrogen), C_{tot} (Total soil carbon), P_{tot} (Total soil phosphorous), and water content, could enhance available nutrients for plants and, consequently, biomass accumulation (Scotti *et al*. 2015). Increase in CEC value could be driven by the presence of cation exchange sites on the biochar surface (Sohi *et al*. 2010). Vaccari *et al*. (2015) reported that this could contribute to retaining NH_4^+, leading to improved N nutrition in biochar-amended soils (Hollister *et al*. 2013). This would confirm a direct role of biochar in the nutrient supply to plants (Clough *et al*. 2010). Additionally, the role of biochar in improving soil structure and nutrient retention, as well as its impact on microbial activity and the phenylpropanoid pathway activation during ripening, is corroborated by Lehmann *et al*. (2011) and Koes *et al*. (2005).

According to studies published in the literature (Amlinger *et al*. 2007; Diacono and Montemurro 2011), lettuce plants in compost-added soil demonstrated the highest total biomass accumulation, assimilation rate, and water use efficiency, likely because of the increased soil nutrient availability (soil C_{tot}, N_{tot}, P_{tot}, content). This high soil nutrient status could also have enhanced the activity of enzymes involved in phosphorus and nitrogen cycling (phosphohydrolase, chymotrypsin, and trypsin), which got increased due to soil amendment as compared to those in the unamended soils. Tyrosine and phenylalanine (precursors of phenolic compound) production is enhanced due to the corresponding increase in enzyme activity. Such changes have been reported to be the result of the increased organic matter and the activation of the shikimate pathway (Benhammou *et al*. 2009). The application of organic manure boosts the synthesis of soluble sugars, amino acids, phenolic volatiles and flavonoids in guava fruits by modulating major metabolic pathways. Specifically, organic manure increases the essential nutrients that activate signaling cascades, contributing to the upregulation of genes culpable for the biosynthesis of these compounds. This reprogramming of the regulatory network of transcripts and metabolites prompts accelerated yields of these valuable components, like flavonoids and phenols, which are antioxidants and contribute to the improved quality of guava fruits (Wu *et al*. 2024). The effects of vermicompost, which provides organic nitrogen and reduces nitrate accumulation, favoring secondary metabolite synthesis, align with findings from Celikcan *et al*. (2021).

The introduction of jaggery substantially augmented the microbial population in the soil, thus boosting enzymatic activity. The addition of jaggery makes this change more effective by increasing biochar's ability to boost microbial activity by making it better at keeping moisture in and letting air flow through. Moreover, biochar amplifies the effects of vermicompost by increasing nutrient concentrations in the soil, hence enhancing antioxidant activity (Gabhane *et al*. 2012; Waqas *et al*. 2018). Jaggery’s contribution to the phenolic content further boosts the antioxidant properties (Choudhary *et al*. 2022).

One hypothesis is that the addition of biochar to vermicompost complexes may cause alterations in the structure, abundance, and activity of the microbial community in soils (Lehmann 2007). The proposed modifications have the potential to enhance the bioavailability of nutrients to plants and may trigger the secretion of plant growth promoting hormones. On the other hand, vermicompost and biochar are generally alkaline in nature with biochar having a high ion exchange capacity and vermicompost has a specific acid-base buffering capacity (Zhao *et al*. 2015; Li *et al*. 2018). Hence, both organic additions have the ability to elevate the pH level of the soil. Our investigations have demonstrated that the introduction of organic amendments resulted in a significant increase in soil pH, converting acidic soil to mildly alkaline soil. This finding aligns with prior research outcomes. The material cycle and energy flow within the soil closely interconnect with soil organic carbon, the fundamental component of soil fertility. Dissolved Organic Carbon (DOC) is a highly reactive component of organic carbon that exhibits rapid oxidation, low stability and susceptibility to degradation (Kaiser and Kalbitz 2012; Guillaume *et al*. 2022; Zhang *et al*. 2022). Research has demonstrated that applying biochar and vermicompost significantly increases the soil's organic matter content (Liu *et al*. 2020; Bi *et al*. 2021). Although organic matter constitutes less than 10% of the solid phase composition of soil, it plays a crucial role in stimulating microbial activity and facilitating mineral transformation. Furthermore, their composition also serves as an indicator of the nutritional condition and soil quality (Wang *et al*. 2022). The synergistic effects of these amendments, as noted in studies by Premuzic *et al*. (2001, 2002), demonstrate the enhancement of antioxidants, phenolic and flavonoid contents in treated guava fruits. The positive correlation between flavonoid content and antioxidant activity during ripening is highlighted by Bhandari and Lee (2016).

As for the results, the consistent and slightly higher values for antioxidant activity in 2023-2024 compared to the previous year may suggest a cumulative or enhanced effect of organic manure over time. The persistent application of organic manure likely improved soil fertility and microbial activity, leading to a more robust nutrient uptake by the plants, which in turn intensified the production of antioxidants. The similar trend observed in phenolic content further supports this hypothesis, indicating that the positive effects of organic manure on guava fruit quality are not only sustained but also amplified over consecutive years. The authors could conclude that the consistent increase in these values over two years underscores the effectiveness of organic manure in enhancing the nutritional quality of guava fruits, likely due to the sustained improvement in soil health and plant metabolic activity.

CONCLUSIONS

1. During the course of two growing seasons, the application of treatment T_6 including vermicompost (5 kg/tree), biochar (7.5 kg/tree), and jaggery (1.25 kg/tree) considerably increased the antioxidant activity, total phenolic content, and total flavonoid content in guava cv. L-49. The highest results were consistently obtained by treatments T_6 followed by T_5 and T_7, showing their greatest efficacy.
2. The increased availability of nutrients and improved biosynthetic processes made possible by the organic amendments are responsible for the increase in these beneficial compounds.
3. These results highlight the possibility of utilising biochar, vermicompost, and jaggery together to increase the antioxidant and nutritional value of guava, providing a sustainable method of enhancing fruit quality.

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