Sustainable Composting of Vegetable Waste, Cow Dung, Grasses, and Food Wastes into Soil Amendment using Starter Culture and Growth Characteristics in Guava Plant

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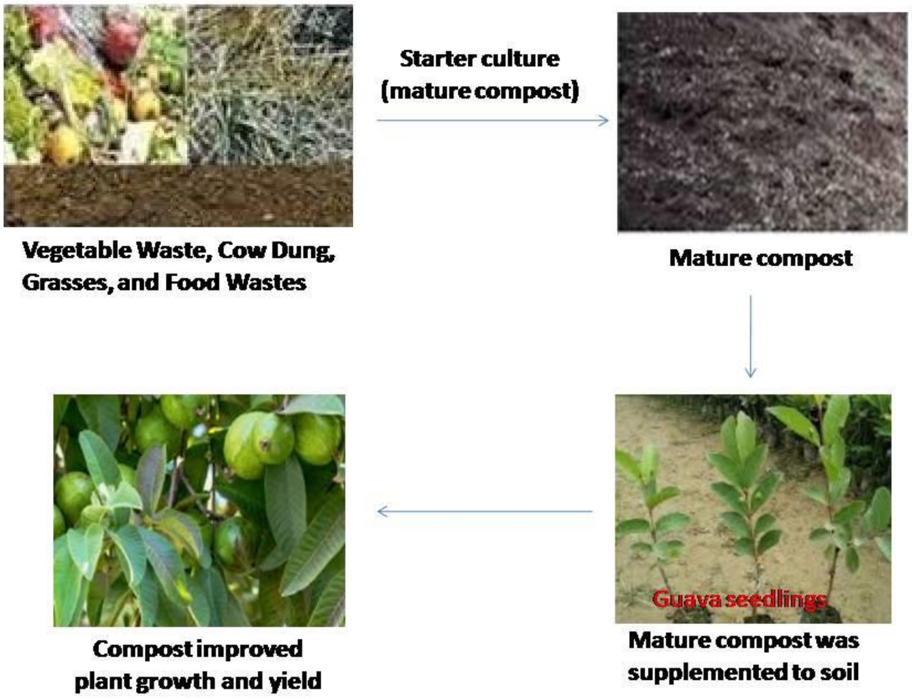
GRAPHICAL ABSTRACT



Sustainable Composting of Vegetable Waste, Cow Dung, Grasses, and Food Wastes into Soil Amendment using Starter Culture and Growth Characteristics in Guava Plant

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The study aim was to optimise the C/N ratio, improve the compost quality, reduce pathogenic bacteria load in the compost, and improve guava yield. Vegetable wastes were mixed with cow dung, grasses, and food wastes in ratios of 4:3:2:1 (w/w) for achieving a C/N ratio of approximately 37. Co-composting is an important strategy because the mixture of bulking agents can help achieve optimal composting conditions. Experimental results were obtained from a pilot-scale rotary drum reactor with forced aeration. In the reactor, the temperature increased during the thermophilic phase (58±2 °C) and decreased after 10 days (54±2 °C). The pH values moderately increased, then decreased, and were near to neutral after maturation. The results indicated that co-composting of bio-wastes at a C/N ratio of 37.6%±1.02% could be effectively decomposed to reduce the residuals to just 13.6%±1.05% after 28 days. The microbial population increased in both mesophilic and thermophilic stages and decreased at the end of the composting, reflecting stability. The stable compost was applied to the growth of guava plant, and the yield was calculated. The organic compost improved plant growth, fruit yield, and enriched phytochemical compounds in the fruit and peels. The phytochemical compounds improved antioxidant activity in the guava fruits.

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Keywords: Municipal solid waste; Compost; Nutrients; Eco-friendly; Plant growth

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INTRODUCTION

Solid waste management constitutes an important challenge for metropolitan cities.Presently, municipal solid waste (MSW) is disposed of mainly in landfills in most countries, causing serious health and environmental impacts. The MSW consists of wastes produced by public institutions, offices, municipal parks and gardens, and households. In developing countries, generation of leachate from dumpsites poses a serious threat to the environment and human health (Chaturvedi and Kaushal 2018). In developing countries, organic waste material (food waste and green waste) generation was more than 50% of total MSW (USEPA 2015). The release of greenhouse gases from the municipal solid waste into the living atmosphere is becoming an important concern. Recently, Zhang *et al.* (2020) used tobacco waste and livestock manure for composting, and gaseous emission was reported during the composting process. Food waste composting is useful to mitigate greenhouse gases by converting vegetable matter generated from municipal solid waste into compost. Additionally, composting is an economically viable method to make a soil amendment to manage aquatic weeds. Green waste (GW) is mainly composed of flowers, branches, leaves, bark of trees, and various material generated by garden maintenance activities (Zhang and Sun 2016). The management of GW is highly complex because it is continuously generated, occupies a large volume, and is hard to degrade. Most countries prefer physical methods (landfill) for the disposal of MSW, but other methods are also suggested for managing these wastes (Odlare *et al*. 2011). The landfilling method is considered as one of the best methods of final disposal of various solid wastes.

Composting of MSW is an alternative method to manage wastes in large quantities; however, this method has some limitations, including the quality of the final product and increased processing time. Several approaches have been employed to optimize the composting process of MSW (Reyes-Torres *et al.* 2018), which include co-composting with various bulking agents, supplementation of the microbial consortium, chemical/physical pretreatment, changes in the oxygen supply in inlet, and changes in the operational conditions of the composting process. Many materials have been used in the co-composting of MSW to enhance the composting process and the quality of the product, to maintain the balanced ratio of C/N, to supply various nutrients, and to enhance the microbial activity (Zhang and Sun 2019). Bulking agents can be defined as substances having excellent water-absorbing potential, a relatively high C/N ratio, a neutral pH, and the ability to maintain a free air space of above 30%. These include woody residues, grasses, agricultural crop residues, and food wastes (Sathya *et al.* 2024). There are various technical challenges in MSW composting because of the low organic matter range and high water content. Thus, bulking agents, such as sawdust or cornstalk, are mainly added with the medium to adjust the ratio of carbon, nitrogen, and moisture content duringthe composting process. These agents can provide interspaces for smooth air diffusion and improve the composting process. Cornstalk has been used for composting processes along with MSW to mitigate leachate production and gaseous emission (Yuan *et al.* 2016).

The vegetable and food wastes, including fruit wastes, have a very low C/N ratio (<20:1) (Esparza *et al.* 2020), and total organic carbon was also low in cattle manure (20.6±0.5%) (Wang *et al.* 2019). The green leaves have a higher C/N ratio than municipal and kitchen wastes. Total organic carbon and total nitrogen content of sea plant wastes, pruning wastes with grass clippings, leaf litter, fibres, and balls of fibrous material ranged from 6.57±0.26 to 52.1±3.1. In the aerobic composting process, aerobes require a higher C/N ratio (25 to 35) because they require high energy for decomposition. But anaerobes require a lower C/N ratio (20 to 30) for composting (Guilabert *et al.* 2021). In most cases, 30:1 is considered the optimum C/N ratio; however, several authors used >40 for the composting process. The increased C/N ratio may slow down the composting process (Kumar *et al.* 2010). In the composting process, the degree of composting depends on the source, moisture content, and C/N ratio. The available C/N content in the compost influences the growth of microorganisms and activity. Cerda *et al.* (2018) reported that the C/N ratio of 25 to 50 was preferred for composting, but different biomasses with different C/N ratios (20 to 50) were used for composting. Vegetable wastes were mixed with cow dung, grasses, and food wastes in 4:3:2:1 (w/w) to achieve a C/N ratio of 37 in order to limit the odour.

Co-composting of municipal solid waste with saw dust improved the quality of the compost and provided optimum C/N ratio for composting process. This process enhanced water-holding capacity and reduced heavy metal concentrations. In some fermentation processes, green waste and compost of mushrooms were supplemented to enhance lignin degradation (Zhang and Sun 2015; Mohammadipour *et al.* 2020), whereas sugar cane bagasse was added to enhance the availability of oxygen and to control pH. In the composting process, an initial C/N ratio should be 25to30, and this ratio is generally considered as optimum. In addition, water is essential for microbial activity and should be at an optimum level. In the composting process, different types of microbial consortia are associated with various degrees of degradation (López-González *et al.* 2015). Co-composting of municipal solid waste with bulking agents, such as yard trimming waste, agricultural waste,and wood shavings enhanced the quality of the compost and increased microbial enzyme activities. The co-substrates (processed food waste and unprocessed food waste) selected in the composting process increases the amount of nitrogen and improves the degradation rate. Thus, a feedstock mixture consisting of vegetable waste, cow dung, grasses, and food wastes were selected at a 4:3:2:1 ratio. Vegetable waste and grasses were rich in cellulose, hemicelluloses, and lignin, whereas cow dung and food waste were enriched with these cellulosic materials, as well as minerals, enzymes, proteins, fat, and ions. The objectives of the study were to analyze the suitability of the selected MSW in the composting process, to determine optimum factors on composting and to analyze the microbial activity during composting. The research hypothesis was that the degree of maturity of the compost applied to the field will determine the growth and antioxidant properties of guava fruit.

EXPERIMENTAL

Materials and Methods

Composition of bulking agents for composting

Organic wastes, such as vegetable waste (remaining of uncooked cabbage, potato peel, cucumber peel and flesh, onion waste, *etc*.), cow dung, grasses, and food wastes (cooked rice, cooked vegetable waste, bread, fruit peels and flesh), were collected from Tamilnadu, India. Food and vegetable wastes were collected at Kanniyakumari, India; grasses and cow dung were collected from an agriculture farm. These wastes were dried for several days and mechanicallyblunted, and the maximum size of the particle was fixed as 0.75 mm. The biomass (vegetable waste, cow dung, grasses, and food wastes, w/w) was mixed at a 4:3:2:1 ratio, and the initial moisture content of the bulking agent was maintained at 70% (Al-Dhabi *et al.* 2019). The composition of the bulking agent was fixed based on C/N ratio of the bulking agents. The carbon and nitrogen ratio of the substrate was fixed as 37. To maintain C/N ratio as 37%, a total of 25 kg mass was used for composting, including vegetable waste (10 kg), cow dung (7.5 kg), grasses (5 kg), food waste (2.5 kg), and mature compost (starter; 2.5 kg).Mature compost was prepared earlier by composting vegetable wastes, grasses, food wastes and cow dung for one month. After one month, the maturity was determined, and used as a starter for composting in a rotary drum reactor.

Composting of MSW

Composting was performed using a pilot-scale rotary drum reactor with forced aeration, as previously described (Villaseñor *et al.* 2011). This reactor was operated as a fixed bed batch bioreactor and was capable of holding up to 100 L. The drum of the reactor was made of polypropylene with 0.7 m length and 0.5 m internal diameter and coated with a thermally insulating material. The reactor was turned using a rotary system connected with an engine at the centre of the drum. A rotameter was applied to control the inflow of air, and a valve was used to pump atmospheric air. The air was moistened to prevent the bulking agent from drying, and to maintain the optimum temperature an electrical heater was used to warm the reactor. The internal bioreactor temperature was monitored using a thermocouple and the initial reactor temperature was approximately 32±1 °C. The drum was rotated manually to recycle leachate that was accumulated at the bottom of the reactor; rotation also helped to maintain the uniform moisture content into the reactor. Seed compost was used as the starting material for effective composting. Composting was performed for 28 days.

Analytical Experiments

The moisture content of the compost was measured by drying the sample at 105 °C for 24 h in an oven with circulation (Coslab, India). The compost was extracted with double distilled water (1:10 ratio, w/v; compost:water) (Campitelli and Ceppi 2008) and pH was measured using a digital pH meter (HANNA, Mauritius). The temperature of the compost within the reactor was recorded using a thermocouple. Total carbon was evaluated using combustion method (550 °C, 5 h), as suggested by Ogunwande *et al.* (2008). The Kjeldahl digestion method was followed for the determination of Total Kjeldahl Nitrogen as suggested by the American Public Health Association (APHA 1989). Total phosphorus content of the compost was determined after acid digestion of the compost using the method of stannous chloride (Zucconi *et al.* 1985). The compost was digested using acid and used for the detection of potassium using flame photometry by the standard method (Dipietro *et al.* 1988).

Total Viable Count of the Compost

Approximately 1 g compost was mixed with 9 mL phosphate-buffered saline (PBS; pH 7.2, 0.1 M), and serial dilutions were performed. The enumeration of the bacteria from the compost was performed, and the total viable count was measured after performing the spread-plate method on nutrient agar plates (Himedia, Mumbai, India). These plates were incubated at 37 °C for 24 h, and CFU was calculated.

Experimental Design, Harvest of Guava, and Analysis

The field experimental trials were performed between June 2022 and August 2023 at Kanniyakumari, India, in which mature compost was applied at 12.5% (T1), 25% (T2), 37.5% (T3), and 50% (T4) concentrations and mixed with garden soil. The garden soil was considered a control (T5). Approximately a 1-month-old guava sapling (*Psidium guajava*) was planted in the garden soil (2×2 ft^2) and compost manure was supplemented for every 30 days (2 kg manure/square feet area). This experiment was performed to analyze the growth-promoting activity and yield of mature compost on guava plant. The experiment was performed for 15 months, and the yield was calculated. The temperature was approximately 28±2 °C. The fruit yield (fruit/plant), average weight, and diameter were measured manually. The amount of total soluble carbohydrate (TSS, %) was determined by the anthrone method, and titratable acidity (%), non-reducing sugar (%), reducing sugar (%), and vitamin C (mg/100 g wet weight) in guava fruits were estimated by the titration method.

Determination of Polyphenols and Flavonoids from the Guava Fruit

The number of polyphenols in the guava fruit was determined by the Folin Ciocalteu method. Briefly, the guava fruit (50 g) was mixed with 200 mL of double-distilled water and homogenized using a glass homogenizer. The clear supernatant was obtained after centrifugation at 5000 rpm for 10 min. The amount of polyphenols was determined using a calibration curve prepared using gallic acid, and the result was expressed in mg of gallic acid per 100 g.

Analysis of Antioxidant Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of the guava fruit and peel was determined. In this method, DPPH radicals are reduced in the presence of antioxidants, representing a color change in the solution over incubation time. To the control, only a methanol solution was added, and TROLOX was prepared at various concentrations (10 to100 ppm) and used as a standard. A guava fruit and peel sample was prepared in double-distilled water, and a 0.2-mL sample was used for analysis. The result was expressed in μmol Trolox equivalent (TE)/100 g of fresh peel or flesh.

Phytochemical Analysis of Guava Leaves

Guava leaves (100 g) were collected and dried for 10 days under the sun. The dried leaves were ground using a mixer grinder, and the sample was used for the extraction of phytochemicals. About 100 g of finely powdered guava leaves were macerated with 100% methanol, ethanol, chloroform, and n-hexane for three days. The extract was filtered with Whatman number 1 filter paper, and a clear supernatant was obtained. It was then evaporated, and residue was obtained. The bioactive phytochemical components of guava leaves, such as saponins, tannins, phenols, terpenoids, glycosides, and flavonoids, were determined.

Antimicrobial Activity

The antibacterial activity was tested against pathogenic bacteria, such as *Enterococcus faecalis*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Streptococcus pneumoniae*, which were cultured in Mueller-Hinton broth medium and incubated overnight at 37 °C. The inoculum concentration of the bacteria was set to 0.5 McFarland standards. Then, 0.1 mL of culture was spread on Mueller-Hinton agar (MHA) medium. The selected three guava leaf extracts (methanol, ethanol, and chloroform) were placed on MHA medium. Chloramphenicol was used as a positive control. After 24 h of incubation, the zone of inhibition was calculated.

RESULTS AND DISCUSSION

Co-composting of MSW with Starter Culture

Starter culture was added with the bulking agents to enhance the composting process. The results revealed that the starter culture at the thermophilic phase of the compost was suitable for composting. Based on the physicochemical factors of the compost materials described in the following subsections, the applied starter culture was efficient. Co-composting is one of the important processes for improving food waste composting. According to Varma *et al.* (2017), the starter culture from the thermophilic phase was more efficient in composting organic matter. After the initiation process, the bacteria from the compost continuouslyenhanced degradation. Generally, the compost from the thermophilic phase of finished compost is recommended to enhance the composting process. However, individual bacterial and fungal species also can be used as the starting material in any process. Aerobic fermentation is preferred for composting organic wastes at definite proportions. The bulking agents effectively provide free air passage and regulate the moisture content in the composting process. The municipal solid waste consists of various substrates, and these substrates provide nutrients to indigenous microorganisms (Raju and Divakar 2013). The reactor was loaded with vegetable waste (10 kg), cow dung (7.5 kg), grasses (5 kg), food waste (5 kg), and 2.5 kg mature compost. In a study, Awasthi *et al.* (2016) employed enriched nitrifying bacteria at a 10% level for co-composting the organic fraction of the municipal solid waste, sludge from thegelatine industry, and poultry waste at 1:6:0.5. Recently, Zhang and Sun (2016) reported the use of co-composting of the organic fraction of the municipal solid waste and sewage sludge. The composting process was effective when cornstalk was added at 15% with sewage sludge. Food waste and green waste were previously used for organic waste management. These organic wastes were used for the conversion of products through solid-state fermentation. In organic waste composting, mature compost was added to improve composting. The supplemented mature compost consists of microorganisms, which improve composting (Liu *et al.* 2023; Sánchez 2022; Wang *et al.* 2022). The fermented food waste was applied to improve the growth of microorganisms and used in environmental management (Quan *et al.* 2023; Cheng *et al.* 2023).

The application of a starter culture for the initiation of the composting process has been previously suggested. Xi *et al.* (2015) reported that the initial microbial population is insufficient in organic matter composting, and therefore Song *et al.* (2016) reported additional microbes for composting the matters that are rich in lignocellulosic materials. For the complete degradation of cellulose and lignocelluloses materials, the combination of β–glucosidase, exoglucanases, and endoglucanases are required. These enzymes are inducer types, and the starter culture obtained from the composted MSW has the potential properties to degrade cellulose biomass. Individual strains and microbial consortia also have been used as the starter culture in the composting of organic substances. Inoculation of microbial consortium, such as *Trichoderma viride*, *Phanerochaete chrysosporium*, and *Pseudomonas aeruginosa*, with bulking agent reduced the composting period (Awasthi *et al.*2015). Many physical and chemical factors, such as timing of inoculation (stage) and the concentration of inoculum, also influenced the composting process (Xi *et al.* 2015). Generally, mature compost can act as a more potent starter than individual culture, because the latter has less specificity to compost types of substrates.

Changes of Moisture Contents with Time

Moisture content is one of the most important factors affecting composting processes. Generally, the rate of compost of organic waste is associated with moisture loss. The moisture content of the composting material was monitored for up to 28 days and reduced slowly up to 11 days, then decreased dramatically from 12 days onwards. The initial moisture content of the composting material was 67%±4% anddecreased to 40%±3% after 28 days. The average moisture content was 48%±3% (Fig. 1). The optimum moisture level is an important factor for the metabolism of existing bacteria, fungi, and actinomycetes from the composting materials because moisture content indirectly provides oxygen. Adequate moisture content was provided for biodegradation, and the optimum level ranged between 35% and 60%. In a study, Rawoteea *et al.* (2017) used paper waste, vegetable wastes, and bulking agent at different compositions for thecomposting process. The observed moisture level of the compost ranged between 62% and 68%. Moisture content decreased in the first two weeks and increased in the third week. In a study, Sarpong *et al.* (2019) reported 40%to60% moisture level as the optimum for the composting process, and these levels provided optimal aeration. According to Bernal *et al.* (2009), a moisture level of less than 40% decreased microbial activities. High moisture content (>60%) in the composting materials caused environmental odor and nutrient leachate (Dortmans 2015). The moisture content of the compost influences bacterial activity, and the growth of beneficial organisms is severely affected by a very low moisture content. The moisture content was associated with the porosity of the bulking agent, temperature, oxygen uptake and microbial activity. The decreased level of moisture after 28 days inferred optimum maturity of the bulking agents.

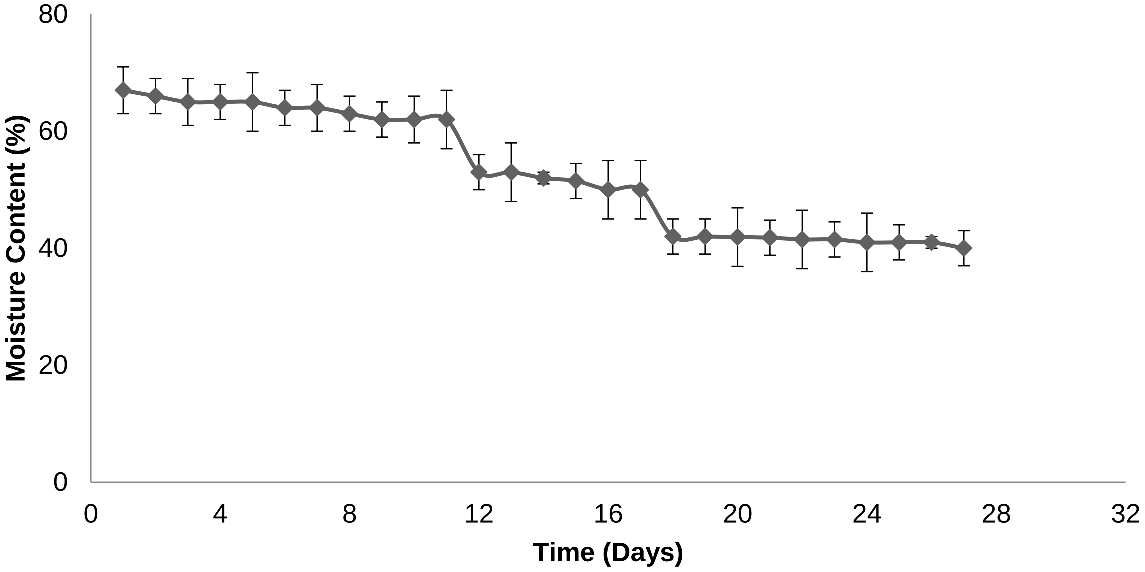

Fig. 1. Moisture content of the compost at various incubation times. Municipal solid waste was used as the composting material and incubated for 28 days. Error bar represents standard deviation (n=3).

Changes of Temperature with Time

The starting temperature of the compost was 28±2 °C, and it increased to 58±2 °C after 8 days (thermophilic stage). The temperature declined to 54± 2 °C after 10 days of the composting process (Fig. 2). Temperature, aeration and moisture content collectively stimulated microbial activity within composting biomass; these factors critically affected the rate of biodegradation of the organic waste during the composting process. Rastogi *et al.* (2019) reported that ambient temperature favored MSW composting. The temperature range from 50 to 55 °C was optimum for microbial composting. These higher temperatures eliminated pathogenic microbial consortium from the compost. Varma and Kalamdhad (2015) reported high heat generation (>70 °C) in the compost due to microbial activity. In MSW composting, the compost temperature reached about 50 °C after 8 days of composting, and this excess heat generation occurred due to quick degradation of fats, proteins, and sugars (Troy *et al.* 2012). The rise in temperature during the composting process confirmed microbial activity in the reactor. Based on the composition of bulking agents, moisture-holding capacity varied and affected the microbial activity. Further, microbial activity directly reflected heat generation in the compost and decreased temperature after two weeks, indicating a cooling phase. Aeration of the compost enhanced aerobic metabolism, and high temperature could reduce the pathogenic bacterial load from the compost (Dortmans 2015).

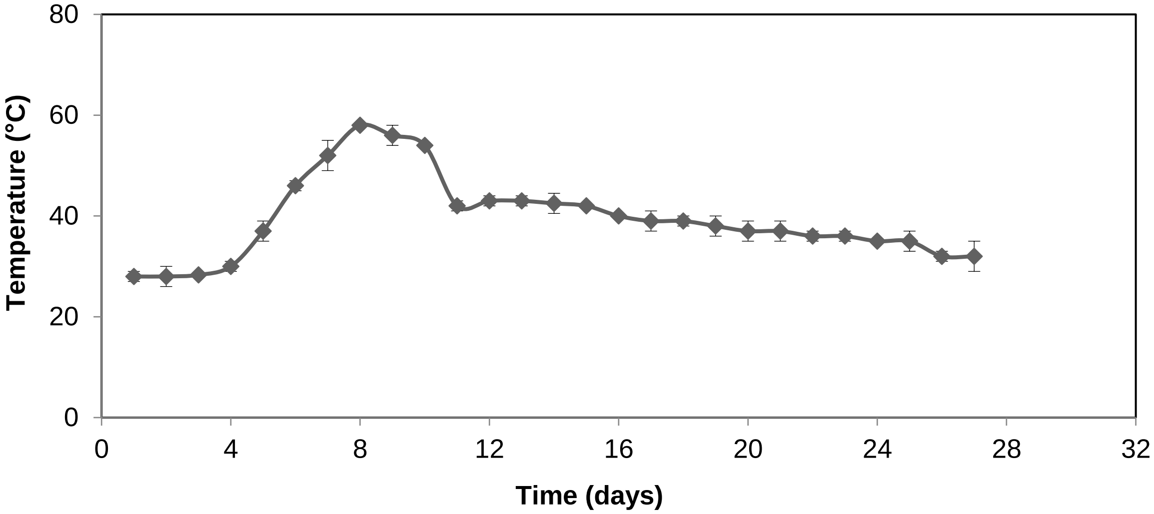

Fig. 2. Temperature of the compost at various incubation times. Municipal solid waste was used as the composting material and incubated for 28 days. Error bar represents standard deviation (n=3).

Changes of pH with Time

ThepH is one of the critical factors affecting the fermentation process. During the composting process, the pH value was observed to be between 7.5 and 8.1. The pH of the compost was decreased considerably after 15 days of incubation (Fig. 3).

Due to protein degradation by the action of proteolytic bacteria, a rise in pH in the compost was reported, which can be attributed to microbial decomposition and volatilization of various nitrogenous substances. At pH values of6.0 or less, the decomposition process was much less; likewise, an unpleasant smell was generated at higher pH values. Previous findings revealed that pH range between 5.5 and 8.0 was suitable for microbial activity (Zhang and Sun 2016). Lim *et al.* (2015) reported that increases in high pH in the compost were due to the presence of various organic matters in municipal waste, other than plant biomass.

At the thermophilic stage of the composting process, the pH of the medium decreased considerably. After two weeks of composting process, pH varied between 7.2 and 7.4. Zhang and Sun (2016) reported the ideal pH value for composting ranged from 5.5 to 8.0; however, Bernal *et al.* (2009) suggested the pH range between 6.7 and 9.0 for an effective composting process. Recently, Vaverková *et al.* (2020) used green waste for composting process and the pH of the compost was determined as 8.89 and 7.40 in two different composting systems.

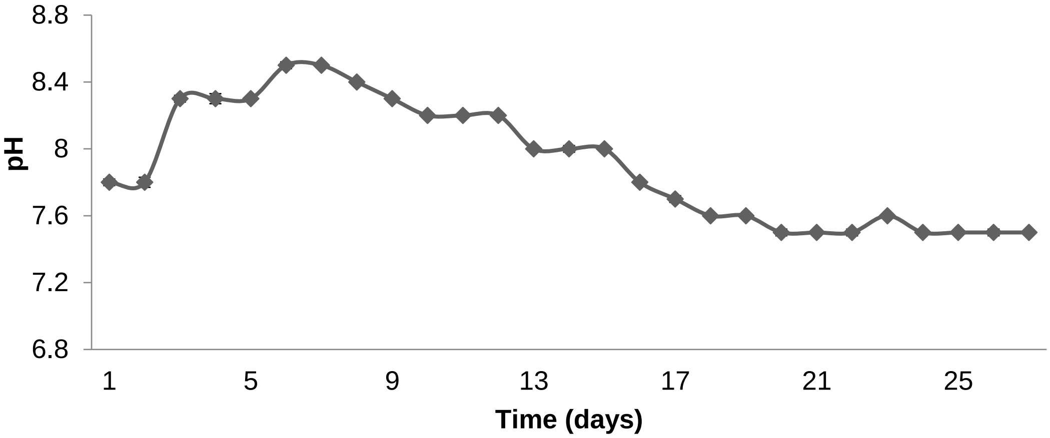

Fig. 3. pH of the compost at various incubation times. Municipal solid waste was used as the composting material and incubated for 28 days. Error bar represents standard deviation (n=3).

Changes of Carbon and Nitrogen Content of the Compost

The maturity of the compost can be identified by analyzing C/N ratio. The moisture content, carbon, nitrogen, and C/N ratio of the bulking agents is described in Table 1. The total organic carbon content of the compost was 37.6%±0.02% after day 1, and it decreased to 34.5%±0.27% after two weeks. After 28 days of composting, carbon content reduced to 27%±2.9%. The total nitrogen content of the compost was 1.78%±1.12% after 1 day of incubation, and it increased rapidly after 8 days (2.8%±0.28%) (Table 2). The increased value of nitrogen content implied the degradation of organic carbon due to the volume reduction of organic materials in the composting unit by microbial action. The recommended ratio of carbon and nitrogen level of the compost should be less than 15. In this experiment, the C/N ratio was 13.6±0.05 after maturation (28 days). The reduced level of C/N ratio (<15) showed growth-promoting activity (Pan *et al.* 2012); hence, the compost obtained was useful for agriculture. The lower C/N ratio (<20) effectively involved in mineralization and was highly preferable for plants (Yadav and Garg 2009). Microbial consortia from the compost have a major role in the reduction of C/N ratio. These organisms utilized carbon sources for their growth and metabolism. Most of the organisms synthesized hydrolytic enzymes, and these reduced the amount of bulking agents in the compost. Degradation of organic materials varied widely, based on the type of substrates used for microbial composting, mainly depending on the amount of hemicelluloses and cellulose in the compost. During composting, process bacteria utilize about 70% carbon sources as CO_2 (metabolic end product) and use 30% for their growth (Yadav and Garg 2009). During this process, the mass of the compost decreased considerably. In addition, the generation of ammonia was reported by Jiang *et al.* (2014) during composting due to the decomposition of amino acids and proteins. Due to the mineralization of various available organic substances, nitrogen content in the compost increased. In this experiment, the C/N ratio was considerably reduced, and the decreased C/N ratio (13.6) was mainly due to the increase of total nitrogen and the reduction of total organic carbon. The initial C/N ratio was approximately 37%, and this clearly showed nutrient balance in the compost mix. In a study, Yang *et al.* (2015) recommended C/N values between 20 and 50 for organic degradation. Nevertheless, Kutsanedzie *et al.* (2015) reported that C/N values ranged from 25to35 for efficient compost mix. A higher C/N ratio decreased nutrient deficiency to microbiota and enhanced degradable N content per C. The declined C/N ratio during the composting process can be attributed to degradation of carbon-rich agro-waste intonutrient-rich, biologically stable soil. Hence, to optimize the C/N ratio, bulking agents (*e.g.*, wood chip, peanut shell, rice husk, and sawdust) are recommended to develop enhanced porosity in the bulking agent (Zhang and Sun 2016).

Table 1. Nutrient Content of Selected Bulking Agents

Nutrients	Vegetable Waste	Cow Dung	Grasses	Food Waste
Moisture	91.38±1.31	13.8±2.7	12.7±4.1	96.2±1.3
Carbon content (%)	46.4±2.15	59.2±2.9	53.4±2.3	36.2±1.64
Nitrogen content (%)	1.08±0.051	1.32±0.31	1.82±0.32	2.64±0.51
C/N ratio	42.96±1.03	44.88±2.81	29.2±0.73	13.71±0.17

Table 2. Total Organic Carbon and Nitrogen Content of the Compost

Time (Days)	Total Organic Carbon (%)	Total Organic Nitrogen (%)
1	37.6 ± 0.02	1.8 ± 0.12
2	37.6 ± 0.03	1.8 ± 0.09
3	37 ± 0.02	2 ± 0.11
4	37 ± 0.03	2.2 ± 0.13
5	36 ± 0.12	2.3 ± 0.13
6	36 ± 0.36	2.31 ± 0.06
7	35.8 ± 0.24	2.3 ± 0.12
8	35.7 ± 0.28	2.6 ± 0.1
9	35.6 ± 0.52	2.8 ± 0.28
10	35.6 ± 0.28	3.1 ± 0.24
11	35.5 ± 0.17	3.2 ± 0.22
13	35 ± 0.01	3.3 ± 0.18
14	34.5 ± 0.27	3.5 ± 0.03
15	34.5 ± 0.49	3.6 ± 0.23
16	38.6 ± 0.61	3.7 ± 0.14
17	38.7 ± 0.82	3.9 ± 0.91
18	38 ± 0.71	3.3 ± 0.13
19	37 ± 0.75	3.2 ± 0.07
20	36 ± 0.82	3.2 ± 0.42
21	35 ± 0.64	3 ± 0.18
22	34 ± 0.28	2.9 ± 0.37
23	33 ± 1.3	3 ± 0.42
24	27 ± 2.6	3 ± 0.28
25	27 ± 0.75	3.2 ± 0.38
26	27 ± 4.1	3.3 ± 0.32
27	27 ± 1.3	3.5 ± 0.14
28	27 ± 2.9	3.6 ± 0.27

Municipal solid waste was used as the composting material and incubated for 28 days (n = 3)

Improvement of Macronutrient in the Compost

The amounts of potassium and phosphorus were monitored during the composting process; generally, these nutrient levels increased during the composting process. On day 1, phosphorus was 3.1± 0.13 g/kg, and it increased to 8.16± 0.8 g/kg after 28 days. Potassium content was 4.2± 1.1 g/kg after day 1, and the maximum amount was detected after 28 days (8.6± 0.48 g/kg) (Table 3). An increasing trend of potassium was reported by Ogunwande *et al.* (2008) in chicken litter compost. Phosphate is a less mobile substance than potassium and forms very strong bonds with Mg, Ca, insoluble phosphate, and organic matter. According to Henry and Harrison (1996), phosphate content in the compost increased. Iqbal *et al.* (2015) also reported improved levels of phosphate and potassium in municipal solid waste compost. In a study, co-composting was performed using cow dung and *Parthenium* at 1:2 ratio, and it was observed that total phosphorus and potassium increased 4.03- to 3.33-fold, and 1.65- to 1.83-fold, respectively (Rai and Suthar 2020).

Table 3. Phosphorus and Potassium Content of the Composting Material (n=3)

Time (Days)	Phosphorus (g/Kg)	Potassium (g/Kg)
1	3.1±0.13	4.2± 1.1
2	3.1± 0.05	4.3± 0.4
3	3.3± 0.12	4.4± 1.3
4	3.5± 0.01	5.3± 0.8
5	3.7± 0.031	5.5± 0.51
6	3.8± 0.032	5.7± 0.27
7	4± 0.031	5.8± 0.27
8	4.1± 0.04	5.9± 0.31
9	3.6± 0.02	6± 0.04
10	5.3± 0.008	6.1± 1.1
11	5.7± 0.31	6.3± 0.9
13	6± 0.031	6.5 ± 1.2
14	6.2± 0.02	7± 1.3
15	6.3± 0.03	7.4± 2.1
16	6.5± 0.41	7.4± 0.9
17	7.3± 0.26	7.5± 1.2
18	7.4± 1.2	7.8± 0.2
19	7.5± 0.98	7.9± 1.1
20	7.6± 1.1	8± 0.41
21	7.6± 0.9	8.1± 1.4
22	7.7± 0.26	8.2± 0.9
23	7.7± 0.87	8.3± 1.2
24	8± 0.71	8.3± 0.71
25	8± 0.96	8.4± 0.11
26	8.02± 1.2	8.5± 0.19
27	8.1± 1.1	8.5± 0.42
28	8.16± 0.8	8.6± 0.48

Bacteria Load on Municipal Solid Waste at Various Maturation Stages

The co-composting process improved the microbial population. The total viable count of bacteria was determined by the Agar plate method. The population kinetics of bacteria in the compost is described in Fig. 4. The initial bacterial count was determined as 3±0.2 × 10^6 CFU/g compost after day 1. After 8 days of incubation, the microbial population was enhanced (72±2 × 10^6 CFU/g compost) and then depleted to 31±0.3 × 10^6 CFU/g compost during the thermophilic stage. The succession or dynamics of a microbial community within composting indicates their degrading ability. In each stage, nutrient availability and temperature varied, thus critically affecting the abundance of microbes. The present findings revealed that at the mesophilic stage, the bacterial population was higher than in other stages. The mesophilic bacterial strains usually dominate in the preliminary decomposition process, and a large amount of organic matter degradation takes place in this phase. The microbial population has been varied widely based on the different phases (Wang *et al.* 2018). In the thermophilic phase, the bacteria population was severely affected, and previous findings revealed that fungal and bacterial communities varied based on temperature. Huhe *et al.* (2017) earlier reported a high bacterial population and reduced fungal population at the thermophilic stage in horse and cattle manure composting. Recently, co-composting of biochar with contaminated soil was performed to improve colonization of bacteria and fungi during composting. According to Vishan *et al.* (2014), the mesophilic bacterial population was high in both mesophilic and thermophilic stages and involved in intense organic matters degradation. Increased microbial population in the compost reflected rapid and extensive degradation of the compost, whereas a decreased microbial population at the end of the compost reflected stability of the compost. This tendency of variation in the microbial population in the compost was also observed by Ghinea and Leahu (2020). Microbiota utilized compost materials and has a great influence on the quality of the final compost. Co-composting of cowdung and *Parthenium* at 1:2 ratio decreased *E. coli* and *Salmonella* in the mature compost and reacheda safer limit (Rai and Suthar 2020).

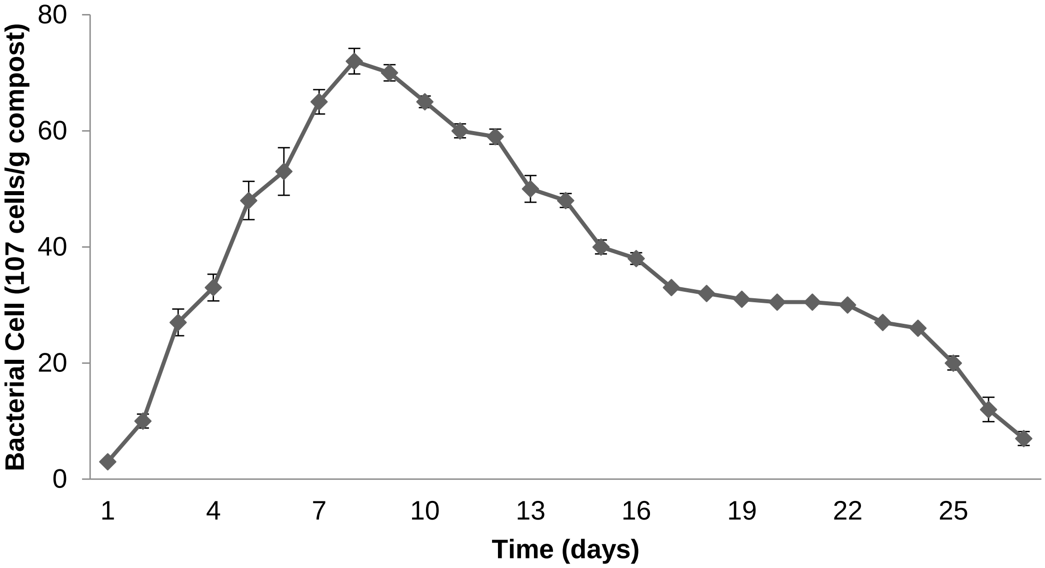

Fig. 4. Bacterial population in the composting materials; Compost was diluted with double distilled water and plated on nutrient agar at various dilutions. All plates were incubated at 37 °C for 24 and the colonies were counted.

Mature Compost Improved the Yield and Bioactive Compounds

The supplemented compost matter was found to significantly influence the guava crop and quality. The improvement in crop yield and good quality of guava observed in the treatment plant with increased concentrations of compost matter showed promise compared to the untreated control plant. The yield of the guava plant increased significantly in the treatment groups; however, no significant variation was observed between the T3 and T4 experiments, except for the for the diameter of the fruits. The number of guava fruits per guava plant was influenced by increasing concentrations of compost ($p<0.05$) (Table 4). Similarly, average fruit was highest with guava plants treated with 50% compost matter. The amount of total titratable acidity, total soluble sugar, and vitamin C were significantly influenced by the amount of compost matter (Table 5). The improvement of these biochemicals in guava fruits due to the supplementation of compost might be due to their plant-growth-promoting properties, available nutrients, and trace elements required for plant growth.

Table 4. Effect of Compost on the Growth and Yield Analysis of Guava Fruit in the Field

Treatment	Yield (Fruit/Plant)	Average Weight (g)	Average Diameter (cm)
T1	19 ± 2^{a}	150.2 ± 4.2^{a}	6.5 ± 0.4a
T2	35 ± 1^{b}	173.5 ± 1.4b	7.4 ± 0.1b
T3	42 ± 2^{c}	188.2 ± 2.3c	7.8 ± 0.2c
T4	43 ± 1^{c}	195.4 ± 1.1d	7.9 ± 0.21c
T5	13 ± 0^{d}	126.2 ± 5.2e	5.9 ± 0.43d

Table 5. Total Titratable Acidity, Total Soluble Sugar,and Vitamin C of Guava Fruit Treated with Compost in the Field

Treatment	Titratable Acidity (%)	TSS (%)	Vitamin C (mg/100 g)
T1	0.342 ± 0.04^{a}	11.4 ± 0.48^{a}	130.5 ± 3.8^{a}
T2	0.386 ± 0.028^{b}	15.3 ± 0.52^{b}	159.2 ± 1.7^{b}
T3	0.475 ± 0.016^{c}	18.2 ± 0.14^{c}	164.8 ± 1.2^{c}
T4	0.482 ± 0.02^{c}	18.5 ± 0.25^{c}	170.1 ± 2.9^{d}
T5	0.271 ± 0.12^{d}	10.3 ± 0.19^{d}	121.4 ± 1.4^{e}

Values are the mean ± standard deviation (n=3), TSS represents total soluble sugar

Polyphenols and Flavonoids From the Guava Fruit Treated with Compost in the Field

The results for the total phenolic content of the peel and flesh of guava fruits are depicted in Table 6. The total phenolic content varied between the treatment and control groups. A great variation was observed between the experimental group (16.4 ± 0.3 mg GAE/g) and the control (8.7 ± 0.1 mg GAE/g) group. The higher amount of phenolic compounds was determined in guava peel than flesh. Likewise, the amount of flavonoid content increased in experimental guava plants treated with organic amendments. The higher amount of flavonoids was determined in guava peel than in the flesh ($p<0.05$).

Table 6. Total Polyphenols and Flavonoid Content from the Peel and Flesh of Guava Fruit

Treatment	Phenols (mg GAE/g)		Flavonoid (mg CE/g)	
	Peel	Flesh	Peel	Flesh
T1	9.3 ± 0.4^{a}	8.5 ± 0.04^{a}	3.41 ± 0.05^{a}	2.91 ± 0.1^{a}
T2	10.4 ± 0.1^{a}	9.5 ± 0.08^{b}	3.94 ± 0.02^{b}	3.2 ± 0.2^{a}
T3	15.2 ± 0.2^{b}	13.7 ± 0.1^{c}	4.15 ± 0.1^{b}	3.9 ± 0.1^{b}
T4	16.4 ± 0.3^{b}	13.9 ± 0.2^{c}	5.2 ± 0.2^{c}	4.2 ± 0.2^{b}
T5	8.7 ± 0.1^{c}	7.9 ± 0.2^{a}	3.2 ± 0.1^{a}	2.82 ± 0.3^{a}

Values are the means ± standard deviation (n=3), GAE represents gallic acid equivalent, CE represents catechin equivalent

2,2 '-Diphenyl-β-Picrylhydrazyl Radical Scavenging Activity of Guava Fruit

Figure 1 shows the antioxidant activity of the methanol extract of guava fruit. The peel showed higher antioxidant activity than guava flesh (Fig. 5).The DPPH activity was 95.1 5 EC50 µg/mL in the control peel, and the T4 exhibited maximum DPPH activity (130.5 EC50 µg/mL flesh). Likewise, control flesh showed 84.2 EC50 µg/mL of DPPH activity, and T4 exhibited significant DPPH activity (109.2 EC50 µg/mL).

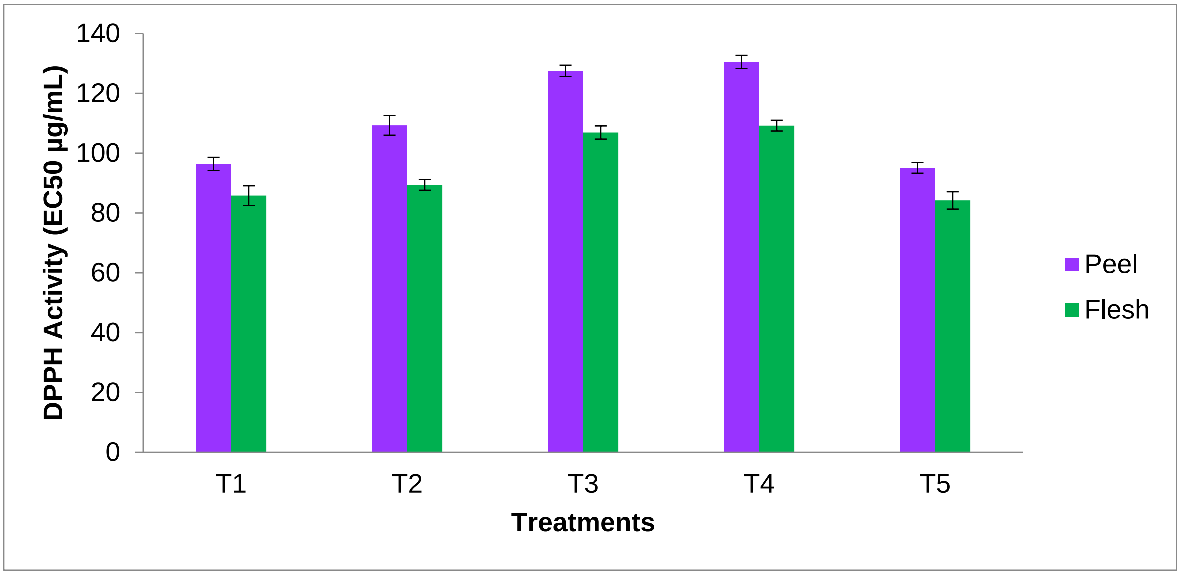

Fig. 5. DPPH scavenging activity of methanol extracts from guava fruit crop cultivated with organic compost

Compost Improved Phytochemical Compounds of Guava Leaves

The phytochemicals of guava leaves are summarized in Table 7. A qualitative analysis revealed the presence of tested phytochemical compounds in the solvent extract. The findings revealed the presence of active compounds in the solvent extract. The ethanol solvent exhibited all tested phytochemicals, whereas methanol showed all compounds except glycosides.

Table 7. Phytochemical Components of Guava Leave Extract

Extracts	Saponins	Tannins	Phenols	Terpenoids	Glycosides	Flavonoids
Methanol	+	+	+	+	-	+
Ethanol	+	+	+	+	+	+
Chloroform	-	+	+	+	+	+
n-Hexane	-	-	+	-	-	-

Antibacterial Activity of Guava Leaves

The antibacterial activities of crude guava extract *vs*. six bacterial pathogens were analyzed by determining the zone of inhibition (mm), as shown in Table 8 and Fig. 6.

Table 8. Antibacterial Activity of Guava Leaves Extract *vs.* Bacterial Pathogens

Bacteria	Zone of Inhibition (mm)		
	Methanol	Ethanol	Chloroform
E. faecalis	17 ± 1^{a}	14 ± 1^{b}	10 ± 0^{c}
B.subtilis	13 ± 1^{a}	15 ± 0^{b}	12 ± 2^{a}
K.pneumoniae	17 ± 0^{a}	15 ± 2^{b}	13 ± 1^{c}
S. pneumoniae	17 ± 0^{a}	14 ± 1^{b}	12 ± 0^{c}
P. aeruginosa	17 ± 1^{a}	12 ± 0^{b}	9 ± 0^{c}
S. typhi	15 ± 2^{a}	13 ± 1^{b}	9 ± 0^{c}

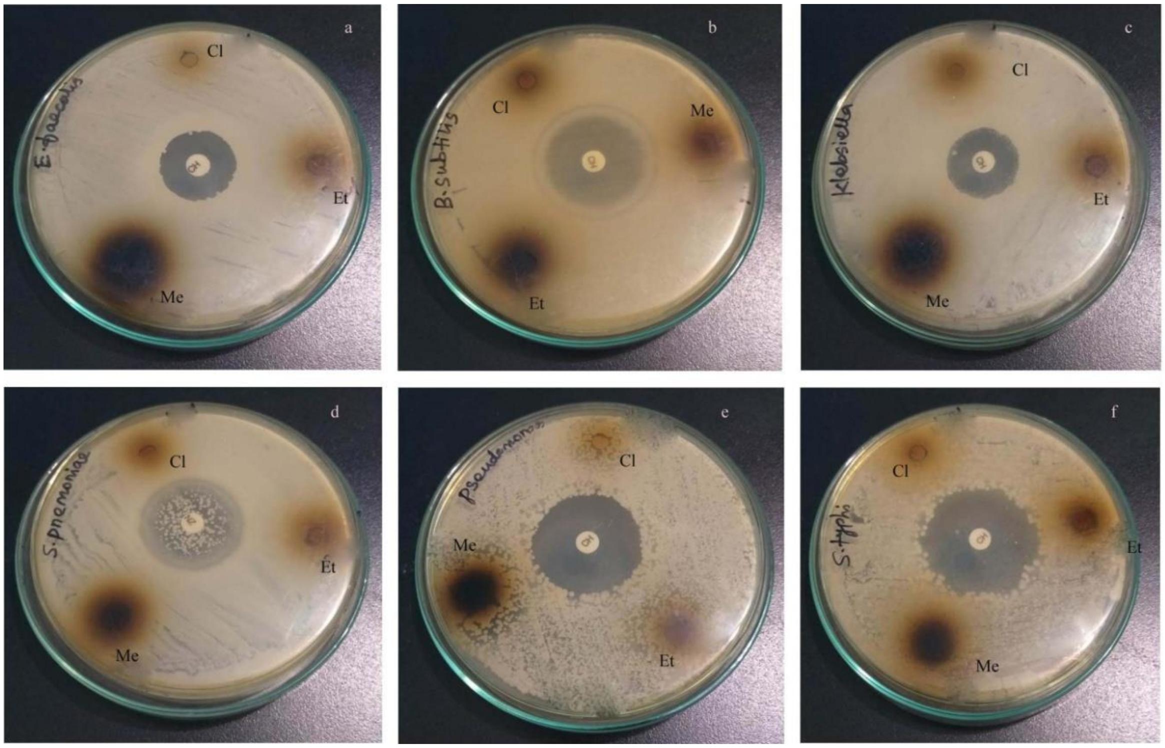

Fig. 6. Antibacterial activity of guava leaves extract against bacterial pathogens (Me-methanol extract; Et-ethanol extract, and Cl-chloroform extract).A total of 20 µg extract was loaded on the disc and antibacterial activity was tested after 24 h incubation. A: *E. faecalis*; B: *B. subtilis*; C: *K. pneumoniae*; D: *S. pneumoniae*; E: *P. aeruginosa*; and F: *S. typhi*

Psidium guajava peels showed potential activity against *E. faecalis*, *K. pneumoniae*, and *P. aeruginosa*. The methanol extract exhibited better activity than ethanol and chloroform. The available phytochemical components in the methanol extract were high, which influenced the antibacterial activity of the extract.

Application of mature compost improved the vegetative growth, nutrient factors, and bioactivities of guava leaves and fruits. The guava plants treated with compost improved total soluble sugar, titratable acidity, and vitamin C concentration. Improvement of these bioactive compounds in the guava fruits can be attributed to the supplementation of compost, which acts as a plant growth promoter. The supplemented organic compost improved phytochemical compounds. The amount of phenols and flavonoids in guava fruit was higher in plants grown in supplemented compost than in control plants. The compost manure consists of carbon, nitrogen sources, growth factors, and minerals. The organic compost allows plants to readily utilize the available nutrients. Hence, the supplementation of organic amendments improves the growth, yield, and bioactive compounds in plants and crops (Rady *et al.* 2016; Afriyie and Amoabeng 2017). The antioxidant activity was determined by the DPPH method, and the guava fruit peel exhibited more antioxidant activity than the flesh. The antioxidant property of guava fruit was previously reported (Liu *et al.* 2018; Almulaiky *et al.* 2018), and the increased level of phenolic compounds influenced antioxidant activity. The organic compost supplements improved plant growth, nutrient contents, phytochemical compounds, and antioxidant activities in fruits (Machado *et al.* 2020; Machado *et al.* 2021). The guava leaf extract showed antibacterial activity, and the antibacterial activity effect was directly related to the available phytochemicals. The antibacterial effect of phytochemicals in guava leaves was described previously (Mailoa *et al.* 2014; Elchaghaby *et al.* 2022).

CONCLUSIONS

1. Composting of the municipal solid wastes, such as vegetable waste, grasses, and food waste, with mature compost constitutes a feasible method for the generation of mature compost, and environmental management.
2. The use of these locally available bulking agents reduced the degradation rate of organic matter, nitrogen loss, and reduced the temperature during the process of composting.
3. The decrease in the ratio of C/N indicated the maturity of the compost within 28 days.
4. The microbial population increased in both mesophilic and thermophilic stages and decreased at the end of the composting, reflecting stability.
5. The mature compost improved guava yield and phytochemical compounds increased in the fruit and peels.
6. The phytochemical compounds improved antioxidant activity in the guava fruits.

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