

Co-composting of Food Waste and Leaves as a Way to Improve Soil Microbial Activity and Green-gram Growth

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Mixtures of vegetable waste and leaves were used to prepare compost in piles. The carbon/nitrogen ratios of the biomass varied among the selected agricultural waste. Co-composting was carried out for six weeks with a starter culture. The organic matter of the leaves declined after four weeks of composting, and a stable compost was obtained after 42 days of composting. The compost temperature reached its maximum after 12 days and moisture content declined continuously up to 42 days. The pH value of the compost increased slowly during composting and reached a maximum after six weeks. The electrical conductivity of the compost was suitable for plant nutrition. The organic matter, as organic carbon, declined, and organic nitrogen was increased. The carbon/nitrogen ratio of the mature compost was decreased. The decreased levels of carbon/nitrogen ratio reflected the maturity of the compost. The organic matter (%) was maximum (>50%) before composting process and it decreased gradually. The seed germination index was higher after 42 days of composting. The compost-treated plant improved yield and green gram seeds showed the presence of antioxidant enzymes.

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INTRODUCTION

Fermentation of foods with probiotic *Lactobacillus plantarum* has attracted increasing interest in recent years. Bacteria can improve the availability of nutrients and improve the health benefits of fermented food derived from organic amendments. Composting is a useful method to convert organic matter into useful products (Azim *et al.* 2018). The presence of inappropriate nutrient compositions in the selected biomass, moisture content, carbon, and nitrogen sources, pretreatment, and composition of wastes influence composting (Chen 2016; Arokiyaraj *et al.* 2024). The increased urbanization, human population, and economic growth influence the steep increase in organic waste (Gouveia and Prado 2010). Composting with more than one substrate allows for an optimum carbon-nitrogen ratio in the compost mixture. Hence, kitchen waste and wasted food items are mixed with leaves to improve the nutrients of the compost. The details of the composting operation may be adjusted in terms of duration, structural characteristics, chemical properties, and leachate formation (Reyes-Torres *et al.* 2018). Biomass, such as wheat straw, wood chip, agro-residues, and rice husk, are used as an effective substrate in compost (Adhikari *et al.* 2008). The nutrient status of the compost is based on the nutrient

composition of the biomass. The variation of available organic nutrients in the bulking agent can depend on the organic waste, season, and country of origin (Bandara *et al.* 2007).

Analysis of composting material, including physicochemical properties, microbiological populations, and soil microbial activity, is useful to predict compost maturity. Microbial communities degrade organic matter and improve soil nutrients and availability. The mature compost consists of a cell wall of dead microorganisms and easily utilizable compounds; a cell wall of bacteria, including sugar molecules; and several intermediate compounds. The developing intermediate compounds are called humic acids. The available humic acids improve soil ecology, soil fertility, soil structure, and plant growth-promoting agents. The humic acid level in the composting affects the maturity of the bulking agents. The maturity of the compost has been evaluated by analytical methods and microbiological observation (Huang *et al.* 2006). The bulking agents, such as green waste, municipal solid waste, cattle manure, and sewage sludge, can be characterized by analytical and microbiological methods (Al-Dhabi *et al.* 2019). In the composting process, maturity is affected by various factors, including time, pH, particle size, porosity, moisture content, carbon and nitrogen ratio, temperature, water content, and oxygen level (Li *et al.* 2013; Sathya *et al.* 2024). Determination of bulk density, temperature, porosity, nutrient content, moisture, and oxygen supply are important criteria to monitor compost (Iqbal *et al.* 2015). The microbial population in the bulking agent, organic matters, and environmental factors regulate composting (Hueso *et al.* 2012). The main objective of this study was to analyze the co-composting of agricultural residues and food waste. The matured compost was applied to improve the growth of the green gram plant. The green gram seed was harvested and antioxidant potential was analyzed and used for the preparation of probiotic food by *Lactobacillus plantarum*.

EXPERIMENTAL

Feedstock

Agricultural residues (AR) and food waste (FW) were collected and used for composting. The food waste was composed of raw and cooked vegetables, including the remnants of brinjal, carrot, beetroot, cabbage, lady finger, onion, coconut husk, cucumber, and potato. (Chen 2016). The agro-residues were composed of neem tree leaves, lemon tree leaves, and cucumber leaves. The green leaves were cut into small pieces and stored for further use. To improve composting, mature compost was used as a starter. The starter culture was prepared in the laboratory. Vegetable waste and leaves mixture (100 g each) were mixed at 1:1 ratio in an Erlenmeyer flask. Co-composting was performed for 60 days without any prior sterilization. The co-composting conditions were: pH6.41, 74.3% moisture content, incubation time-two months and 30±1 °C incubation temperature. This allowed for the growth of indigenous microbial communities. The sample was withdrawn every 10 days, and the germination index was analyzed. The compost with >80% germination index was considered for mature compost (Zhang *et al.* 2018). During the composting process, 20% mature compost was used as an effective inoculum concentration (Yeh *et al.* 2020).

Composting

A mixture of AR and FW at a 1:1 ratio was used for compost preparation. The initial moisture content was 67%, the organic matter concentration was 58%, and the

carbon/nitrogen (C/N) ratio was 29.5. A pile was constructed (0.5 m high, 1.5 m wide, and 2.5 m long), and the composting process was initiated after the inoculation of starter material. The maturity of the compost was determined after 42 days of the composting process (Day and Shaw 2001).

Analytical Methods

The moisture content of the compost was determined by drying the sample at 105 °C for 6 h. The sample (500 g) was mixed with 5 L of double-distilled water. It was filtered and used for pH and electrical conductivity (EC) determinations. The mineral content of the sample was determined by the photometric method. Total Kjeldahl nitrogen, organic content, total phosphorus, and total organic carbon were determined as described earlier (Zucconi *et al.* 1985; Thompson *et al.* 2002).

Seed Germination Analysis

The filtered compost (10 mL) was added to a Petri dish, and filter paper was placed on it. Then, 50 green gram seeds ($n = 50$) (*Vigna radiata*) were placed on the wet filter paper and incubated for 72 h at 28 ± 1 °C. Tap water was considered a control sample. The germination index was calculated using the following formula:

$$GI(\%) = \frac{\text{Seed germination} \times \text{root length of treatment} \times 100}{\text{Seed germination} \times \text{root length of control}} \quad (1)$$

Determination of Soil Microbial Activities

Green gram seeds ($n = 10$) were surface sterilized with a sodium hypochlorite solution (2.5%) for 3 min. An earthen pot was used for this experiment, and each pot was filled with 2 kg of sterilized soil. The seeds were sown in a pot and maintained for two weeks. After two weeks, compost at various stages (7 to 42 days) was added. It was maintained for 90 days, and plant growth and antioxidant activities were determined.

Determination of Soil Enzyme Activities

The earthen pot soil (2 g) was treated with 0.5 mL of toluene. An enzyme reaction was performed at the optimum pH value (pH 6.8), and acid phosphatase activity was determined. Alkaline phosphatase activity was determined by adding 0.5 mL of *p*-nitrophenyl phosphate solution (0.025 M) to the filtered sample and incubating for 60 min at 37 °C. In this reaction mixture, 0.5 M CaCl₂ was added in an alkaline condition and incubated for 15 min. The sample was filtered, centrifuged at 5000 rpm for 10 min, and the absorbance was read at 410 nm. To determine soil dehydrogenase activity, the soil sample was mixed with a CaCO₃ solution. To the preweighed soil (10 g), 0.2 mL of 2,3,5-triphenyltetrazolium solution and 10 mL of deionized water were added. The mixture was incubated at 32 °C, and methanol (10 mL) was added. The material was filtered, and the absorbance of the filtrate was read at 485 nm.

Antioxidant Enzymes

The antioxidant properties of seeds were determined. Briefly, the harvested seeds (5 g) were ground with buffer (phosphate buffer, pH 7.2, 0.1 M) and centrifuged at 10,000 rpm for 5 min. To the sample (0.5 mL), 1% (v/v) guaiacol was added and mixed. After 5 min, 0.2 mL of hydrogen peroxide was added, and the absorbance of the sample was read at 470 nm. The amount of seed phenylalanine ammonia lyase activity was determined by the spectrophotometer method. To determine polyphenol oxidase activity, the sample was

mixed with proline. To this reaction mixture, catechol was added. The sample was read at 546 nm against a blank (Malar *et al.* 2020).

Preparation of Fermented Green Gram Milk

Probiotic culture

The *Lactobacillus plantarum* (MTCC 1325) strain was used for the fermentation of green gram. Bacterial culture was inoculated in deManRogosa Sharpe (MRS) broth medium, incubated for 18 h, and used for fermentation.

Preparation of fermented green gram milk and fermentation

A green gram (5 g) was harvested from the plant cultured in the greenhouse. It was cleaned, rinsed with tap water, and soaked for 10 h. The green gram was drained and homogenized with sterile water. The mixture was filtered using a muslin cloth. It was placed for 2 h at 4 °C, and the raw green gram milk was obtained. About 20 mL of milk was autoclaved, cooled, and inoculated with *L. plantarum* (1×10^7 CFU/mL). To this Erlenmeyer flask, sucrose (2%) was added to improve *L. plantarum* growth. The culture was incubated for 28 h, and uninoculated milk was used as a control. The final pH of the fermented medium, total viable *L. plantarum*, phenolic content, and antioxidant activity were determined.

pH and Viable Cell Counts

The pH of the fermented milk was determined using a digital pH meter. Viable LAB cells from the fermented medium were carried out using MRS agar plates as described previously. Briefly, the fermented milk was diluted appropriately and spread on the MRS agar medium. It was incubated for 28 h at 37 °C, and the viable cells were counted. The final results were expressed as log₁₀ CFU/mL.

Polyphenol and Antioxidant Activity

The total polyphenol content of the milk was determined by the Folin-Ciocalteu method, as suggested earlier. Total phenolic content was expressed as milligrams of gallic acid equivalent (mg GAE) per 100 mL of fermented milk. The DPPH free radical scavenging activity (mmol Trolox/100 mL), ABTS activity (mmol Trolox/100 mL), and ferric-reducing antioxidant power (mmol Fe (II)/100 mL) were performed as described previously (Al-Dhabi *et al.* 2019, 2020).

RESULTS AND DISCUSSION

Analysis of Agricultural Residues and Food Waste

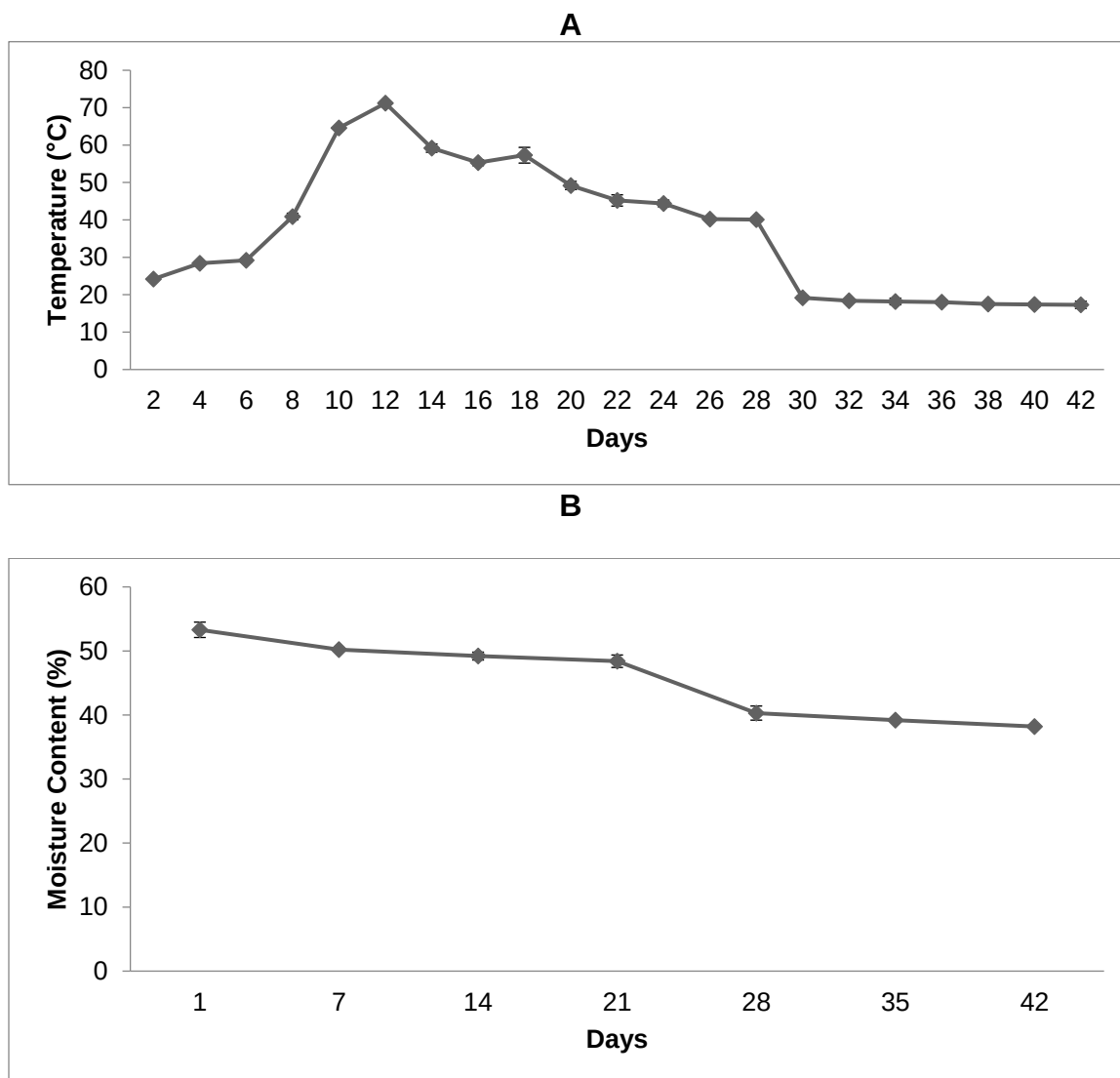
The agricultural residues and food waste were subjected to physical factor and nutrient value analyses. The amount of moisture ($56.2 \pm 1.1\%$) and pH (6.1 ± 0.02) revealed the suitability of this mixture for composting. The total carbon content of the leaf sample was $45.3 \pm 0.9\%$, and an increased level ($47.3 \pm 0.1\%$) was observed in the raw food waste. Total Kjeldahl nitrogen content was also higher in the food waste ($2.62 \pm 0.2\%$) than that in leaves ($0.51 \pm 0.1\%$). The C/N content of mixed organic waste was $32.2 \pm 0.4\%$ and was judged to be suitable for composting (Table 1).

Table 1. Physical and Nutrient Components of Agricultural Residues and Food Waste

Analysis	Agri-residues	Food waste	Mixture
Moisture (%)	57.2±0.1	54.1±0.2	56.2±1.1
pH	6.1±0.02	6.2±0.01	6.1 ± 0.4
Total carbon (%)	45.3±0.9	47.3±0.1	45.1±0.4
Kjeldahl Nitrogen (%)	0.51±0.1	2.62±0.2	1.4±0.2
C/N ratio (%)	88.8±0.2	18.0±0.1	32.2±0.4

Influence of Temperature and Moisture Content

The compost temperature reached its maximum after 12 days (71.2 ± 0.3 °C) (Fig. 1a).

**Fig. 1.** Co-composting of vegetable waste leaves in a pile for 42 days: The pile temperature (A); and moisture content (B)

The compost temperature influenced the microbial community, and the variation affected the microbial population (Azim *et al.* 2018). The microbial population varied in mesophilic, thermophilic, and cooling phases (Franke-Whittle *et al.* 2014). The increased temperature in the pile effectively removed bacterial pathogens from the compost. The microbial activity in the compost influenced the temperature of the pile. The pile temperature can be used to analyze the compost stability (Liu *et al.* 2011). Figure 1b shows the variation of moisture content in the pile over 42 days. On day 1, the moisture content was $53.3 \pm 1.2\%$, and it declined continuously (Fig. 1b). The changes in moisture content in the compost were attributed to microbial activity and the evaporation of water. The moisture content was $38.2 \pm 0.17\%$ after 42 days. The selected vegetable wastes and leaves adequately supported the growth of bacterial populations and improved soil microbial activity. The physico-chemical properties of biomass provide a broad surface area, and the improved temperature leads to evaporation (Zhang *et al.* 2016).

Variation of pH and Electrical Conductivity

The pH of the compost varied during the study period, and the result is depicted in Fig. 2a.

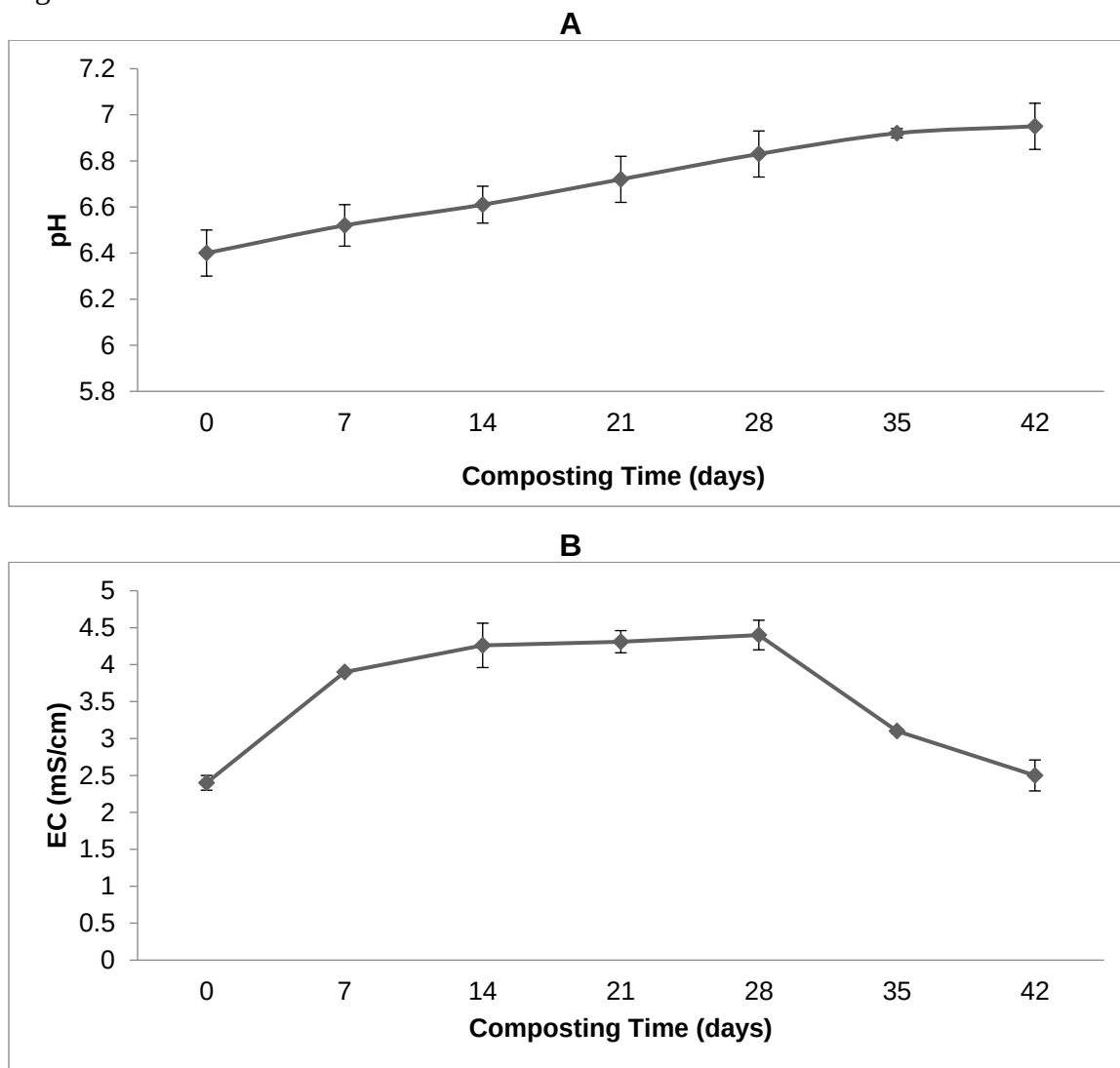


Fig. 2. Variations of pH (A); and EC (B) during composting process in the pile

Based on input from Bustamante *et al.* (2008), the pH value between 6 and 8 was judged to be suitable for the composting process. After three weeks of composting, the pH value was 6.72 ± 0.1 , and it increased to 6.95 ± 0.2 after six weeks. The pH of the compost varied based on the microbial activity, organic acid production, and type of biomass (Awasthi *et al.* 2014). The EC was 2.4 ± 0.1 mS/cm before starting the composting process, and it reached 2.5 ± 0.21 mS/cm after 42 days. A stable EC value was achieved after 35 days of composting (Fig. 2b). This finding revealed that the selected biomasses are easily degradable, and the present finding was corroborated by a previous study (Hosseini and Aziz 2013). The EC value of the mature compost was <3 mS/cm, indicating suitability for plant growth. The EC value of <3.0 mS/cm is preferred for plants (Gao *et al.* 2010).

Analysis of Organic Matter

The organic matter content was $51.2 \pm 1.1\%$ before the composting process, and it decreased gradually. The organic matter content was $29.7 \pm 1.1\%$ after 42 days of treatment (Fig. 3). Analysis of organic matter is useful to determine the rate of microbial composting (Zhao *et al.* 2016). Microorganisms in compost degrade organic matter, and the decreased pH value in the compost affects microbial activity (Tran *et al.* 2015). Microorganisms used can easily degrade matter rapidly (starting phase), and microbial enzymes, especially lignin peroxidase and cellulases, reduced organic matter in the compost (Xi *et al.* 2012).

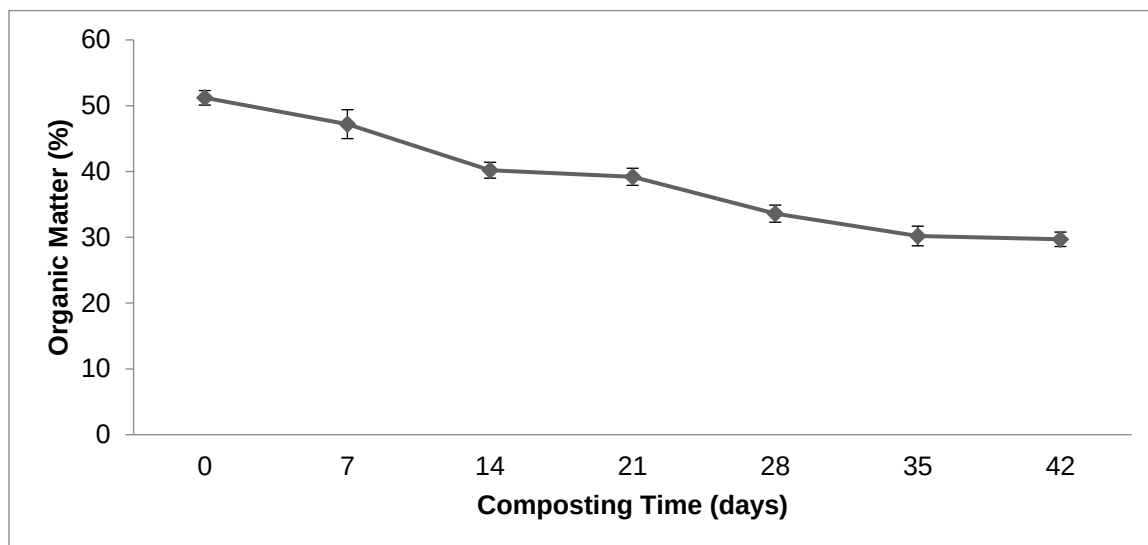


Fig. 3. Organic matter (%) of the compost in a pile for six weeks

Carbon and Nitrogen Content of the Compost

The organic carbon content was $27.2 \pm 0.7\%$ before the composting process, and it declined continuously. After 42 days of composting, it reached $5.5 \pm 0.58\%$ (Fig. 4a). Karatas *et al.* (2014) reported the loss of organic carbon in the compost, and its content directly reflects compost maturity. The organic nitrogen of the biomass was $0.93 \pm 0.09\%$, and it continuously increased. The organic nitrogen reached its maximum ($1.49 \pm 0.08\%$) after 42 days of composting (Fig. 4b). The increase in Kjeldahl nitrogen during composting was previously reported by Seng *et al.* (2013). The nitrogen and carbon ratios of the compost varied based on the available carbon and nitrogen levels. Figure 4c reveals the C/N ratio, and after 42 days of composting, the ratio was 13.8 ± 0.01 . The rapid microbial

decomposition of organic matter results in a decrease in the C/N ratio (Awasthi *et al.* 2018). The decreased level of the C/N ratio reflected the maturity of the compost.

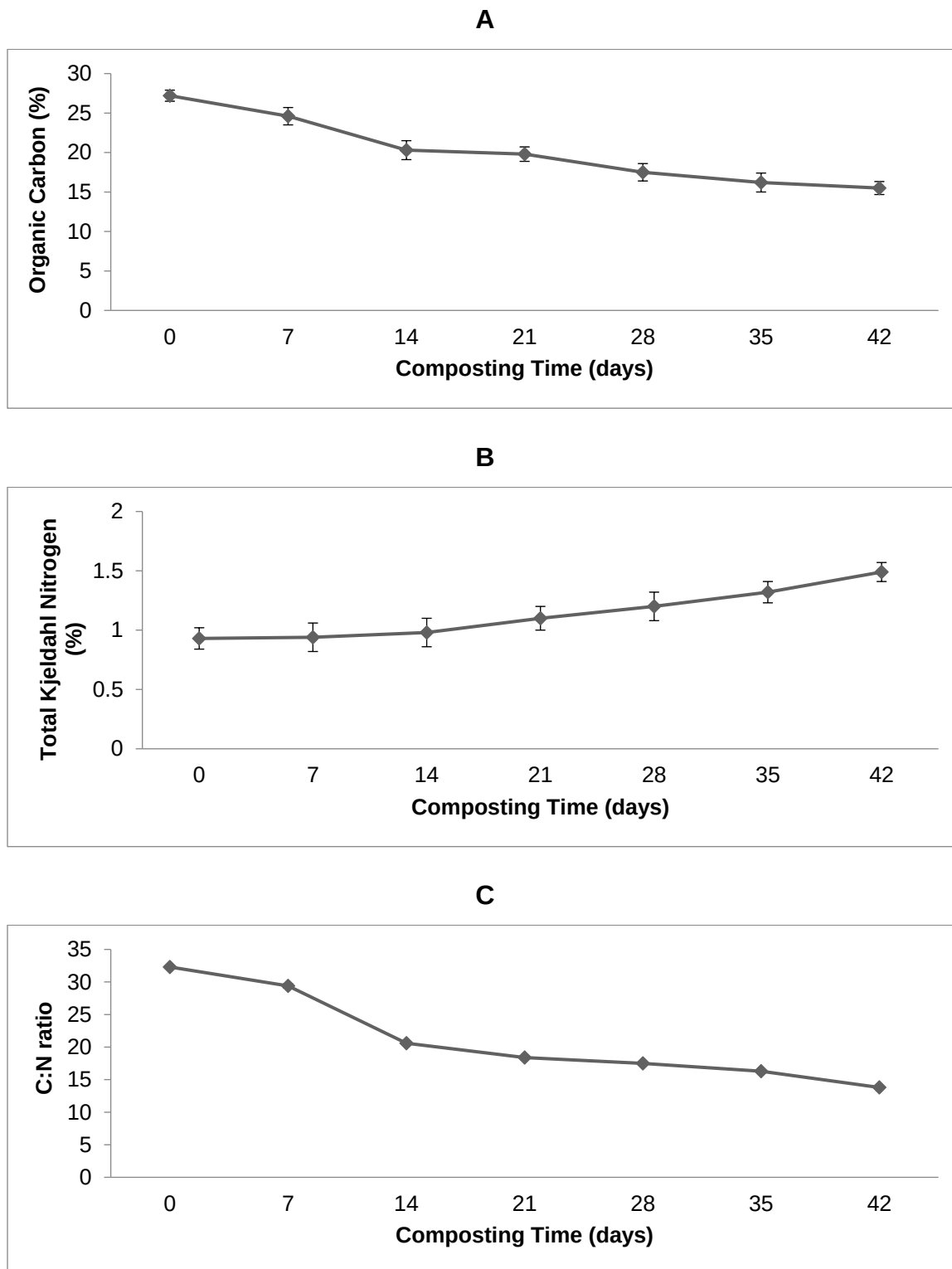


Fig. 4. Carbon and nitrogen content of the compost for 42 days: (A) organic carbon, (b) total Kjeldahl nitrogen, and (C) C/N ratio

Mature Compost and Seed Germination Index

The seed germination index is widely used to analyze the maturity of the compost. The seed germination index was $24.5 \pm 1.1\%$ in the seeds treated with zero days of mature compost. The seed germination index increased to $50.2 \pm 1.1\%$ in the seeds treated with 21 days of mature compost. This result was similar to previous findings. Awasthi *et al.* (2018) reported that the toxic substances in the compost affected the seed germination index of plants.

The seed germination index was $80.4 \pm 1.2\%$ in the 35 days of compost, and the seed germination index increased after 42 days of compost (Fig. 5). The toxic effect of the compost material varied based on the substrate used for composting. The maximum seed germination index was achieved after 84 days (Rashad *et al.* 2020).

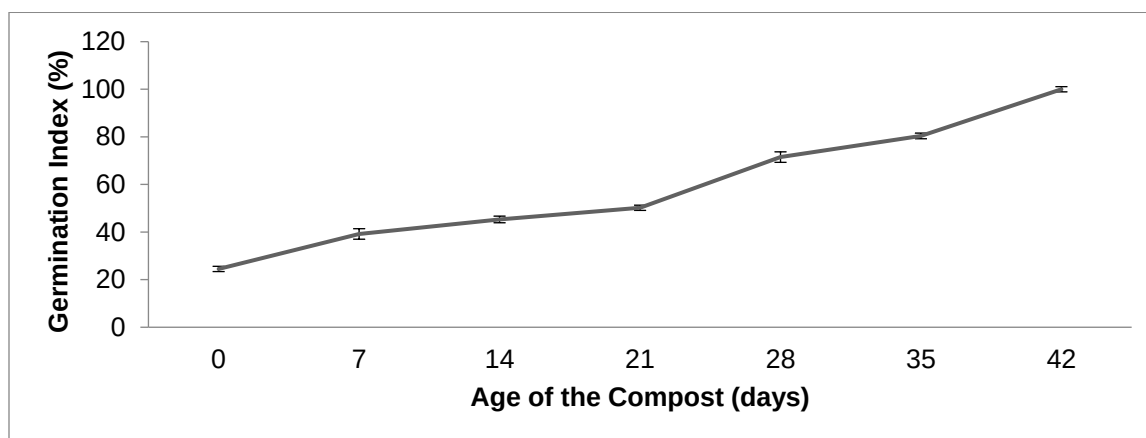


Fig. 5. Analysis of seed germination index using compost. The compost was collected from 0 to 42 days and was used for analysis.

Microbial Activities in the Greenhouse Pot Soil

The compost was used as soil amendment, and the effect on improving microbial activities in the pot was analyzed. In the control pot, soil enzyme activity was less than in the experimental pot. Acid phosphatase ($187 \pm 1.8 \mu\text{g } p\text{-nitrophenyl/g/h}$) and alkaline phosphatase ($319 \pm 40.1 \mu\text{g } p\text{-nitrophenyl/g/h}$) activity were increased in the pot treated with mature compost.

The soil dehydrogenase activity was directly proportional to the soil bacterial activity. The compost improved soil dehydrogenase activity (Figs. 6A and B). Soil microbial enzymes contribute to organic residue decomposition, nutrient cycling, and geochemical cycles. Soil microorganisms, especially bacteria play a major role in ecological processes such as the formation of soil aggregates, and nutrient cycling through the decomposition of organic matter. The microbial communities contributed to nitrification, de-nitrification and nitrogen fixation. Organic amendments can effectively improve the soil microbial population.

Changes in microbial composition and activity can influence plant growth by improving nutrient turnover and mitigating disease incidence (Balasubramanian *et al.* 2021). The supplemented compost improved the soil environment and soil microbial activity (Baazeem *et al.* 2021; Vijayaraghavan *et al.* 2021).

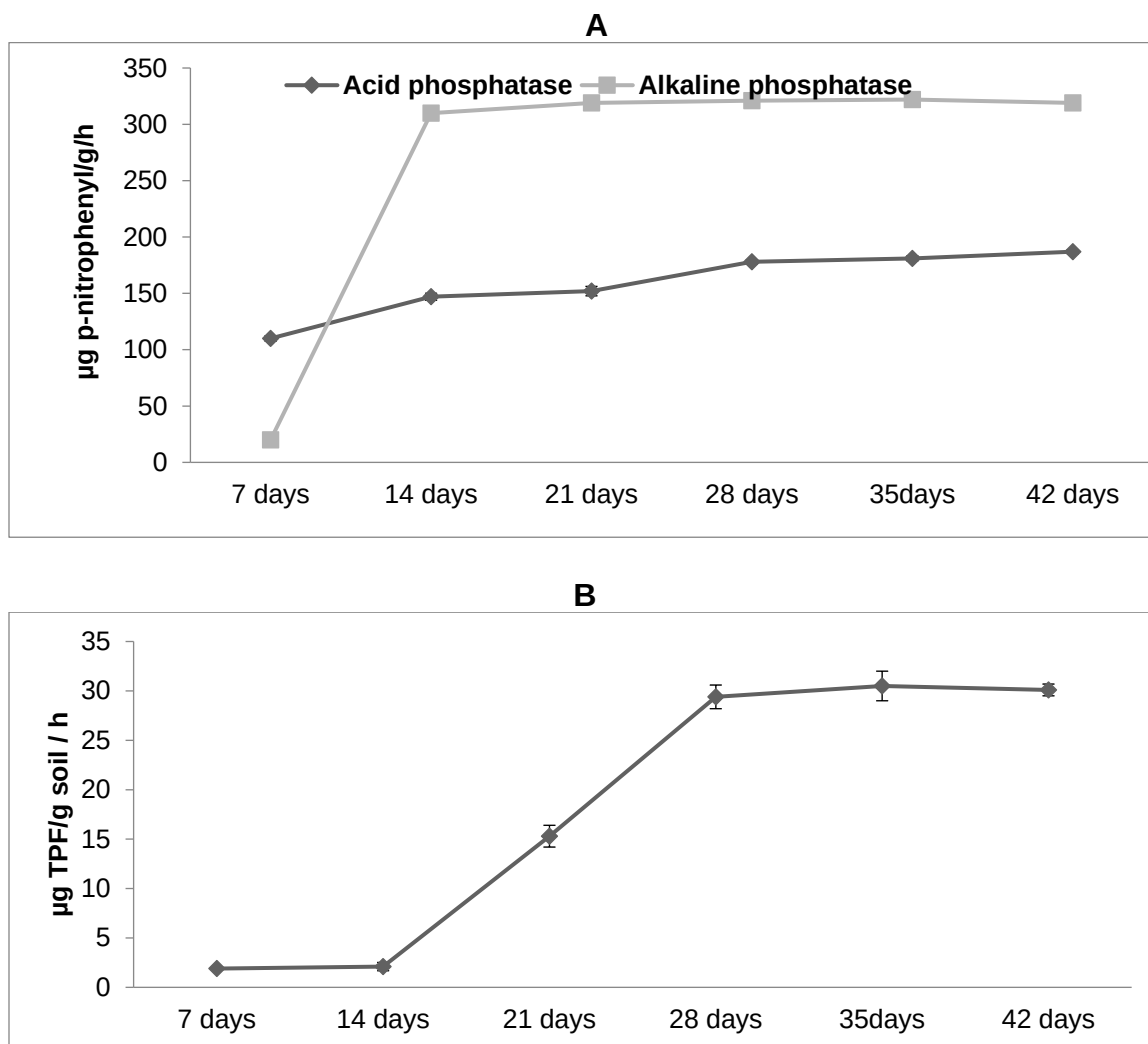


Fig. 6. Soil microbial activity of compost in the earthen pot at various stages after planting green gram: Soil alkaline and acid phosphatase (A); and dehydrogenase activity (B) in the pot soil

Analysis of Antioxidant Enzymes from Green Gram

The compost-treated green gram plant showed improved growth and antioxidant enzymes. The amount of peroxidase increased in the green gram seeds treated with mature compost (42 days old) compared to the control. Phenylalanine ammonia lyase and polyphenol oxidase levels were increased 1.5-fold in the green gram plant treated with compost (Table 2). The improvement activity in of antioxidant the seeds was mainly due to the improvement of nutrients, minerals, and soil microbial activity. Biochar addition and soil bacteria improved enzymatic properties and antioxidant enzymes in plants (Turan 2021). In chickpeas, phosphorus and *Pseudomonas putida* inoculums improved plant growth and defense enzyme activity (Israr *et al.* 2016). The organic amendments in soil improved the antioxidant content and nutritional quality in plants and soil health. Compost effectively mineralizes soil organic content and improves nutrient availability, crop growth and soil health (Verma *et al.* 2015). The present findings showed that the plants supplemented with organic amendment improved antioxidant content. The present finding also validates the positive impact of organic compost on green gram quality in terms of antioxidant defense enzymes.

Table 2. Effect of Compost Manure on the Improvement of Antioxidant Enzymes in Green Gram Seeds

Treatment	POD (U/g seed)	PAL (U/h/g seed)	PO (U/g seed)
T01	49.8 $\pm$ 0.9	14 $\pm$ 2.5	37.8 $\pm$ 0.32
T02	53.8 $\pm$ 2.2	20.5 $\pm$ 0.7	45.2 $\pm$ 0.4
T03	59.4 $\pm$ 0.8	24.9 $\pm$ 0.1	64.93 $\pm$ 1.1
T04	60.8 $\pm$ 0.9	31.5 $\pm$ 2.2	68.2 $\pm$ 0.2
T05	74.2 $\pm$ 0.3	70.5 $\pm$ 0.4	65.9 $\pm$ 2.2
T06	75.5 $\pm$ 0.08	70.9 $\pm$ 0.5	60.3 $\pm$ 1.2
Control	74.9 $\pm$ 0.15	65.5 $\pm$ 1.1	58.1 $\pm$ 0.9

POD-Peroxidase; PAL- Phenylalanine Ammonia Lyase; PO-Polyphenol Oxidase. Compost was supplemented to green gram plant in a greenhouse and the seeds were harvested, and assayed for antioxidant activity.

Assessment of pH in Fermented Green Gram Milk from Green-House

The pH of the green gram milk medium is described in Fig. 7.

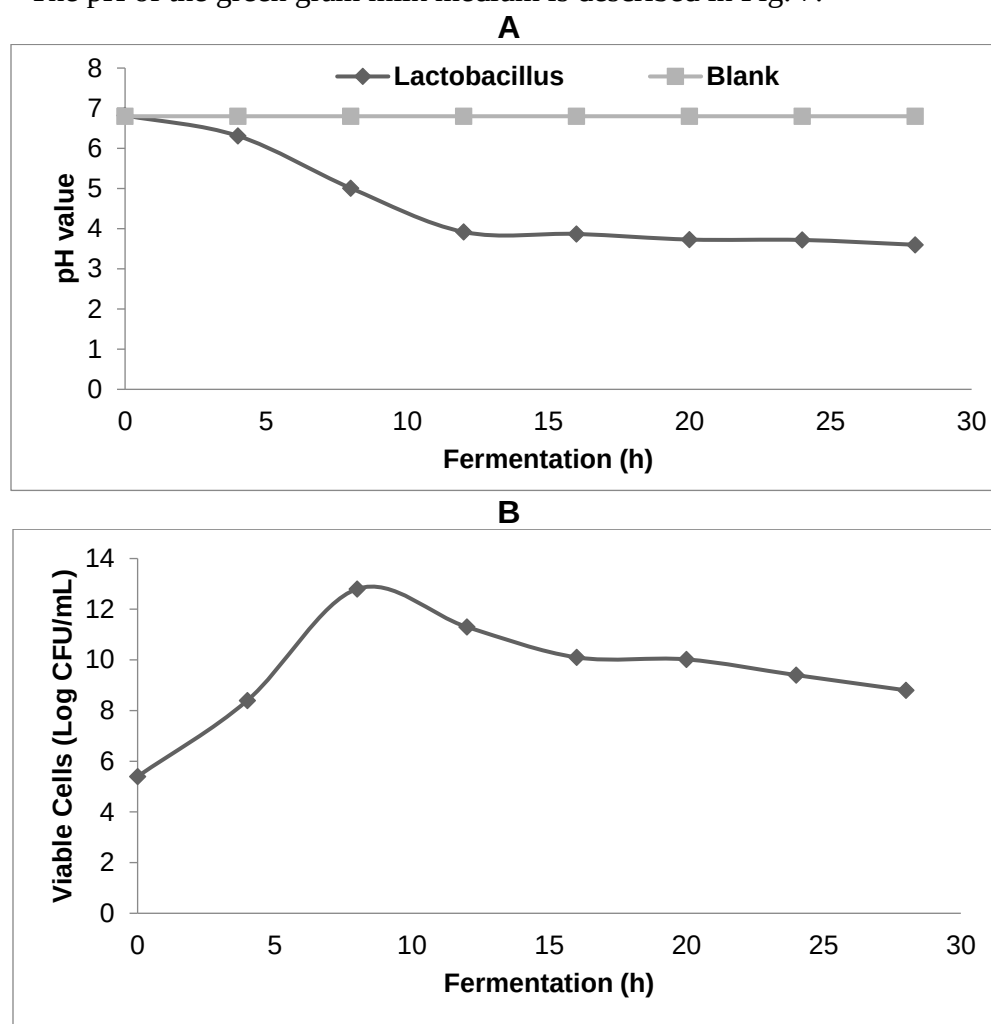


Fig. 7. Variation of viable pH (A); and viable count of *Lactobacillus* cells (B) from the fermented green gram milk

The pH value was found to be 6.82 ± 0.3 at 0 h of fermentation and it decreased drastically after 8 h (5.01 ± 0.3). The growth of *L. plantarum* reached its maximum after 8 h of fermentation, and the pH dropped at this time. The decreased rate of pH value was high within 8 h, and this rate slowly declined thereafter. The decrease in pH value during the fermentation process could be due to the production of various organic acids by *L. plantarum*.

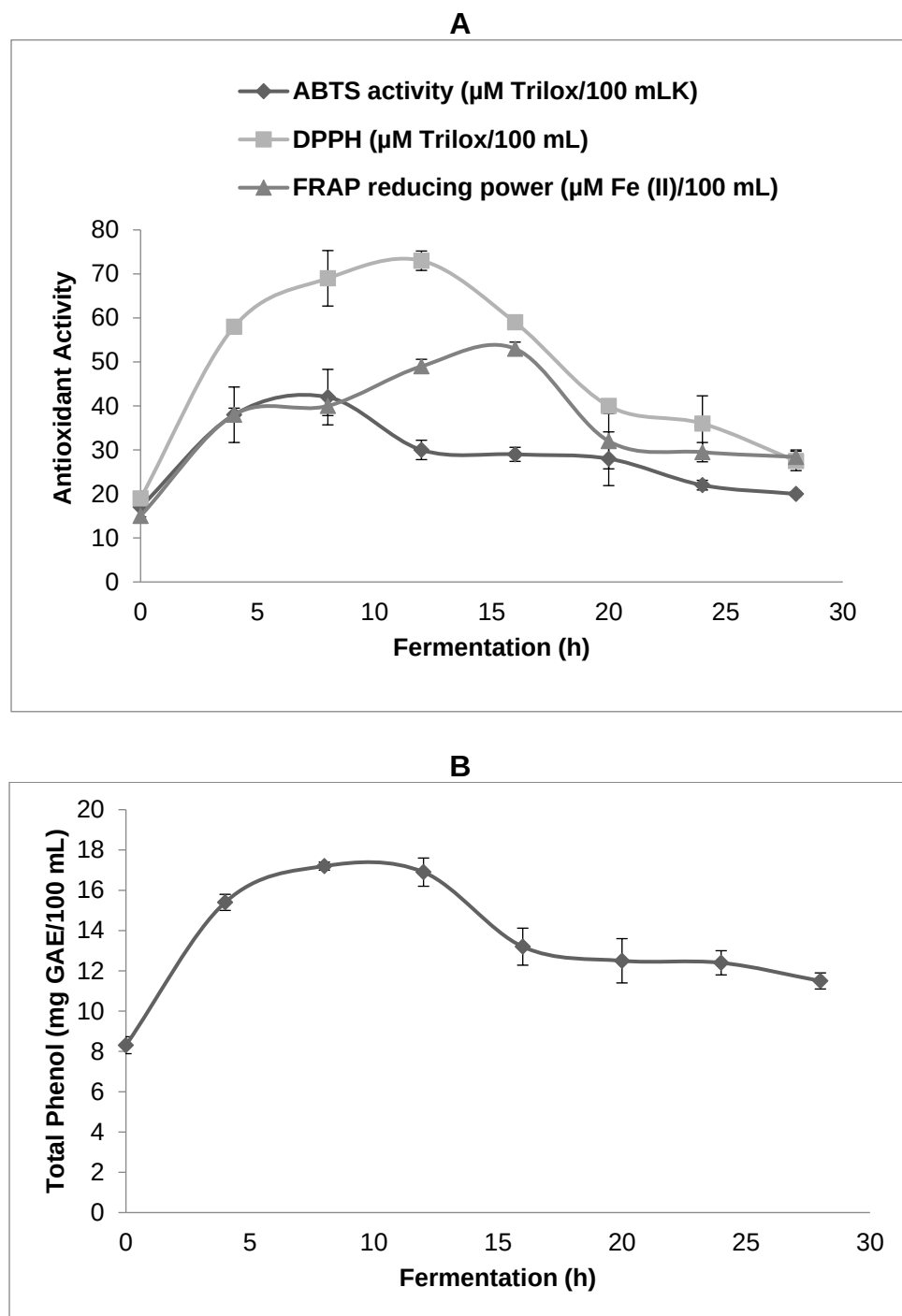


Fig. 8. Antioxidant activity (A); and polyphenol content (B) of fermented green gram milk with *L. plantarum* for 28 h

The viable cells increased and reached maximum within 8 h of incubation in green gram milk and gradually declined, which was almost similar to the results achieved with *L. plantarum* B1-6, which reported maximum growth within 10 h (Xiao *et al.* 2015). The poor viability of cells after 12 h of fermentation was attributed mainly to the extreme acidic environment. Most of the *Lactobacillus* species released a number of organic acids during the fermentation process, and the released organic acids reduced the pH. The acidic environment prevents the growth of other microorganisms.

Polyphenol Content Improved Antioxidant Activity of Fermented Green Gram Milk

The ABTS, DPPH, and FRAP reducing powers of fermented milk were analyzed, and the results are described in Fig. 8A. The ABTS activity was increased after 8 h of incubation in green gram milk, and it gradually decreased from 8 h to 12 h. The kinetics of DPPH activity was different from ABTS activity. The DPPH activity increased after 12 h and declined from 12 h to 28 h gradually. The observed DPPH value in this study was comparatively high compared to ABTS results and FRAP-reducing power. Zhao and Shah (2014) reported increased DPPH free radical scavenging activity in fermented food. The increased antioxidant activity in the fermented milk could be associated with increased antioxidant vitamins. The FRAP activity assay has been widely used to determine antioxidant properties in liquid samples. Fermentation with *L. plantarum* improved FRAP activity, and the result is described in Fig. 8A. Compared with 0 h fermentation with either 8 h or 12 h fermentation, FRAP activity increased significantly. The polyphenol content level was improved after fermentation with *L. plantarum*. Total polyphenol content was 8.3 ± 0.42 mg GAE/100 mL in the raw milk, and after fermentation, the amount of polyphenol content increased (Fig. 8B). Earlier reports stated that fermentation with *Lactobacillus* species could significantly increase total phenolic content (Xiao *et al.* 2015), and the improved polyphenol in the fermented medium attributed to high antioxidant activity (Awasthi *et al.* 2022).

CONCLUSIONS

1. Co-compost was prepared using vegetable waste and leaves. Co-compost improved microbial activities in the greenhouse experiments.
2. The co-compost improved peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase activities.
3. The high polyphenol content in the fermented green gram milk is correlated with the high antioxidant capacity of the green gram milk fermented by *L. plantarum*.
4. Total viable count, pH, polyphenol content, and antioxidant activity in fermented green gram milk with *L. plantarum* showed improved health benefits.

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