

Effect of Biochar, *Azolla* and Plant Growth-Promoting Rhizobacteria on Physical and Chemical Properties Changes of Trees Residues During Composting

Zahra S. Abd-Elhameed, A.A. Abobaker and H.M. Farrag *

Soil and Water Department, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt.

Abstract

The aim of this investigation is to study the effect of adding biochar, *Azolla* combined with acid producing bacteria and plant growth promoting Rhizobacteria (PGPR) on the chemical and physical properties of the tree residues compost during aerobic decomposition. Five composting heaps were carry out in this study as follows; 1-control (T0), 2- heap supplied with bio char (T1), 3- heap supplied with *Azolla* (T2), 4- heap supplied with mixture of bacterial cultures (*Bacillus megatherium, Azospirillum lipoferum, Azotobacter chroococcum*) (T3) and 5- heap supplied with biochar, *Azolla* plus mixture of bacterial cultures (T4). All compost heaps showed an rising in temperature after start of the composting reached to the highest peak values of 56, 60.2, 61, 58.7, and 67.6 °C in day 10 for T0, T1, T2, T3 and T4, respectively. Gradual decreasing of the organic matter, organic carbon and C/N ratio with increase of composting period process, on the other hand, progressive increases in % Ash, Total N, available P, total K, pH and EC with increasing of composting period process. Compost supplied with biochar, *Azolla* and mixed bacterial cultures (T4); recorded higher values of N, P and K contents compared to other treatments. These results indicated that, adding biochar, *Azolla* and bacterial cultures to trees residues during composting improve properties and nutritional value of the produced compost.

Keywords: Azola; biochar; composting; properties; residues; rhizobacteria.

1. Introduction

Gardens, parks and streets in Egypt contain a large number of wooden trees, in addition, Egypt plans to plant 100 million trees nationwide to increase green spaces and reduce greenhouse gases to combat climate change. Trees of all kinds produce waste such as twigs and branches from the pruning of tree plants. This can be troublesome to dispose of in a way that is kind to the environment. Unlike burning, shredding and composting is the ideal solution, resulting in a usable organic material for the agricultural fields. But, being rich in carbon but low in nitrogen, it

*Corresponding author: Hosny M. Farrag

Email: <u>hosnyfarraj@agr.svu.edu.eg</u>

will take typically 3-4 years for woody chippings to break down into crumbly compost. So. shredded woody materials will need to be mixed with some materials to reduce the time required for compost to mature and improve the characteristics of the compost resulting from this type of waste. Azolla is an aquatic fern present in the tropical and temperate regions. It possesses a cavity in the dorsal leaves for the blue-green algae (Anabaena algae) that is capable of fixing atmospheric nitrogen through the symbiotic association with Azolla. Azolla in symbiosis with cyanobacterium can fix 2-4 kg N ha/day, its incorporation also enhanced the effectiveness of other chemical fertilizers. EL-Zeky et al. (2005), EL Shimi et al. (2015) and Hanafy and El-Emery, (2018). Bindhu (2013) found that, Azolla can

Received: December 22, 2022; Accepted: December 29, 2022; Published online: December 30, 2022. ©Published by South Valley University. This is an open access article licensed under ©050

growth-promoting releasing nutrients and compounds such as gibberellins, cytokinins, auxins, abscisic acid, vitamins, antibiotics, and amino acids, moreover minerals such as calcium, phosphorus, potassium, ferrous. copper, magnesium, and others are abundant in Azolla. Azolla has a protein concentration of 25-30%, a mineral content of 10-15%, and bioactive compounds, However, the carbohydrate and lipid content is extremely minimal, also, Azolla can serve as good nitrogen supplement to plants (Choudhury et al., 2004; Emam et al., 2022). Biochar is a highly carbonaceous solid residual produced from the pyrolysis of organic waste at a high temperature (300-700 °C) and under the absence of oxygen (Qu et al., 2020). Biochar has porous structure, large surface area, and anion exchange capacities so has shown its beneficial role in nutrient retention, enhancing compost maturity and improving carbon sequestration (Wu et al., 2017). Also, the addition of biochar to compost could affect the microbial-mediated activities, such as organic matter mineralization, biochar could be added to compost for enhanced performances of microorganisms (Bong et al., 2021). Several mechanisms have been proposed to provide an explanation for the obvious retention of N in biochar-amended soils and reduction of N leaching, these include adsorption of NH3 or organic-N onto biochar, cation or anion exchange reactions, and enhanced immobilization of N as a consequence of labile C addition in the biochar (Clough et al., 2013)..Also, experiments showed that biochar not only improved the chemical and biological properties of the soil, including increasing soil pH, CEC, and microbial activity, but also improved the physical properties of the soil, such bulk density, hydraulic conductivity, aggregate stability, and erosion resistance (Jien and Wang, 2013). The application of biochar significantly increased the soil organic matter, soil bulk density, total nitrogen, available phosphorus and available potassium contents of soil, however, the pH of the soil did not differ significantly, but increased with increasing rates of biochar application (Timilsina et al., 2017). Rock phosphate is a naturally occurring ore of marine sedimentary origin. rock phosphate is sparingly soluble and little of the phosphorus is available to crops. Rock phosphate the backbone of the Pfertilizer industry (Adnan et al., 2017). Adding rock phosphate is A possible means of improve nutritional value of the resulting compost (Rasslan et al., 2021). One of the major problems with the application of rock phosphate is that because it is slowly released, acid produced bacteria inoculation improved P release, Olsen extractable P content progressively increased with the incubation duration in all inoculated treatments (Adnan et al., 2017). Bacillus megathirum and Bacillus polymyxa are acid produced bacteria have the ability to dissolve phosphorus from phosphate rock (Zhong and Huang, 2005; Farrag and Bakr, 2021). The aim of this investigation is to study the effect of adding biochar, Azolla combined with acid producing bacteria and plant growth promoting Rhizobacteria (PGPR) on the chemical and physical properties of the tree residues compost during aerobic decomposition.

2. Material and methods

2.1. Raw materials

2.1.1. Tree residues

Tree stalks and leaves were collected from gardens, parks, farms and streets in south valley university, Qena, Egypt. The tree residues were air dried and chopped into small pieces (2-3 cm length) by the waste mincer. Some Characteristics of tree residues are presented (Table 1).

2.1.2. Farmyard manure

Farmyard manure was obtained from the Animal Production Farm at the Faculty of Agriculture, South Valley University. It was added at rate 200 kg/ton tree residues as a source of degrading microorganisms. Some Characteristics of farmyard manure are presented (Table 1).

2.1.3. Biochar

Biochar derived from tree residues, which oven dried (70 °C) and converted into biochar through slow pyrolysis using a furnace at temperatures of 450 °C for 4 hours; biochar samples were crushed and ground to pass through a 2-mm. Biochar used at the rate of 10 kg / ton of tree residues. Some characteristics of Biochar are presented (Table 1).

2.1.4. Azolla

Were obtained from the research farm of the Agricultural Botany Department, Faculty of Agriculture, South Valley University, Qena, Egypt; the samples were air-dried; grinding and used at the rate of 10 kg dry *Azolla* / ton of tree residues. Some characteristics of *Azolla* are presented (Table 1).

Table 1. Some characteristics of tree residues, farmyard manure, biochar and Azolla.

Character	Tree residues	Farmyard	Biochar	Azolla
		manure		
pH (1:10)	7.52	8.40	8.4	5.82
EC (1:10), (dS/m)	0.52	6.35	1.98	4.91
Organic matter (%)	89.20	39.52	67.39	64.23
Organic carbon (%)	51.73	22.04	39.08	37.25
Total N (%)	0.59	1.13	1.2	3.21
C/N ratio	87.67	19.5	32.57	11.60
Total P (%)	0.18	0.91	0.29	1.22
Total K (%)	0.9	0. 74	0.83	0.98

2.1.5. Rock Phosphate

Rock phosphate (RP) contain 24 % P2O5 obtained from El-Sebaiya site mines (25°10'30"N 32°40'43"E) Aswan Governorate, Egypt. The ground rock phosphate was crushed and sieved to pass through a (270 mesh Rock phosphate was added at a rate 100 kg/ton tree residues. RP composition is presented in Table 2.

2.1.6. Plant growth promoting rhizobacteria (PGPR)

Three types of PGPR: *Azospirillum lipoferum*, *Azotobacter chroococcum* as a nitrogen fixers and *Bacillus megatherum* as a phosphate dissolver used in this investing were obtained from Microbiological Resources Center, Faculty of Agriculture. Ain Shams University, Cairo, Egypt.

Chemical composition	Contents (%)	
P ₂ O ₅	28%	
SiO ₂	9.2 %	
MgO	0.65 %	
AL ₂ O ₃	0.81 %	
Fe ₂ So ₃	1.2 %	
Na ₂ O	0.4 %	
K ₂ O	0.05%	
MnO	0.05 %	
F	2.7 %	
CaO	46.2 %	
CaCO ₃	8.82%	
SO ₃	0.6 %	
pH	8.6	
EC	3.55 dS m ⁻¹	

Table 2. The chemical	composition of	rock phospha	te (RP)
- doite	eomposition or	room phooping	

2.1.7. Chemicals activator for compost processing

chemicals activator mixture was added at a rate 35 kg ammonium sulphate, 20.5% + 7 Kg superphosphate 15.5 % + 15 Kg Calcium carbonate + 100 kg of fertile soil collected from the surface layer of soil from a farm close to the Nile River, Qena Governorate, Egypt /ton tree residues.

2.2. Experimental procedures

2.2.1. Composting treatments

Compost-1(T0): was used as a control (tree residues + Farmyard manure + rock phosphate + chemical activator).

Compost-2 (T1): (tree residues + Farmyard manure + rock phosphate + Biochar + chemical activator).

Compost-3 (T2): (tree residues + Farmyard manure + rock phosphate + Azolla + chemical activator).

Compost-4 (T3): (tree residues + Farmyard manure + rock phosphate + (mixed cultures *B.megatherum, A.lipoferum, A. chroococcum*) + chemical activator).

Compost-4 (T4): (tree residues + Farmyard manure + rock phosphate + Biochar+ *Azolla* + (mixed cultures. *B.megatherum, A.lipoferum, A. chroococcum*) + chemical activator).

2.2.2. Preparing of PGPR inoculants

Bacterial strains (*A.lipoferum, A. chroococcum and B.megatherum*) were separately grown in three fermenters each containing 10 liters of medium (50 ml molasses + 5 g peptone/1000 ml distilled water), at 28-30 °C for 7 days. Equal volumes of the three bacterial liquid cultures were mixed before adding to the compost heap (T3 and T4); which added at a rate of 20 litters/ton residues when the temperature had steadied around 30 - 35 °C (60 days of composting period). (Rasslan *et al.*, 2021).

2.2.3. Heap formation

Tree residues were thoroughly mixed for homogenization purpose, and five heaps were prepared for five treatments. A heap of the tree residues of 2 m long, 1.5 m width and 1.0 m height were made for composting process. In all heaps the moisture content was adjusted to reach about 65 % of their water holding capacity and the heaps were turned for aeration every 15 days. The composting process was allowed to continue for 90 days until maturity. Finally, the compost heaps were covered after maturity with plastic tarpaulin for later use in production of different crops.

2.3. Methods of analysis

2.3.1. Compost physical properties:

2.3.1.1. Composting materials temperatures

The composting piles temperature was measured every 5 day at different parts in the morning at a depth of 50 cm by using a stainless-steel temperature probe with Ni-Cr-Ni component connected to a digital thermometer (Juárez *et al.*, 2015; Rasslan *et al.*, 2021).

2.3.1.2. Bulk Density (BD)

Bulk density of all materials was determined using the mass per unit volume with hard plastic cylinder of 10 cm diameter and 40 cm in height was used for collecting compost samples and drying at 105°C in a drying oven. dried bulk density (BD) was calculated using the following equation according to (Huerta-Pujol *et al.*, 2010). BD= dried mass material (g) / cylinder volumecm³.

2.3.2. Compost chemical properties

pH value was determined in a suspension compost/distilled water ratio 1:10 (w/v) using glass electrode Jenway 3510 pH Meter according to (Smith and Hughes, 2002). The electrical conductivity was determined in a water extract compost/distilled water ratio 1:10 (w/v) using 4510 conductivity meters (JENWAY, UK) according to (Sánchez-Monedero *et al.*, 2001). The organic matter content of the compost and compost raw materials was analyzed after drying at 105 °C for 24 h by weight loss on ignition at 550°C for 4h as recommended by (Møller et al., 2000). The total organic carbon (TOC) was determined based on the average organic matter containment of 58% total carbon Nelson and Sommers (1996) according to the following procedure. $(TOC) = (O.M \times 58/100)$. The total nitrogen was determined in the fresh compost samples was digested using a 20: 5 mixture of sulfuric acid to hydrogen peroxide (Agiza et al., 1985; Lowther, 1980). The total nitrogen was estimated based on dry weight after subtracting the moisture content. Raw materials and compost samples were determined using the Kjeldahl digestion method as described by (Jackson, 1973). The ratio of total carbon to total nitrogen is calculated depending to the following equation: *C/N ratio* = (% *total carbon/*% *total nitrogen*). The available phosphorus was determined in the fresh compost samples was extracted by 0.5 M NaHCO3 at pH 8.5 (Olsen et al., 1954) and spectrophotometrically determined according to Jackson (1973). and available phosphorus was estimated based on dry weight after subtracting the moisture content. Total potassium was determined by flame photometer method in acid solution of digested compost and compostable material samples according to (Jackson, 1973).

3. Results and discussion

3.1. Changes of Physical and chemical characteristic during composting process

3.1.1. Changes in Temperature during composting

The data in figure (1) showed that, changes in temperature of different composting heaps during composting process. The results revealed that temperature increased in all treatments, the material temperature reached to highest temperature and recorded 69.3, 64.3, 62, 61 and 56.9 °C for T4, T2, T3, T1 and T0 respectively until 10 days then it gradually decrease until 60

days. This decrease in temperature can be explained by the gradual decrease in microbial activity during the composting period, also during compost heap turning process some temperature may lose. These results agree with (Qian *et al.*, 2014; Rasslan *et al.*, 2021). In addition, application mixture of PGPR with treatments T3 and T4 after 60 days recorded an increase in temperature. The increase in temperature in the treatment T3 and T4 compared to other treatments may be due to the activity of bacterial strains added to compost heaps.

3.1.2. Bulk Density

The data shown in Figure (2) show the amount of change in compost bulk density during composting process, bulk density was observed gradual increase on all compost heaps during composting until the end of composting, The bulk density in all compost heaps recorded the highest value at the end of the composting period This result agrees with (Jain et al., 2018). There is an inverse relationship between the compost period and bulk density due to the decomposition of the organic components and the loss of substrates during the compost, as reported by (Larney et al., 2000). Also, at the end of composting period T2 recorded lowest value (0.99 g cm⁻³) compared to composting treatments T0, T1, T3to and T4, for which it was 1.08, 1.08, 1.05 and 1.08 g cm⁻³, respectively. very little bulk density can imply extreme substrate aeration indirectly and a droplet in the available water portion. In contrast, Higher rates of bulk density indicate an increase in mass and a reduction of porosity and air capacity. (Nappi and Barberis, 1993; Raviv et al., 1987) mentioned that as the long composting process time, the general particle size shifted from larger to smaller particles.

3.1.3. pH

The results in Figure (3) indicate that the initial pH depended on the composition of the ingredient used in composting production, where the highest pH values of 7.48 and 7.35 were found with T1 and T4 respectively with applying biochar, and

6.8, 6.5 and 6.4 for T0, T2 and T3 respectively, this could be due to the effect of biochar in increasing value of pH. (Carvalho, 2015; Syuhada et al., 2016; Timilsina et al., 2017). pH was recorded minimum value starting from the beginning time of the composting process until day th10. The reduction in the pH, depends upon the progress of decomposition and oxidation of organic matter and production of inorganic and organic acids and CO₂ produced from the microorganism's activity. These results are in an agreement with Aqarab et al. (2021) that they found that, decay of organic matter, and microorganisms' exudates including bacteria and fungi may be the cause decreased pH by producing large quantities of organic acids. Then the pH values started to increase gradually in all treatments until the end of composting processes after 90 th day. The pH values after the 90th day

of composting were 8.01, 8.16, 8.02, 8.10 and 8.04 with T0, T1, T2, T3 and T4 treatments, respectively. This indicates a good quality produced compost and within the suggested range of 6-8.5, as has been reported by (Fogarty and Tuovinen 1991). an increase in the value of pH with an increase the composting period This could be due to, the rock phosphate adding to compost heaps containing a high percentage of calcium carbonate. with microorganism exudates and production of inorganic and organic acids and produced from the CO_2 activity of microorganisms, this encourages the dissolution of calcium carbonate, with the released carbonate anion leading to increases in the pH value. Farrag and Bakr (2021) found that, an increase in calcium carbonate content (CaCO₃) It can cause an increase in the pH value.



Figure 1. Changes in temperature during composting process.



Figure 2. Changes in Bulk density of compost heaps during composting



Figure 3. Changes in pH of compost heaps during composting

3.1.3. Electrical conductivity (EC)

EC described the contents of soluble salt through the composting, data of EC changed according to composition of the ingredient used are given in Figure (4). The data show that the initial EC value for all compost heaps being 1.80, 2.05, 1.95, 1.98 and 2.05 ds m⁻¹ for composting treatment T0, T1, T2, T3 and T4, respectively. Also, the results in figure (4) show that, increases in EC through the composting period in all compost heaps with increased the composting period. The EC values at end of composting period were 3.74, 3.68, 4.06, 3.23 and 4.39 ds m-1 for treatments T0, T1, T2, T3 and T4, respectively. The increase in the EC of all treatments may be due to the mineralization of organic matter, which increased concentration

of soluble salts (Chan *et al.*, 2016; Rasslan *et al.*, 2021). Also, an increase in EC in all treatments could be attributed to the activity of microorganisms producing organic acids and CO_2 during the decomposition of organic matter, it helps accelerate the rate of dissolution of rock phosphate and release of the excess salts from rock into the solution, this effect increases with increasing rate of rock phosphate used.

3.1.4. Organic Matter and Total Organic Carbon

The value of organic matter and total organic carbon decreased in all treatments with increasing composting period, Figure (5 and 6). similar results were found by (Seoudi, 2013; Rasslan et al., 2021). The initial values of organic matter were 80.21, 81.20, 81.26, 81.05 and 80.70% for T0, T1, T2, T3 and T4 respectively, while the initial values of total organic carbon were 46.52, 47.10, 47.13, 47.01 and 46.81% for T0, T1, T2, T3 and T4 respectively. At maturity stage, the organic matter values decreased to 42.33, 42.46, 42.90, 42.31 and 44.12 % for treatments T0, T1, T2, T3 and T4 respectively. Moreover, the total carbon values decreased to 24.55, 24.63, 24.88, 24.54 and 25.60% for treatments T0, T1, T2, T3 and T4 respectively. From the results in figure 5 we observed that, the different treatments had no clear effect on the rate of carbon loss from compost heaps. In generally, large total carbon losses suggest pronounced microbial activity in the former. Diaz et al. (2020) found that during composting, carbon is a source of energy for microorganisms to build up cells.

3.1.5. Total Nitrogen

The N content in compost is one of the critical indices for determining composting quality. Figure 7 show changes in total nitrogen percentage through the different times of the composting process. The nitrogen value increased in all treatments with the increase in the composting period. After compost maturity the values of total nitrogen were 1.78, 2.51, 2.48, 2.52 and 2.53 % for treatments T0, T1, T2, T3 and

T4 respectively. Mineralization of organic materials may be responsible for increasing the total nitrogen. (Farrag and Bakr, 2021; Rasslan et al., 2021). Also, (Rasslan et al., 2021; Zhong et al., 2018) found that, the increase in total nitrogen percentage After compost maturity may be caused to a condensation impact caused by active decomposable of the degradation carbonaceous substrates, resulting a decrease in the mass of the compost mass. Also, the results in figure 6 show that, addition of Biochar, Azolla and PGPR plant growth promoting Rhizobacteria to compost heaps led to increase in total nitrogen percentage compared to control (T0). It was also found that using the three treatments together is better than using these materials alone. The addition of biochar to compost could affect of increases of total nitrogen by effect of the microbial-mediated activities, such as organic matter mineralization, biochar could be added to compost for enhanced performances (Bong et al., 2021). Also, biochar can helpe to retention of N by Several mechanisms. These include adsorption of NH3 or organic-N onto biochar, cation or anion exchange reactions, and enhanced immobilization of N as a consequence of labile C addition in the biochar (Clough et al., 2013). In addition, Azolla can releasing nutrients and growth-promoting compounds such as gibberellins, cytokinins, auxins, abscisic acid, vitamins, antibiotics, and amino acids, moreover Azolla can serve as good nitrogen supplement to plants (Choudhury et al., 2004; Emam et al., 2022). Moreover, Rasslan et al. (2021) found that, the increase in nitrogen content in compost was shown in the treatment inoculated with (A. *lipoferum* + A. chroococcum + B.megatherum) which showed an increase of 25.94% in nitrogen over the control, these increases may be due to non-symbiotic nitrogen fixing activities affected by A. chroococcum and A. lipoferum Madusari et al. (2020) showed that the total nitrogen content in compost with the addition of Azotobacter is slightly higher than compost without the addition of Azotobacter.



Figure 4. Changes in EC of compost heaps during composting



Figure 5. Changes in Organic Matter % of compost heaps during composting.



Figure 6. Changes in Organic Matter % of compost heaps during composting.

3.1.6. C/N Ratio

The carbon-to-nitrogen ratio of organic matter means the amount of carbon relative to the amount of nitrogen present. The initial values of C/N ratio were 44.73, 31.82, 35.71, 44.77 and 30.39 % for T0, T1, T2, T3 and T4 respectively. The carbon to nitrogen (C/N) ratio is significant in composting because microorganisms need a good balance of carbon and nitrogen (ranging from 25 to 35) in order to remain active, decomposition slows down If the C:N ratio is too low (excess nitrogen) you will end up with a stinky pile. (Meijide et al., 2007; Brust, 2019). In addition, from the data in figure 8 we show that, in all treatments during the composting process, a decrease in C/N ratio with increase of composting period, The decrease in C/N values due to the mineralization of organic matter (Rasslan et al., 2021). The final values of C/N ratio after the 90 days were 13.79:1, 9.81:1, 10.03:1, 9.74: 1 and 10:1 for treatments T0, T1, T2, T3 and T4, respectively. Compost can be characterized as mature only when the C/N ratio is below 20, The lower the C:N ratio, the more rapidly nitrogen will be released into the soil for immediate crop use (Rasslan et al., 2021; Meijide et al., 2007).

3.1.7. Available Phosphorus

The data in Figure 9 show that, available phosphorus increased significantly in all treatments during composting, this may be due to production of organic and inorganic acids, as well as CO_2 that react and dissolve the rock phosphate and this helps to increase of the available phosphorous. (Farrag and Bakr, 2021; Rasslan *et al.*, 2021).

The final values of available phosphorus after the 90 days were 789.02, 839.50, 989.33 and 989.45 mg/kg for treatments T0, T1, T2, T3 and T4, respectively. This may be attributed to the effectiveness of microbial strains added to compost heaps (T3 and T4) on the degree of phosphorus dissolution from rock phosphate, this depends upon the progress of decomposition and oxidation of organic compounds and production of inorganic and organic acids and CO_2 produced from the activity of microorganisms; these products help to convert the insoluble forms of P into soluble ones (Chen *et al.*, 2006; Sugihara *et al.*, 2010; Xiao *et al.*, 2017).



Figure 7. Changes in Total nitrogen % of compost heaps during composting



Figure 8. Changes in C/N ratio of compost heaps during composting



Figure 9. Changes in available phosphorus of compost heaps during composting

3.1.8. Total Potassium

Figure 10 shows the changes in total potassium in compost heaps in all treatments during composting, which revealed that a gradual increases in total potassium found with increasing of composting period, these increases may be due to the increasing rate of decomposition of organic matter and potassium released along the composting period (Rasslan *et al.*, 2021). In addition, the final values of total potassium after the 90 days were 0.29, 0.41, 0.41, 0.32 and 0.42 % for treatments T0, T1, T2, T3 and T4,

respectively. From these results, we conclude that, there is an increase in the value of total potassium in the treatments T1, T2 and T4 compared to control (T0), This increase in the total potassium value can be attributed to the materials added to the compost heaps, which are biochar and *Azolla* which improve composting process condition. Bindhu (2013) found that, *Azolla* can releasing nutrients such as potassium. also, the application of biochar significantly increased the available potassium contents (Timilsina *et al.*, 2017).



Figure 10. Changes in total potassium of compost heaps during composting

4. Conclusion

In conclusion, the results of this study indicated that adding biochar, *Azolla* combined with plant growth-promoting rhizobacteria to trees residues during composting improve properties and nutritional value of the producing compost and could be considered an option of waste management that is cheap, environmentally friendly and produces high value compost It can be used as alternative fertilizers or a supplement to chemical fertilizers.

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Authors' Contributions
All authors are contributed in this research.
Funding
There is no funding for this research.
Institutional Review Board Statement
All Institutional Review Board Statements are
confirmed and approved.
Data Availability Statement
Data presented in this study are available on fair
request from the respective author.
Ethics Approval and Consent to Participate
Not applicable
Consent for Publication
Not applicable.
Conflicts of Interest
The authors disclosed no conflict of interest starting
from the conduct of the study, data analysis, and
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writing until the publication of this research work.

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