New York Science Journal

Websites:
http://www.sciencepub.net/newyork
http://www.sciencepub.net

Emails:
newyorksci@gmail.com
editor@sciencepub.net

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Evaluation of *Bacillus Subtilis* Inoculation for Bioconversion of Organic Solid Wastes (Maize Husk, Cowpea Husk and African Spinach) Into Biofortified Compost

[1]Oladapo Zainab Abosede and [2]Bukola Catherine Akin-Osanaiye

[1]Department Of Microbiology, Faculty Of Science, University Of Abuja, P.M.B. 117, Abuja, Nigeria
[2]Department Of Biochemistry, Faculty Of Science, University Of Abuja, P.M.B. 117, Abuja, Nigeria
[1]**Email:** oladapozainababosede@gmail.com

Abstract: This study focused on the Evaluation of *Bacillus subtilis* inoculation for bioconversion of organic solid wastes (maize husk, cowpea husk and African spinach) into biofortified compost. Ten (10) kilograms of carbon-based organic wastes comprising of maize husk wastes, 5 kg of nitrogen based organic wastes which comprises of beans husk wastes and the cofactor which is vegetable (African spinach) wastes combined in the ratio of 2:1:1, 3:2:1 and 4:3:2 were used for the composting set up. The organic waste with 4:3:2 combination had the highest ($p<0.05$) proximate values of 24.70±3.2 % for ash content and the lowest lipid recorded 3.01±0.1 % as the least. The biofortified compost temperature was monitored during the period of composting. The initial temperature of the three organic wastes composition (2:1:1, 3:2:1 and 4:3:2) inoculated with *Bacillus subtilis* as well as their controls after mixing was 26.5 °C for day one and rose to 43.2 ^{0}C at day 30 while the control temperature was 29.4 ^{0}C. The initial pH of the biofortified compost (day 1) was 6.4 for all ratios. For the 2:1:1 combination, the pH increases from 6.4 to 6.5 at day 40 up till day 60 and for 3:2:1 and 4:3:2 combinations ratio the pH increases from 6.4 to 6.7. As the composting progress, the organic matter and organic carbon decrease from the day one to day 60 for all the combinations. The substrates mixtures showed an initial electrical conductivity (EC) of 1.8 dSm^{-1} from the first day. It reached up to 2.19 $dS\ m^{-1}$ for 2:1:1, 2.23 $dS\ m^{-1}$ for 3:2:1 and 2.35 $dS\ m^{-1}$ for 4:3:2 with progressive degradation up to 60 days meanwhile, the control only increased from 1.8 $dS\ m^{-1}$ to 1.9 $dS\ m^{-1}$. Compost analysis of the organic waste inoculated with *Bacillus subtilis* showed a good C:N ratio and increased value of phosphorus and nitrogen up till day 60 which are required for enhancement of soil fertility. The compost generated from the degradation can be applied to increase the fertility of the soil.

[Oladapo Zainab Abosede, Bukola Catherine Akin-Osanaiye. **Evaluation of *Bacillus Subtilis* Inoculation for Bioconversion of Organic Solid Wastes (Maize Husk, Cowpea Husk and African Spinach) Into Biofortified Compost.** *N Y Sci J* 2025;18(5):1-10]. ISSN 1554-0200 (print); ISSN 2375-723X (online). http://www.sciencepub.net/newyork. 01. doi:10.7537/marsnys180525.01

Keywords: Organic waste; *Bacillus subtilis;* biofortified compost; bioconversion

1.0 Introduction

Compost is a rich source of minerals (carbon, nitrogen, and phosphorus) with a slow mineralization rate, making it ideal for long-term soil fertility (Ishii *et al*., 2020). Compost significantly enhanced soil microbial biomass and nitrification in acidic soil by decreasing the toxicity of soil microorganisms and inhibiting soil heavy metals. Even at the lowest compost rate, such favorable effects are more significant than those induced by mineral fertilizer; this indicates that this type of compost can be particularly suitable for the reclamation and re-vegetation of polluted soils (Esse *et al*., 2020). Composting can degrade a broad range of hazardous organic pollutants and generate a soil amendment or fertilizer.

The term "biodegradable waste" refers to waste that can decompose naturally. In Nigeria's urban municipal solid waste, the proportion of organic matter ranges from 75 to 85% (Saha and Santra, 2020). Municipal solid waste (MSW) is typically made up of biodegradable trash. According to Bandara *et al.* (2021), the amount of organic matter in waste fluctuates according to the economic health of the country where the garbage is produced. As a result of the swift growth in population, economy, and urbanization over the past few decades, trash creation has been rising noticeably each year (Gouveia and Prado, 2022). According to Gupta and Arora (2021), urban India produces 68.8 million metric tons of municipal solid trash annually, or 1.88 million metric tons of trash each day. Dumping trash in a designated disposal yard is the most cost-effective disposal method. Because of this, diseases in people, plants, and cattle begin to appear (Forastiere *et al.*, 2020). Finding good bacterial strains is made easier because the soil microorganisms at waste sites are exposed to a variety of substrates and contaminants. Microorganisms with higher enzyme activity are required to quickly break down complicated polymers into more easily digestible molecules. Inoculating waste with microorganisms that produce extracellular enzymes at higher levels, such as cellulase, amylase, protease, pectinase, and lipase, speeds up waste disintegration and aids in maintaining the waste degradation rate at the disposal rate (Saha and Santra, 2020).

2.0 Materials and Method

2.1 Sample Collection

About 15 kilograms of organic waste comprising of maize, cowpea, and vegetable husk were collected from food vendors' waste bins in Gwagwalada, FCT. The samples were packed into a sterile polythene bag and brought to the Microbiology Laboratory, Department of Microbiology, University of Abuja, Gwagwalada, for preparation.

2.2 Compost Preparations

The composite was prepared by combining the organic waste (maize husk, cowpea husk and african spinach) at different ratios 2:1:1, 3:2:1, and 4:3:2.

2.3 Test organism

The test organism *Bacillus subtilis* (ATCC 6633) used for this evaluation was obtained from the Diagnostic Division of the National Veterinary Research Institute (NVRI), Vom, Jos. The organism was resuscitated by streak inoculation on Tryptic soy agar, incubated at 37 ^oC for 24 hrs, tested for purity by microscopy following Gram staining, subjected to conventional tests, and preserved on fresh nutrient agar slants in a refrigerator at 4 ^oC.

2.3.1 Identification of test organisms

The test organism was identified based on microscopy following Gram staining, and the characteristics used included growth patterns, colonial characteristics, color, shape, arrangement, and the entire surface of pure isolates, which were observed by visual examinations. Catalase tests and spore staining were performed to further confirm that *Bacillus subtilis* (ATCC 6633) is *Bacillus subtilis* (Cheesbrough, 2019).

2.4 Proximate Analysis of Organic Waste before Composting

Before composting, the maize husk, bean husk, and spinach wastes in the ratios 2:1:1, 3:2:1, and 4:3:2 were examined for their proximate composition, which comprises of moisture, protein, carbohydrate, nitrogen, fat, fiber, and ash. The procedure of Association of Official Analytical Chemists (AOAC, 2019) was used to determine the total ash, crude fiber, and ether extract. Micro Kjeldahl's method as Pearson (2018) was used to measure the nitrogen concentration. The protein content was then determined by multiplying the nitrogen concentration by 6.25. Carbohydrates contents was determined differences.

2.5 Standardization of *Bacillus subtilis*

Mcfarland turbidity standard number 0.5 was used to standardize the *Bacillus subtilis*. Barium Chloride (1.175 g) was dissolved in 100 mL distilled water. One (1) milliliter of sulphuric acid was measured into 100 mL of water. For the turbidity standard of 0.5; 0.5 ml of Barium Chloride solution was added to 99.5 mL of H_2SO_4 Solution. This is equivalent to a bacteria density of approximately $1x10^{5.}$ The isolate was diluted in a test tube using sterile water and then compare with the Mcfarland turbidity (Clinical and Laboratory Standards Institute, 2022).

2.6 Biofortification of Organic Waste

The biofortification of the organic waste composite was done as described by Payel and Rounak (2021). Briefly, the nitrogen-based organic waste and the carbon-based organic waste were sterilized by autoclaving for 15 minutes at 121°C and a pressure of 15 psi. After autoclaving, 1kg mixtures of the nitrogen-based organic waste and the carbon-based items were layered in a 5-liter container perforated at the top, middle, and base areas, and 5% (v/v) equivalent to 50 mL of 24-hour-old broth cultures of test *Bacillus subtilis* were then thoroughly mixed, and 100 milliliters of distilled water were also added to keep the pile moist and then incubated at ambient temperature (25 ± 2^{o}C) to observe the degradation. The composting pile was mixed periodically to maintain aeration every 5 days for 60 days until the compost resembled the color of healthy soil, had a loamy consistency, and had a pleasant aroma. Un-inoculated organic waste kept under similar experimental conditions serves as a control.

2.6.1 Chemical analysis of biofortified compost from organic solid waste

The resulting biofortified compost from organic solid waste was examined before and after composting by quantitative analysis using parameters such as pH, moisture content, temperature, electrical conductivity (E.C.), organic matter, total organic carbon (TOC), total nitrogen, C: N ratio, and phosphorus. This test was performed to observe the ability of the degraded organic solid wastes to be used as manure to increase the fertility of the soil.

2.6.2 Determination of biofortified Moisture Content

The compost sample was taken in a pre-weighed container, and the initial weight was recorded. The sample was dried in a hot air oven at a temperature of 110 ^oC while being weighed at regular intervals until a constant weight was achieved. The moisture content of the sample was calculated using the formular below:

$$MC (\%) = \frac{W - w}{w} \times 100$$

Where MC is the moisture content, W is the original weight, and w is the constant weight after oven drying.

2.6.2 Determination of biofortified compost temperature

The temperature was continuously monitored using a thermometer. The temperature was monitored every ten days during the process by inserting the thermometer into a depth of about 30 cm in the compost pile. The temperature was read and recorded.

2.6.3 Determination of pH

Using a digital pH meter, the potentiometric method determined the pH of the compost sample. Twenty (20) grams of the compost samples were transferred to a clean 250 mL beaker, to which 40 mL of distilled water was added. The contents were stirred intermittently, and the sample suspension was again stirred just before taking the reading. The electrode was immersed into the beaker containing the sample, and the meter reading was recorded (Tejada and Gonzalez 2021).

2.6.4 Determination of Electrical Conductivity (EC)

The electrical conductivity of the compost samples was determined according to the procedure described by Joshi *et al.* (2020). Electrical conductivity is the measurement of the total amount of soluble salts present in the sample and is expressed as millisiemens per cm (mS/cm). To five grams of the compost sample, 50 mL of distilled water was added, stirred well, and the suspension was allowed to settle for eight hours. The electrode of the conductivity cell was immersed into the sample solution, and the EC was read and expressed in millisiemens per cm (mS/cm).

2.6.5 Estimation of Organic Carbon

The determination of organic carbon was carried out according to the empirical method described by AOAC (2019). Exactly 1g of finely ground oven-dried compost sample (at 105 ºC) was placed in a constant-mass silica crucible and heated in a muffle furnace at 550 ºC for 2 hours. The crucible was allowed to cool down in desiccators and was again weighed.

$$\text{Organic matter (\%)} = \frac{\text{Initial mass - Final mass}}{\text{Initial mass}} \times 100$$

The ratio of carbon content to volatile substance content remains, to some extent, constant for a particular type of organic waste. The volatile substance in the sample was determined for organic matter estimation.

$$\text{Organic Carbon (\%)} = \frac{VS}{A} = \frac{\text{Organic matter (\%)}}{1.724}$$

Where A is a constant of 1.724.

VS = volatile substance percent (organic matter percentage).

2.6.6 Available Phosphorus Determination

Two grams of compost were weighed and dispensed into 20 mL of a 0.025 N HC1 + 0.03 N NH_4F solution, shaken for 5 minutes, and then filtered. After filtration, 3 mL of the filtrate preparation was put into a test tube, followed by 3 ml of 0.87 N HC1, 0.38 N ammonium molybdate, and 0.05 % H_3BO_3 solution, and 5 drops of 2.5 g of 1-amino 2-tetraoxosulfate (vi) acid, 5.0 g Na2SO3, and 146 g $Na_2S_2O_5$ solution were sequentially added to the preparation. A colorimeter (at a wavelength of 540 nm) was used to take the readings (AOAC, 2019).

2.6.7 Total Nitrogen Determination

Kjeldahl method as described by AOAC (2019) was used to estimate the sample's total nitrogen content. About 20 mL of concentrated tetraoxosulfate (VI) acid was added to 1g of compost. A catalyst known as Kjeldahl TAB was also added, and the solution was then digested. After digestion, a clear solution was observed, and the clear solution was distilled and subsequently titrated with 0.01M HCL.

2.6.8 Statistical Analysis

Data obtained in this study was statistically analyzed using Analysis of Variance (ANOVA) and the test applied was F-test statistic at $p < 0.05$.

3.0 Results

3.1 Proximate Composition of Organic Waste before Inoculation

Table 1 showed the proximate composition of the organic waste before inoculation with *Bacillus subtilis*. The organic waste with a 4:3:2 combination recorded highest ($p < 0.05$) proximate values of Ash, Moisture, Lipid, Fiber and Crude protein while the organic waste with a 2:1:1 combination recorded low proximate values of Ash, Moisture, Lipid, and Fiber. There is no significant different ($p < 0.05$) in the moisture and crude protein contents of the three organic waste samples.

Table 1: Proximate Compositions of the combined Organic Wastes

Proximate	2:1:1	3:2:1	4:3:2
Ash (%)	22.11±2.2[b]	23.10±2.1[c]	24.70±3.2[a]
Moisture (%)	10.03±1.1 [a]	10.04±1.3 [a]	10.34±2.2 [a]
Lipid (%)	1.21±0.12 [a]	1.22±0.2 [a]	3.01±0.1[c]
Fiber (%)	10.40±1.3[a]	10.44±1.2[a]	20.10±2.1[b]
Crude Protein (%)	17.05±2.2 [c]	17.02±2.4 [c]	17.39±2.3[c]
Carbohydrate (%)	37.20±3.4 [a]	38.18±4.6[b]	23.62±4.1[c]

Data are the Mean ± standard deviation of triplicate determination. Data having different lowercase superscript letters along the same column differ significantly (p <0.05).
Key: 2:1:1 = Maize husk + Cowpea husk + African spinach
3:2:1= Maize husk + Cowpea husk + African spinach
4:3:2 = Maize husk + Cowpea husk + African spinach

3.2 Physicochemical Characteristics of the Biofortified Compost

The physicochemical characteristics of the biofortified compost are represented in Figures 1 to 2 respectively.

3.2.1 Temperature of the biofortified compost

The compost temperature was monitored during the period of composting as represented in Figure 1. The initial temperature of the three organic wastes composition (2:1:1, 3: 2:1, and 4:3:2) inoculated with *Bacillus subtilis* as well as their controls after mixing was 26.5°C for day one. All the biofortified temperatures increased to 43. 2 ºC at day 30 while the control temperature increased at 29.4ºC. The temperatures of the biofortified composts from different composition ratios of organic waste were not significantly different (p< 0.05).

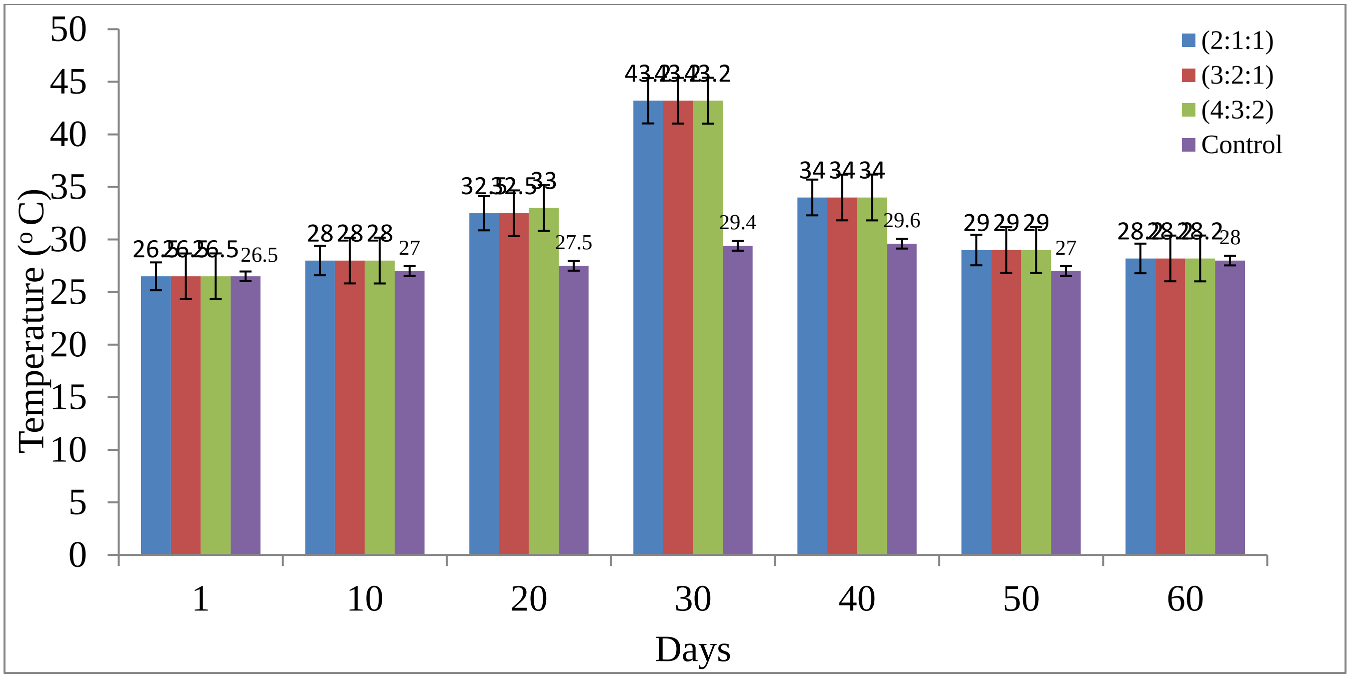

Figure 1: Temperature of the Biofortified Compost during composting

3.2.2 pH of the biofortified compost

At day one (1), the pH of the biofortified compost was 6.4 for all ratios. For the 2:1:1 combination, the pH increases to 6.5 from day 40 to day 60 and for the 3:2:1 and 4:3:2 combinations ratio, the pH increases from 6.4 to 6.7 respectively presented in Figure 2.

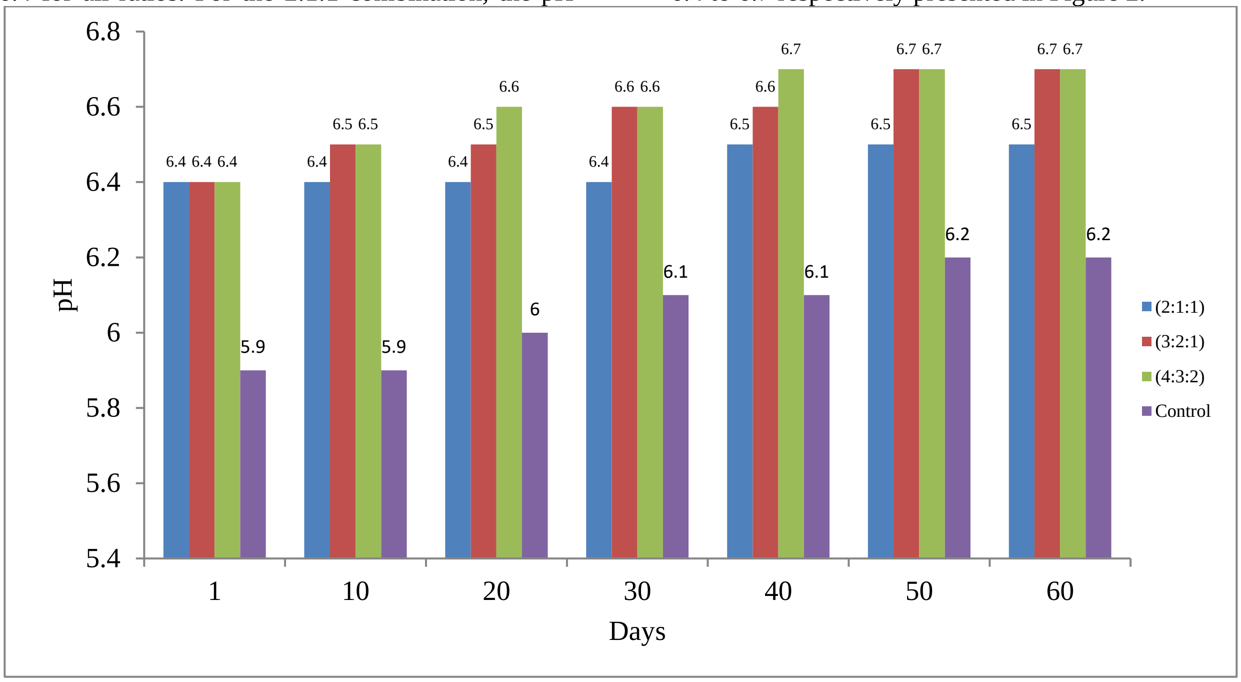

Figure 2: pH in the biofortied Compost during composting

3.2.3 Organic Matter of the biofortified compost

As the composting progress, the organic matter and organic carbon decrease from the day one to the day 60 for all the combinations as presented in Figures 3 and 4 respectively. The organic carbons of the biofortified compost from different combinations of the organic wastes differ significantly (*P*< 0.05).

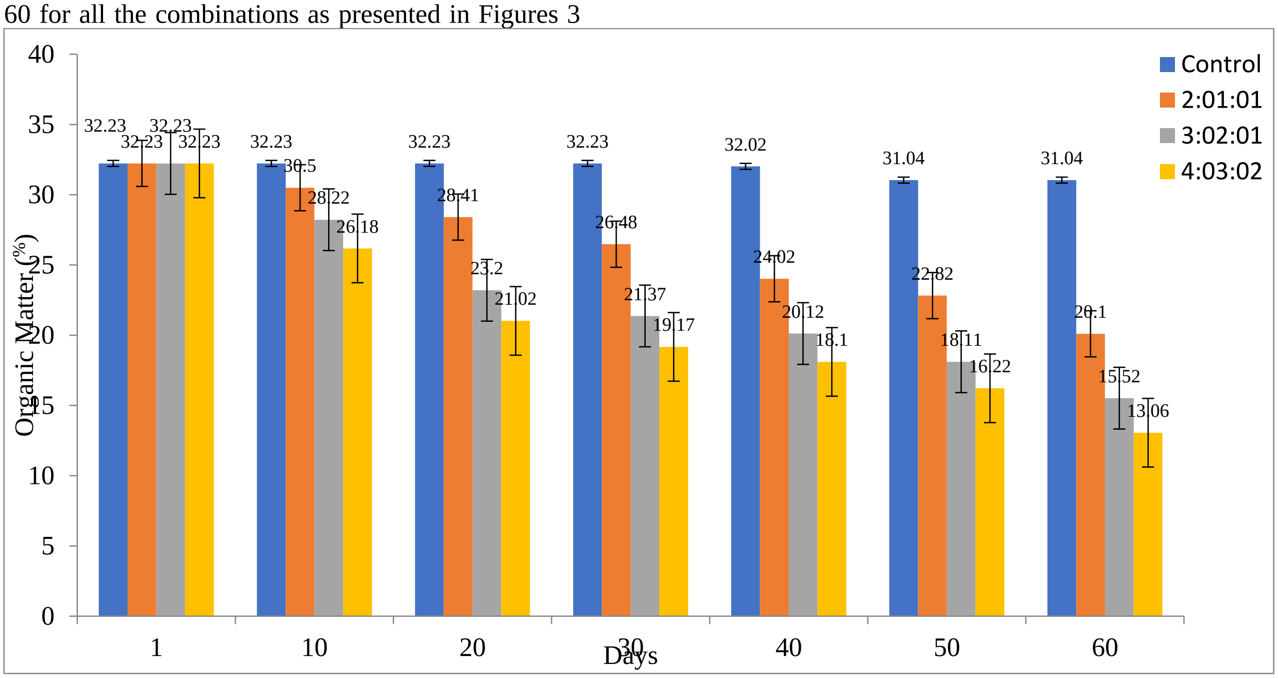

Figure 3: Organic Matter of the biofortified Compost during composting

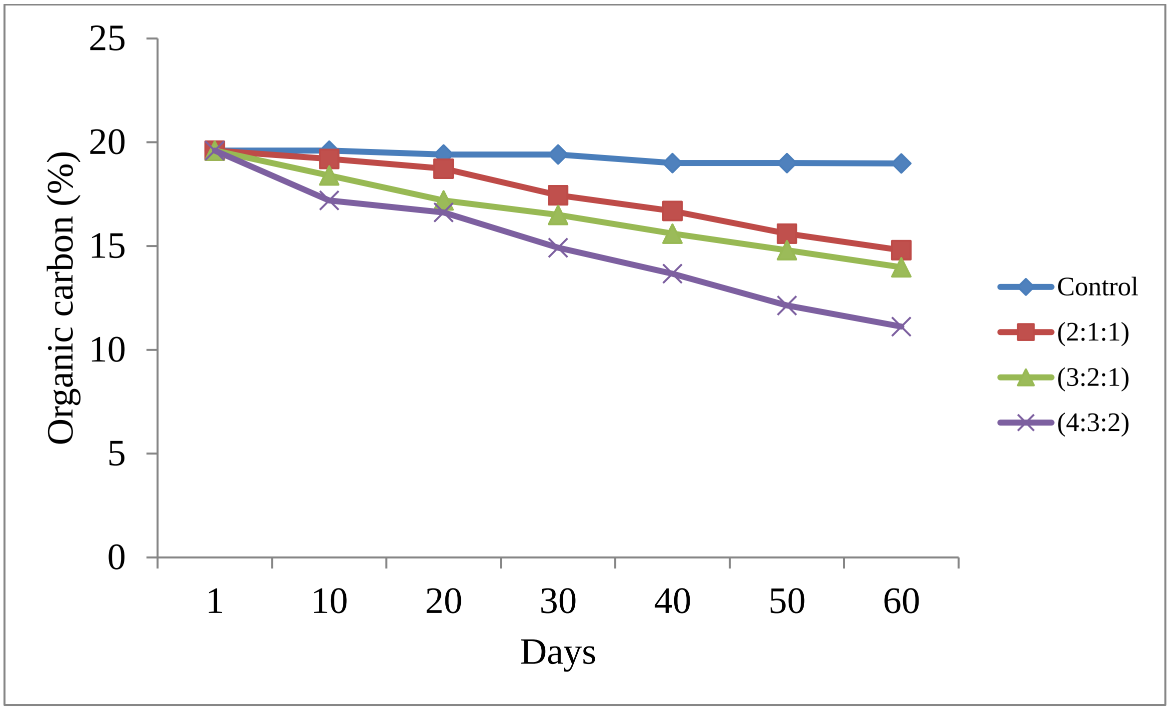

Figure 4: Organic Carbon of the biofortified Compost during composting

3.2.4 Available Phosphorus

The available phosphorus at the initial stage of the composting (day 1) was 1.4 mg/kg which later increase up till the day 60 as presented in Figure 5. The available phosphorus in the compost heap further in the composting period of different combinations ratio of organic waste was significantly different (*P*< 0.05).

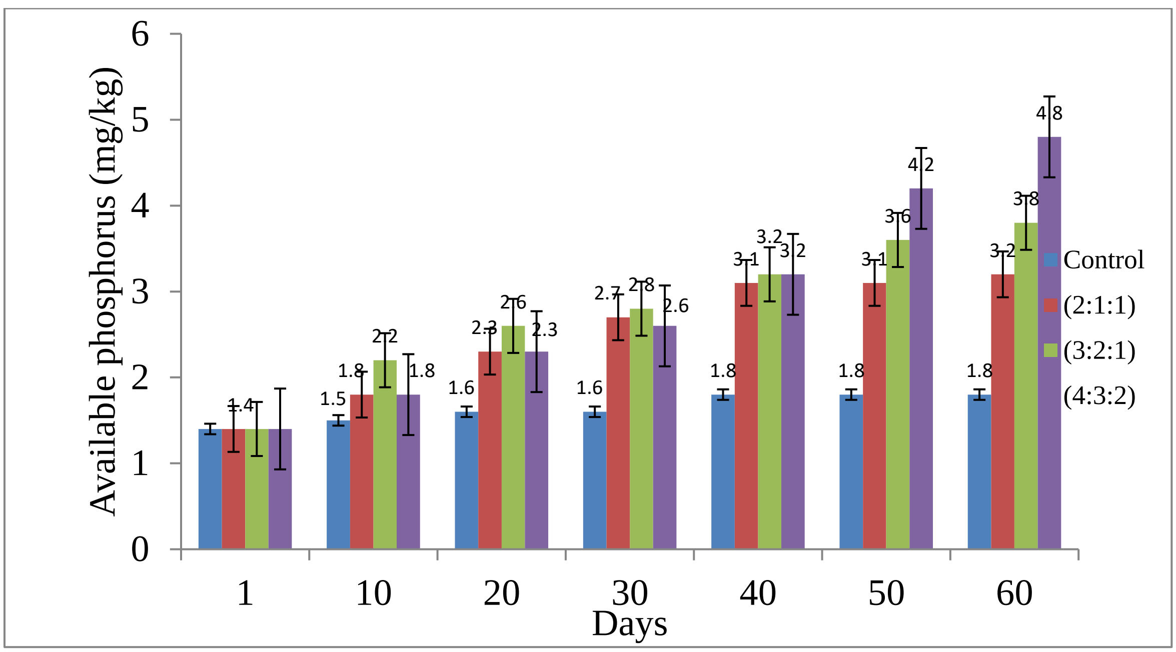

Figure 5: Available Phosphorus in the Compost Heap during Composting Period

3.2.5 Available Nitrogen

The total nitrogen increases from the first day to the 30th to 40th day and remains static up till the day 60 as presented in Figure 6. The total nitrogen of the biofortified compost from different combinations of the organic waste differ significantly ($P < 0.05$).

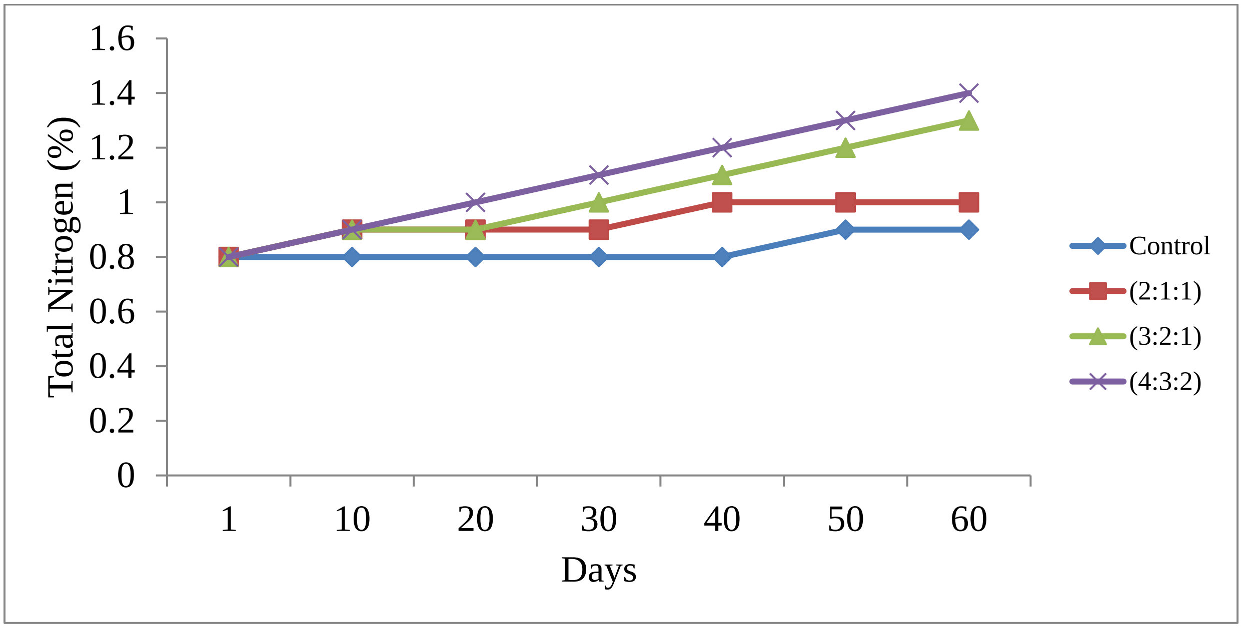

Figure 6: Total Nitrogen of the biofortified Compost during composting

3.2.6 Electrical conductivity

During the present study, the substrate mixtures showed an initial electrical conductivity (EC) of 1.8 dSm^{-1} with subsequent increases with progressive degradation up to 60 days as presented in Figure 4.7. The electrical conductivity of the biofortified from different combinations of the organic wastes differ significantly ($P < 0.05$).

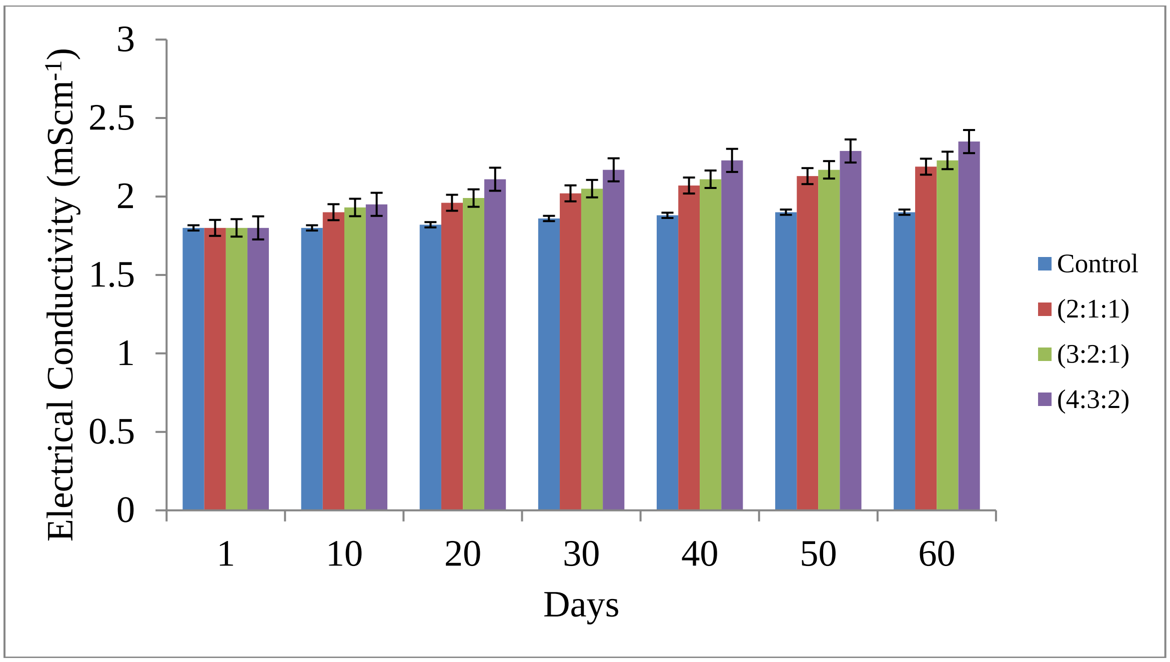

Figure 7: Electrical Conductivity of the biofortified Compost during Composting

3.2.7 Carbon: Nitrogen (C: N) Ratio

The C: N of the biofortified compost with a 2:1:1 combination ratio decreased from 25:1 to 15:1, biofortified compost with h 3:2:1 combination ratio decreased from 25:1 to 11:1 and biofortified compost with h 4:3:2 combination ratio decreased from 25:1 to 8:1 as presented in Table 2. Meanwhile, the, C: N of the biofortified compost for the control decreased from 25:1 to 21:1. The C: N biofortified compost using *bacillus subtilis* from different combinations of the organic wastes were significantly different ($P < 0.05$).

Table 2: C: N of the Biofortified Compost using *Bacillus subtilis*

Days	Control	2:1:1	3:2:1	4:3:2
1	25:1[a]	25:1[a]	25:1[a]	25:1[a]
10	25:1[a]	21:1[b]	20:1[c]	19:1[d]
20	24:1[a]	21:1[b]	19:1[c]	17:1[d]
30	24:1[a]	19:1[b]	17:1[c]	14:1[d]
40	24:1[a]	17:1[b]	14:1[c]	11.1[d]
50	21:1[a]	16:1[b]	12:1[c]	9:1[d]
60	21:1[a]	15:1[b]	11:1[c]	8:1[d]

The C: N biofortified compost using *bacillus subtilis* from different combinations of the organic wastes were significantly different ($P < 0.05$). Key: C =Carbon, N = Nitrogen. Each Value Represent Mean ± standard deviation from three replicate values. Values with the same superscript across the same row are not significantly different ($P < 0.05$).

4.0 Discussions

Composting is an integrated sustainable process that offers some advantages over inorganic fertilizer. This study focused on co-composting using different types of food waste. In this study, the organic wastes made up of maize husk, cowpea husk and African spinach in combination of 4:3:2 had the highest proximate values of ash content (24.70±3.2 %), moisture content (10.34±2.2 %), fat (3.01±0.1 %), fiber (20.10±2.1 %), crude protein (17.39±2.3 %) and carbohydrate (23.62±4.1%) followed by 3:2:1 combination while 2:1:1 recorded the lowest. The proximate compositions of the organic waste before inoculation determine the development of *Bacillus subtilis* during the composting. The *Bacillus subtilis* secrete different enzymes to hydrolyse the complex organics matter to a stable and simple form and eventually produce a product such as humus (Goyal *et al*., 2020).

Ibrahim *et al*. (2020) reported that one of the principal sources of microbial enzymes is produced by bacteria from the genus *Bacillus*. For this reason in the present study, The *Bacillus subtilis* was screened for production of amylase, protease, pectinase and cellulase. Amylase was the highest with 9.21±0.12U/ml enzyme unit, followed by protease activity of 8.8 ±0.44U/ml. Pectinase enzyme produced by the *Bacillus subtilis* was 6.40±0.13U/ml while cellulase recorded by *Bacillus subtilis* was 5.11±0.22U/ml. These results demonstrate that this *Bacillus subtilis* synthesize different type of enzymes over time, suggesting that this strain has the capacity to degrade cellulose, starch, pectin and protein substances.

Similar result was reported by Gopinath *et al*. (2020) who constructed five different microbial consortia and the compatibility of the consortia to degrade organic waste was checked by enzyme assays of four substrates (starch, cellulose, casein and tributyrin).

Temperature is a significant parameter in the composting process. Composting has a typical temperature profile of a quick increase in temperature of up to 43.2 °C in the first 30 days for all the combinations in this study. Composting involves a rapid transition from a mesophilic to a thermophilic microbial community and followed by a slow decrease in temperature. At the thermophilic stage, thermophilic bacteria used in this study degraded the complex material such as pectin, protein, starch and cellulose. During this process, the particulate organic matter was disintegrated by enzymatic hydrolysis (Lauwers *et al*., 2020). These hydrolytic enzymes including protease, pectinase, cellulase and amylase, are secreted by the test organism *Bacillus subtilis.* This finding suggested that application of *Bacillus subtilis* in agricultural waste could reduce the dependency on the industrial enzyme, chemical and thermal pretreatments during composting.

As the composting progress, the organic matter and organic carbon decrease from the initial day one to day 60 for all the combinations in this study. The organic matter content of the test compost was 20.1 % and was lesser than that of the control compost (31.04 %) after 60 days. Organic matter content decreases with composting as the degrading organisms *Bacillus subtilis* utilize the organic carbon for energy generation and biosynthesis. This reduction of organic matter in the test compost leads to reduction in volume and mass of the compost.

The carbon nitrogen ratio (C: N of the biofortified compost with 2:1:1 combination ratio decreased from 32:1 to 22:1, biofortified compost with 3:2:1 combination ratio decreased from 32:1 to 20:1 and biofortified compost with 4:3:2 combination ratio decreased from 32:1 to 18:1. Higher C: N ratio causes initial N immobilization and very low C: N ratio causes overabundance of N, resulting in accumulation of ammonia (Huang *et al*., 2020). The C: N ratio decreases during composting, as organic carbon is converted to CO_2 by degraders and lost in its gaseous phase, whereas the nitrogen is converted to other forms by the test organism. The C: N ratio of the test compost was reported to be 22:1, suggesting it to be effective for the enhancement of soil fertility. Jeyapriya and Saseetharan (2021) reported a reduction in the C: N ratio of municipal solid waste from 30:1 to the final compost C: N ratio of 19:1. Jilani (2021) observed a huge reduction in the C: N ratio from 40 to 26 during municipal solid waste composting. A final C: N ratio of less than 26:1 is considered suitable for the compost for its applicability to increase the fertility of soil.

Initially at day one in this study, the pH of the biofortified compost was 6.4 for all ratios. For the 2:1:1 combinations, the pH increases to 6.5 at day 40 to day 60 and for 3:2:1 and 4:3:2 combinations ratio, the pH increases from 6.4 to 6.7, an ideal property of the compost to be used to increase soil fertility. Tejada and Gonzalez (2021) reported that production of organic acids and other degradation products lead to the generation of foul odour and a decrease in pH.

During the present study, the substrates mixtures showed an initial electrical conductivity (EC) of 1.8 dSm^{-1} from the first day. It reached up to 2.19 dS m^{-1} for 2:1:1, 2.23 dS m^{-1} for 3:2:1 and 2.35 dS m^{-1} for 4:3:2 with progressive degradation up to 60 days meanwhile the control only increased from 1.8 dS m^{-1} to 1.9 dS m^{-1}. Janakrinam and Sridevi (2020) also reported an increase of EC content from 7.39 to 10.77 m mho/cm during municipal solid waste composting. The difference in the EC value may be due to the difference in the composting materials. There was a slight increase in the percentage of phosphorus and nitrogen in the test compost as compared to fresh waste. Phosphorus of the biofortified compost with organic waste ratio of 4:3:2 decreased from 1.4 to 4.8 mg/kg. The available phosphorus at the initial stage of the composting was 1.4 mg/kg which later increase up till day 60 as presented in Figure 5. The un-inoculated organic waste set up increase from 1.4 to 1.8, biofortified compost with organic waste ratio of 2:1:1 increased from 1.4 to 3.2, biofortified compost with organic waste ratio of 3:2:1 increased from 1.4 to 3.8 and biofortified compost with organic waste ratio of 4:3:2 increased from 1.4 to 4.8 mg/kg available phosphorus. Similar results were obtained by Janakrinam and Sridevi (2020), where phosphorus increased from 0.29 to 0.40% and nitrogen increased from 1.61 to 1.78%. *Bacillus subtilis* can serve as a biological tool for removal of organic solid wastes from the environment and the compost generated from the degradation can be applied to increase the fertility of the soil.

4.1 Conclusion

It can be concluded from the study that among the three organic wastes composition (2:1:1, 3:2:1 and 4:3:2) inoculated with *Bacillus subtilis* for composting, the 4:3:2 was the most degraded and compostable organic waste after 60 days of degradation with dark colour and lacked foul smell.

References

1. AOAC (2019). *The Association of Official Analytial Chemists*. The Official Methods of Analysis of AOAC International, 18th ed. Arlington, U.S.A. pp. 23-56.
2. Bandara, N.J., Hettiaratchi, J.P., Wirasinghe, S.C. and Pilapiiya, S. (2021). Relation of waste generation and composition to socio-economic factors: a case study. *Environmental Monitoring and Assessment,* 135(1):31–39.
3. Cheesbrough, M. (2019). *District Laboratory Practice in Tropical Countries Part Two*. Cambridge University Press, Pp. 23-140.
4. Clinical and laboratory standards institute (CLSI) (2022). Performance standards for antimicrobial susceptibility testing; 27th informational supplement. M100-S28. Clinical and Laboratory Standards Institute, wayne, PA

5. Esse, P.C., Buerkert, A., Hiernaux, P. and Assa, A. (2020). Decomposition of and nutrient release from ruminant manure on acid sandy soils in the Sahelian zone of Niger, West Africa, Agriculture. *Ecosystem Environment,* 83: 55-63.
6. Forastiere, F., Badaloni, C., Hoogh, K.D., Kraus, M.K., Martuzzi, M. and Mitis, F. (2020). Health impact assessment of waste management facilities in three European countries. *Environmental Health,* 10:53-57.
7. Gopinath, L.R., Christy, P.M., Mahesh, K., Bhuvaneshwari, R. and Divya, D. (2020). Identification and evaluation of effective bacterial consortium for efficient biogas production. *Journal of Environmental Science and Toxicological Food Technology,* 8(3):80–86
8. Gouveia, N. and Prado, R.R. (2022). Health risks in areas close to urban solid waste landfill sites. *Journal of Environmental Microbiology,* 44(5):1–8
9. Goyal, S., Dhull, S. K. and Kapoor, K.K. (2020). Chemical and biochemical changes during composting of different organic wastes and assessment of compost maturity. *Bioresource and Technology,* 96: 1584-1591.
10. Gupta, B. and Arora, S.K. (2021) Municipal solid waste management in Delhi—the capital of India. *International Journal of Innovative Research Science and Engineering Technology,* 5(4):5130–5138.
11. Huang, G. F., Wong, J. W. C., Wu, Q. T. and Nagar, B. B. (2020). Effect of C/N on composting of pig manure with sawdust. *Waste Management,* 24: 805-813.
12. Ibrahim, S.E., El-Amin, H.B., Hassan, E.N. and Sulieman, A.M.E. (2020). Amylase production on solid state fermentation by *Bacillus* spp. *Food and Public Health,* 2:30–35.
13. Ishii, K., Fukui, M. and Takii, S. (2020). Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. *Journal of Applied Microbiology,* 89: 768-777.
14. Janakrinam, T. and Sridevi, K. (2020). Conversion of waste into wealth: a study in solid waste managent. *Electronic Journal of Chemistry,* 7(4):1340–1345.
15. Jeyapriya, S.P. and Saseetharan, M.K. (2021). Study on municipal solid waste reuse characteristics and leachate samples of Coimbatore city. Journal of Nature, Environmental Pollution and Technology, 6(1): 149–152
16. Jilani, S. (2021). Municipal solid waste composting and its assessment for reuse in plant production. *Pakistan Journal of Botany,* 39(1): 271–277
17. Joshi, N., Sharma, S. and Kangri, G. (2020). Physico-chemical characterization of sulphidation press mud composted press mud and vermicomposted pressmud. *Report and Opinion,* 2: 79-82.
18. Lauwers, J., Appels, L., Thompson, I.P., Degrève, J., Van Impe, J.F. and Dewil, R. (2020)., Mathematical modelling of anaerobic digestion of biomass and waste: Power and limitations. *Progress in Energy and Combustion Science,* 39 (4): 383-402.
19. Payel, S. and Rounak, C. (2021). Bioconversion of organic solid wastes into biofortified compost using a microbial consortium. *International Journal of Recycling Organic Wastes and Agriculture,* (2021) 6:321–334.
20. Pearson, D. (2018). The Chemical Analysis of Food (7th Edition.). Churchill Livingstone. New York. Pp. 11-15.
21. Puyuelo, B., Ponsá, S., Gea, T. and Sánchez, A. (2020). Determining C/N ratios for typical organic wastes using biodegradable fractions. *Chemosphere,* 85: 653-659.
22. Saha, A. and Santra, S.C. (2020). Isolation and characterization of bacteria isolated from municipal solid waste for production of industrial enzymes and waste degradation. *Journal of Microbiology Experts,* 1(1):1–8.
23. Tejada, M. and Gonzalez, J.L. (2021). Influence of organic amendments on soil structure and soil loss under stimulated rain. *Soil Tillage Research,* 93(1): 197–230.

3/22/2025