# Changes in microbial communities and nutrient content of rice straw compost caused by sterilization and their impact on plant growth

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**Abstract.** Compost, which plays a crucial role in sustainable farming systems, is produced through composting, an aerobic process mediated by microorganisms that decompose organic matter and typically involves a thermophilic phase. Compost functions as both a source of nutrients and organic matter and as a reservoir of beneficial microorganisms. However, the impact of compost, whether containing microorganisms or not, remains unclear. This study evaluated the impact of sterilization on rice straw (RS) compost and its influence on the growth of rice, maize, and peanut. RS-compost was produced over 60 days by adding Azolla, EM-4 and molasses. The compost was divided into sterilized (multistage autoclaving, 3 times over 3 consecutive days) and non-sterilized treatments. Sterilization reduced microbial populations in the compost significantly (0–5.1 × $10^5$ CFU/g vs. 2.9–836.7 × $10^5$ CFU/g) but increased nutrient content, notably total nitrogen (128.57%) and available potassium (84.4%), while reducing organic carbon (4.9%). Greenhouse experiment showed the application of non-sterilized compost significantly boosted dry weights of rice (142.01%), maize (29.22%), and peanut (8.78%) compared to sterilized compost. These findings highlight the critical role of indigenous microorganisms in enhancing RS-compost functionality and plant growth. This approach is critical for fundamental research on compost treatments, which host diverse microorganisms with multifunctional roles that influence the final outcomes.

## 1 Introduction

Nutrients are essential for plant growth and determine plant production in agriculture. Compost is well known as organic fertilizer which functions not only as nutrients or organic matter source, but also as beneficial microorganisms source. Compost is produced by a typical process of an aerobic decomposition and conversion of various solid degradable organic materials by microorganisms that passes through a thermophilic stage, that is used worldwide owing to its eco-friendly [1–3]. Successful composting requires large numbers of different mesophilic and thermophilic aerobic microorganisms to decompose the

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corresponding organic fraction at different stages to finally produce products with a high maturity index [4].

The composting process covered four stages, namely the pre-composting stage, thermophilic, middle, and curing stages which characterized by specific microbial communities as revealed by phospholipid fatty acid (PLFA) analysis [5] and polymerase chain reaction - denaturing gradient gel electrophoresis (PCR-DGGE) followed by sequencing [6]. The high temperatures during the thermophilic stage effectively eliminate the microorganisms originally present in the compost material, allowing new microbial communities to proliferate successively in correspondence with the subsequent composting stages. The sanitation effect during the thermophilic stage suppressed pathogenic microorganisms while simultaneously stimulating the growth of beneficial microorganisms.

The quality of compost product is determined by the quality of materials, the involved microorganisms, and the whole process of composting [1]. Incorporation qualified compost to soil affected to the improvement of the chemical, biological, and physical properties of soil including stimulating mineralization and increasing of available nutrients either macro- and micronutrients [7], increasing population density, diversity, and metabolic activities of soil microorganisms, increasing soil enzyme activity [8–11], suppressing soil pathogen [12], increasing soil cation exchange capacity (CEC) [13], soil water holding capacity and stability aggregate [14,15].

Several studies have reported the effect of compost by separating the function of compost as nutrients/organic matter sources and as microbial sources, that were performed by sterilization treatment to the compost product and continued by evaluating the effect of sterilized and non-sterilized compost products. Saison et al (2006) [16] investigated the sterilization of compost using gamma radiation, it was found that the effect of compost amendments on soil was due to the physical and chemical characteristics of the compost matrix rather than the microorganisms in the compost. Sidhu et al (2001) [17] reported that sterilized compost by autoclaving affected the increase of *Salmonella* growth compared to non-sterilized compost.

There is a lack of comprehensive understanding regarding the varying effects of compost with and without active microbial communities. Investigating the effects of sterilization on compost properties, including nutrient content and microbial communities, as well as its subsequent influence on plant growth, is critical. This research aims to assess the impact of sterilization on the changes of the populations of general and specific bacterial communities and nutrient content in rice straw (RS) compost and to evaluate its effects to the growth of rice, maize, and peanut.

The present experiment is significant as a methodological approach to support fundamental research involving specific treatments that require compost, either solely as a source of nutrients or microorganisms, rather than as a comprehensive input.

## 2 Materials and Methods

### 2.1 Composting

Composting was conducted in the greenhouse of the Faculty of Agriculture, Sebelas Maret University in March-May 2024. The materials used for composting were 300 kg rice straw, 15 kg *Azolla,* and bioactivator EM4 + molasses. Rice straw (RS) was moistened with water and piled on the floor [5,6]. EM-4+ molasses and *Azolla* were spread and mixed on each layer around 20 cm depth until all RS material was piled with 2 x 0.75 x 1 $m^3$ then the surface covered with a plastic sheet.

The developing temperature in the center of compost pile was measured every day in the first week then continued every time before turning with 3 replications. Additionally ambient

temperature was measured. RS pile was turned every 2 weeks to ensure aerobic and homogeneous decomposition [5,6]. Compost harvested on day 60.

### 2.2 Sterilization of Compost Product

Compost product was taken from a depth 50 cm at the center of the compost pile [1]. Sample was taken as much as 5 kg and divided into two parts, a half part was non-sterilized and the other part was sterilized. Sterilization was carried out using multistage autoclaving at 121°C and 1 atm for 15 minutes, repeated 3 times over 3 consecutive days, with a one-day incubation period between each autoclaving [18,19].
Previous study by [17] reported that twice autoclaving at 121℃ for 20 min on two consecutive days showed high regrowth of indigenous *Salmonella* in the composted biosolids. Therefore, the present study tried to apply 3 times autoclaving in 3 consecutive days to increase the effectiveness of sterilization. Multistage autoclaving was performed to ensure the elimination of all present microorganisms, including microbial spores that might break dormancy and germinate during the first or second autoclaving cycle.

### 2.3 Microbial Community Analysis

The microbial community of compost products were analyzed using the spread plate method [20]. Samples were prepared in 10 grams and diluted with physiological saline solution at $10^{-1}$ to $10^{-7}$. A total 0,1 mL from $10^{-1}$, $10^{-3}$, $10^{-5}$, and $10^{-7}$ dilutions were inoculated on nutrient agar (NA) and Pikovskaya media and 0,1 mL of $10^{-1}$, $10^{-3}$, and $10^{-5}$ dilutions were inoculated on yeast extract mannitol agar (YEMA) medium for symbiotic N-fixing bacteria and Jensen medium for non symbiotic N-fixing bacteria. The inoculum was incubated for 2 × 24 hours at room temperature for general bacteria and 5 × 24 hours for specific bacteria, then population density and colony morphotype diversity were observed.

### 2.4 Chemical Characteristics of Compost

Compost products were analyzed for chemical characteristics including Organic-C, total-N and P, available P and K. Organic carbon analysis used Walkley and Black method, total N used Kjeldahl method, total P and available P used $HNO_3$ Extraction, and available K used $HNO_3$ extraction [21].

### 2.5 Greenhouse experiment

A greenhouse experiment was conducted in the greenhouse of the Faculty of Agriculture, Sebelas Maret University in July-August 2024 to test the effect of sterilization compost on the growth of rice, maize, and peanut. A greenhouse experiment used a Completely Randomized Design with two factors: Compost type (non-sterilized and sterilized) and Plant type (Rice var. Inpari 32, Maize var. Bonanza, and Peanut var. local) with 5 replications of each treatment. The soil used was a non-sterilized Alfisol with the characteristics of pH 5.9, organic-C 0.95%, total-N 0.1%, total-P 10.3 ppm, available-P 0.79 ppm, total-K 0.005 me/100g. Each pot contains 200 g of soils and 19 tons/ha of compost or equivalent to 9.5 g/pot. The soil of each pot was moistened by watering and maintained at the level 70% field capacity. Plants were harvested on day 35.
Plant growth parameters observed included plant height, number of leaves, shoot fresh and dry weight, root fresh and dry weight, and the additional variables for peanut including number of nodules and nodules weight.

### 2.6 Statistical analysis

The data obtained in this study were analyzed using a t-test to determine the significance of differences between the treatment group (sterilized RS composts) and the control group (non-sterilized RS composts). The variables analyzed included microbial populations and nutrient content in the RS composts, as well as the growth parameters of rice, maize, and peanut plants.

## 3 Results and Discussions

### 3.1 Temperature during composting

Compost temperature measurements were taken every day in the first week at the center of the compost with 3 replications and continued every time before turning with the following results.

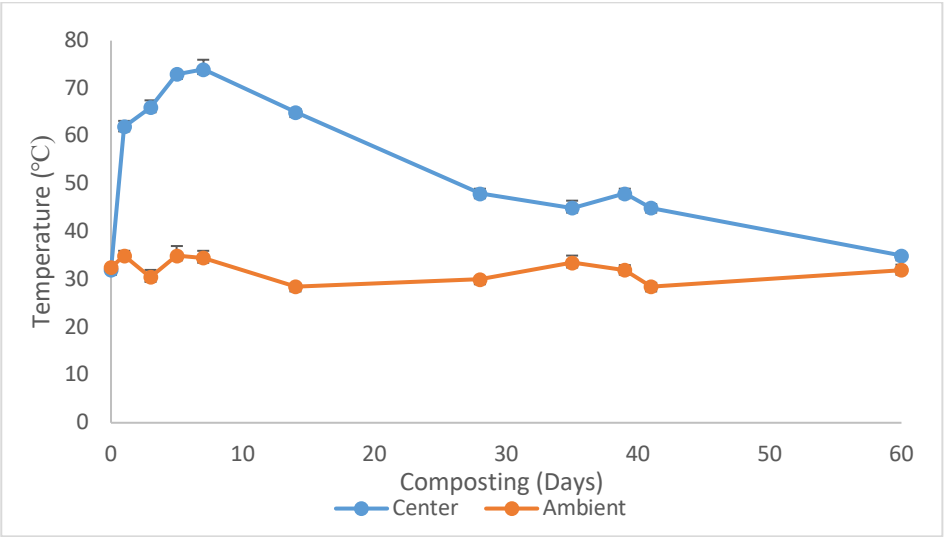


**Fig. 1.** Temperature during composting

The composting process started at the mesophilic phase on days 0-1, then temperature increased until achieved the peak temperature at 74℃ on day 7 and then gradually decrease to level 45℃ on day 28. Afterwards the temperature slightly increased to 47℃ on day 39, then smoothly decreased to ambient temperature (Figure 1). This pattern of developing temperature was similar with the report of composting process by [1,6,22], indicating the composting in the present study underwent sterilization process during high temperature at the thermophilic stage.

### 3.2 *Population density and colony diversity of non-sterilized and sterilized compost grown on 4 media*

As shown in Table 1, in the non-sterilized compost, the highest bacterial population density was shown in NA medium at 836.72 × $10^5$ CFU/g, followed by Pikovskaya media at 548.81 × $10^5$ CFU/g, YEMA media at 3.74 × $10^5$ CFU/g, and the lowest was Jensen's agar media at 2.88 × $10^5$ CFU/g. In the sterilized compost, the highest bacterial population density was shown in NA media at 5.1 × $10^5$ CFU/g, followed by Pikovskaya media at 2.63 × $10^5$ CFU/g, YEMA media at 0.03 × $10^5$ CFU/g while in Jensen media there was no growth of non-symbiotic Nitrogen fixing bacteria. This study showed that the population density either general bacteria or specific bacteria from non-sterilized compost was significantly higher than sterilized compost. Thus, sterilization treatment by steam method of multistage autoclaving at 121℃, 1 atm, 3 times over 3 consecutive days effectively reduced the population of indigenous bacteria in compost. Using the present method, it is suggested that the third autoclaving effectively eliminated the regrowth of microbial propagules that persisted after the first and second autoclaving. This is in line with the findings of [19], who reported that soil sterilization by using autoclave 121℃, 1 atm, 3 times with one day incubation between sterilizations affected significantly in reducing microbial population of bacteria (using NA medium) and fast and slow growing fungi (using potato dextrose agar and rose bengal agar media, respectively). [23] reported that there was a difference of total bacterial colony forming unit (CFU) between autoclave treatment with non-sterilized soil by $1\times10^8$ to $207\times10^8$.

**Table 1.** Population density and colony morphotypes diversity of RS compost

| Medium type | Microorganism group | Population density ($10^5$ CFU/g) | | | Colony morphotypes diversity | | |
|---|---|---|---|---|---|---|---|
| | | Non-sterilized RS Compost | Sterilized RS Compost | *p value* (t-test) | Non-sterilized RS Compost | Sterilized RS Compost | *p value* (t-test) |
| NA | General Bacteria | 836.72 | 5.10 | 0.00** | 6 | 2 | 0.020* |
| Pikovskaya | Solubilizing Phosphate Bacteria | 548.81 | 2.63 | 0.00** | 5 | 2 | 0.035* |
| YEMA | Symbiotic Nitrogen Fixing Bacteria | 3.74 | 0.03 | 0.00** | 5 | 1 | 0.057ns |
| Jensen | Nonsymbiotic Nitrogen Fixing Bacteria | 2.88 | 0.00 | 0.00** | 2 | 0 | 0.074ns |

Note: Significance levels compared to the corresponding control value based on the t-test: **=$p \leq 0.01$, *= $0.01 < p \leq 0.05$, and ns (not significant) = $p > 0.05$

Based on colony morphotypes, bacteria grown on NA medium have the highest colony diversity compared to the other media while the lowest colony diversity is on Jensen medium. In detail, the morphotypes diversity of bacterial colonies in non-sterilized compost grown on NA media was 6 types, on Pikovskaya media 5 types, YEMA 5 types, and Jensen 2 types (Figure 2). On the other hand, the morphotypes diversity of bacterial colonies in the sterilized compost which was grown on NA and Pikovskaya media are 2 types, YEMA 1 type, and there was no growth of bacteria on Jensen medium (Figure 3).

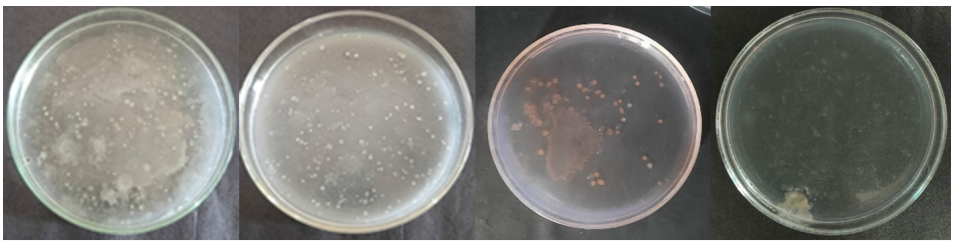


**Fig. 2.** The bacterial colonies from non-sterilized compost grown on: a. NA; b. Pikovskaya; c. YEMA; d. Jensen

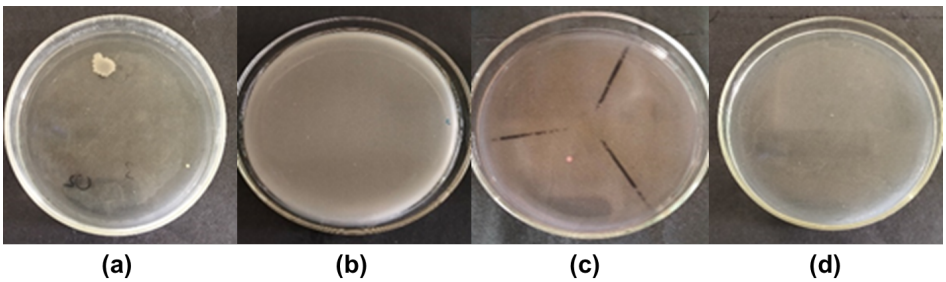


**Fig. 3.** The bacterial colonies from sterilized compost grown on: a. NA; b. Pikovskaya; c. YEMA; d. Jensen.

### 3.3 *Chemical characteristics of non-sterilized and sterilized composts*

It was found significant differences between non-sterilized and sterilized compost on their nutrients content. Total nitrogen and available potassium concentration increased after sterilization treatment. Total N from 0.98 to 2.24% and available potassium from 2.25 to 4.15 ppm. Compost pH and organic carbon decreased after sterilization treatment (pH from 8.9 to 8.6 and organic-C from 27.57 to 26.21%) (Figure 4). [24] reported a similar result that total carbon concentrations decreased after steam sterilization. In the present study, sterilization treatment did not affect significantly toward available and total phosphorus contained in the composts but tended increase.

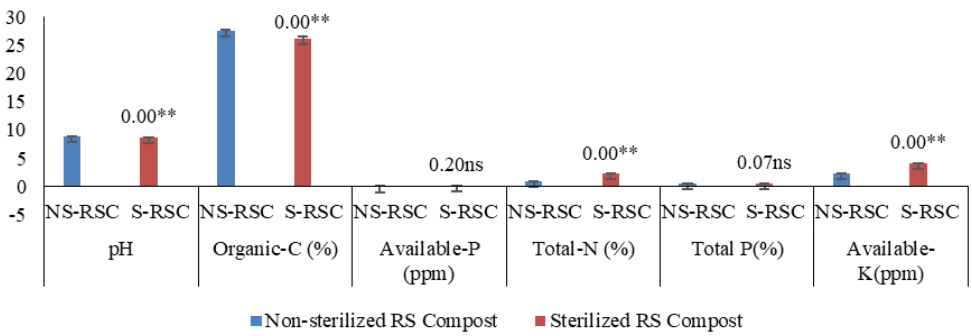


**Fig. 4.** Chemical characteristics of non-sterilized and sterilized RS compost.
Significance levels compared to the corresponding control value based on the t-test:
**=$p \leq 0.01$, *= $0.01 < p \leq 0.05$, and ns (not significant) = $p > 0.05$

The results indicate that sterilization compost has a significant impact on nutrients content. Sterilization increased total nitrogen by 128.57% compared to non-sterilized compost which is in line with other studies that sterilization of soil by autoclaving increased effectively extractable nitrogen using micro-Kjeldahl method by 1.4% [23]. The present study confirmed that multistage sterilization using autoclave stimulates mineralization of nutrients contained in the compost, as represented by the significant increase of total N, available K, while for the available and total P although not significant but tended to increase in the sterilized compost.

### 3.4 *Effect of Non-sterilized and Sterilized RS Compost on Plants Growth*

The effect of non-sterilized and sterilized compost on plants growth are presented in Figure 5.

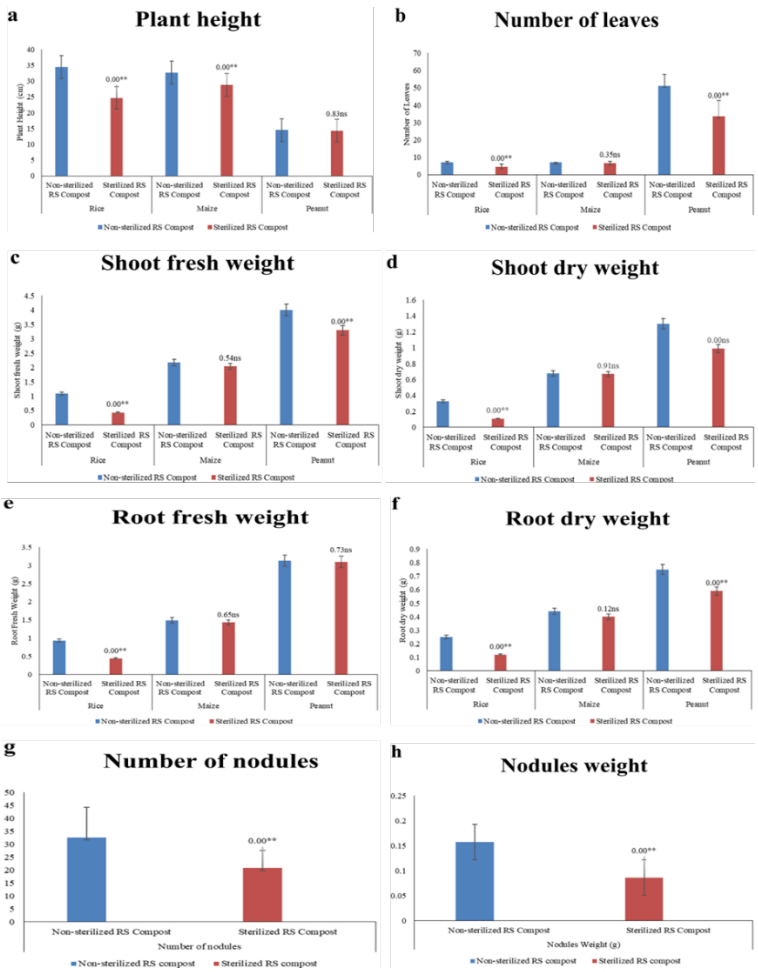


**Fig. 5.** Effects of non-sterilized and sterilized RS compost on the growth of rice, maize, and peanut plants: a. Plant height; b. Number of leaves; c. Shoot fresh weight; d. Shoot dry weight; e. Root fresh weight; f. Root dry weight; g. Number of nodules (peanut); h. Nodules weight (peanut); Significance levels compared to the corresponding control value based on the t-test: **=$p \leq 0.01$, *= $0.01 < p \leq 0.05$, and ns (not significant) = $p > 0.05$

The soil used for the greenhouse experiment was an Alfisol that characterized low fertility with pH 5.9, organic-C 0.95%, total-N 0.1%, total-P 10.3 ppm, available-P 0.79 ppm, total-K 0.005 me/100g. The application of non-sterilized compost affected significantly higher plants growth of rice, maize, and peanut compared to sterilized compost, represented by all variables (Figure 5 a-h). As shown in Figure 4, non-sterilized compost treatment indicated higher organic-C than sterilized compost. This higher C was estimated to contribute in optimizing soil conditions that affect to improve plant growth. [25] reported that the addition of C by application of compost biochar affected increase of total organic carbon that support of plant growth on oats *Avena sativa ssp. Nuda.* The non-sterilized compost treatment in the present study also affected significantly higher number of nodule and nodule weight for peanut compared to the sterilized compost. This phenomenon was considered due to the roles of symbiotic N-fixing bacteria which existed in RS compost. Previous study by [1] also reported the existing of N-fixing bacteria in compost products. Similar report by [26] revealed that application of paddy straw compost significantly increased root nodules on soybean.

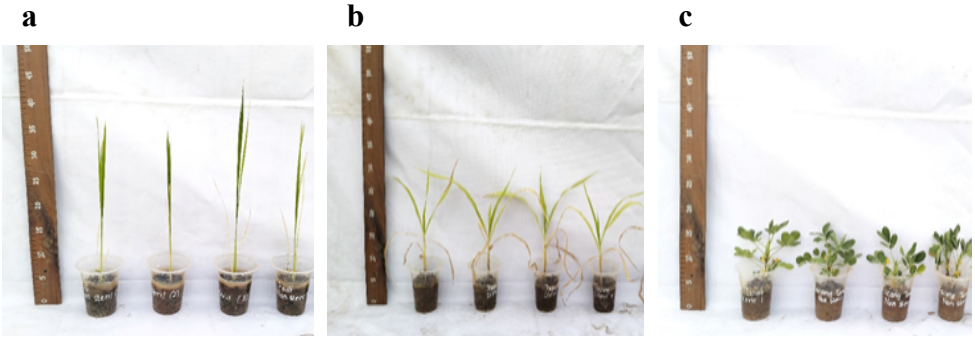


**Fig. 6.** Plant growth on non-sterilized compost treatment (35 days after planting): a. rice, b. maize, and c. peanut

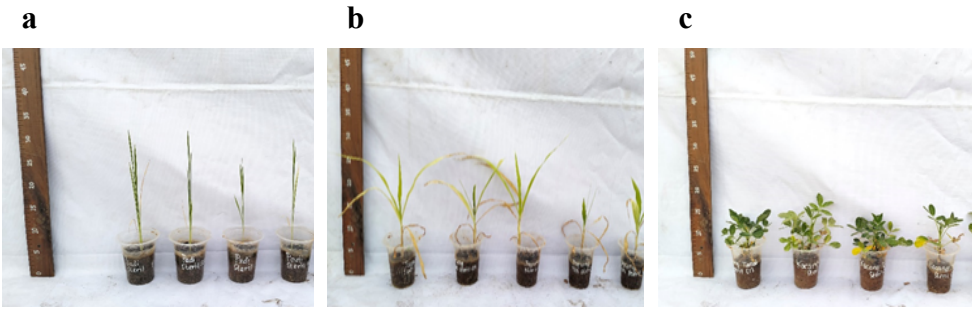


**Fig. 7.** Plant growth on sterilized compost treatment (35 days after planting): a. rice, b. maize, and c. peanut

The non-sterilized compost treatment indicated higher fresh plant and dry weights of rice, maize, and peanut by 5.6 - 131.8% and 4.94 - 142.01% compared to the sterilized compost treatment (Figure 6 a-c and Figure 7 a-c). Indigenous organisms in non-sterilized compost, after its incorporation into the soil, are believed to play vital roles in supporting plant growth by contributing to a wide range of soil functions, such as in decomposition and mineralization of organic matter in releasing available nutrients in soil [25], in increasing soil cation exchange capacity (CEC) [27] in increasing stability soil aggregate and water holding capacity [28], and in suppressing plant pathogen [29]. The similar phenomenon was reported

by [30], that soil sterilization killed indigenous organisms in the soil, and impacted to the inhibition of the growth of pepper plants. The study highlighted the critical role of indigenous microorganisms in specific habitats, where they contribute to essential processes within life cycles.

## 4 Conclusion

Sterilization method using multistage autoclaving at 121°C and 1 atm for 15 minutes, performed 3 times over 3 consecutive days, with a one-day incubation interval between each autoclaving significantly reduced microbial populations in RS compost either for general bacteria or specific bacteria of phosphate-solubilizing bacteria, symbiotic and non-symbiotic nitrogen-fixing bacteria. Sterilization also decreased organic carbon by 4.9% but stimulated mineralization, resulting in significant increases in total nitrogen (128.57%) and available potassium (84.44%), alongside slight increases in total phosphorus (0.08%) and available phosphorus (0.4%). Even with its higher nutrient content, sterilized compost proved to be less effective in fostering plant growth. In contrast, non-sterilized RS compost resulted in significantly higher fresh and dry weights for rice (131% and 142%), maize (5% and 4.9%), and peanut (10.67% and 29%) compared to sterilized compost. This finding underscores the diverse roles of indigenous microbial communities in RS compost after its incorporation into the soil, extending beyond nutrient enrichment to promoting overall plant growth. This methodological approach clarifies key factors in utilizing compost, especially in fundamental research, by considering its multifunctional nature and potential impact on the intended treatments. Further studies are needed to assess the effects of various sterilization methods on compost products with different materials.

### Acknowledgments

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