

## Effect of nitrogen amendments and inoculum on spent mushroom substrate composting for sustainable waste management

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**Abstract.** Spent Mushroom Substrate (SMS) is the medium used in mushroom cultivation and is mostly dumped into the environment as waste. Composting SMS is identified as a sound method of reuse. However, lower N levels and reduced microorganism activity limit aerobic decomposition. Therefore, this experiment was conducted to evaluate the effects of amending different N sources to obtain good-quality SMS compost. Nitrogen sources were mixed to SMS separately based on the C/N ratio of each source, with and without the bio-dynamic inoculum to obtain ten SMS compost mixtures with Urea (T1), Urea + inoculum (T2), *Gliricidia sepium* leaves (T3), *G. sepium* leaves + inoculum (T4), *Tithonia diversifolia* leaves (T5), *T. diversifolia* leaves + inoculum (T6), *Pueraria phaseoloides* leaves (T7), *P. phaseoloides* leaves + inoculum (T8), SMS without inoculum (T9), and SMS with inoculum (T10). Treatments were arranged in a Completely Randomized Design (CRD) with four replicates and composted for 90 days. Final samples were analyzed for total organic matter (%), total N (%), C/N, total K (%), total P (%), pH and EC and data analysis was done using SAS software 9.1.3. According to the results, T7 and T8 have given a significantly higher level of N% (1.71 and 1.89, respectively). The C/N ratio of all treatments except T5, T9 and T10 reached an average of 20-30, and T8 recorded the lowest. In all treatments EC values were within the Sri Lankan Standards (<4 mS/cm), however, the pH values were higher (>8.5). Based on the results, it is concluded that treating SMS with *P. phaseoloides* leaves with the bio-dynamic inoculum could obtain good quality SMS compost resembling the given compost standards.

**Keywords:** compost; compost quality; green leaves; urea

### 1. Introduction

Mushrooms are a highly concentrated protein source with rich nutrient value and as a result, the demand for mushrooms and their value-added products have increased worldwide (Chakravarty, 2011). Statistical data indicate that the world mushroom production has reached a level of 25 million tons, and it plays a vital role in balancing the protein profile of vegetarian diets as well (Ashrafi *et al.*, 2014a). However, after obtaining the harvest, tons of the media used in mushroom cultivation are dumped without any alternative use. This is known as spent

mushroom substrate (SMS), considered as waste. In mushroom cultivation, around 5 kg of SMS is released as waste in the process of 1kg of mushroom production and it was estimated that 70 million metric tons of SMS was generated around the world in 2007 (Ashrafi *et al.*, 2014a). This waste contains lignin-rich sawdust which decomposes slowly and ions such as  $Mg^{2+}$  and  $Ca^{2+}$ , which contribute to water hardening and pollution (Nepfumbada, 2021). Therefore, dumping of this SMS leads to surface runoff and leaching of these ions, which contaminates both surface water bodies and ground water. Consequently, it is important to find an eco-friendly and cost-effective method to reuse SMS.

Many researches have highlighted the potential of using SMS in compost production, which is considered as an environmentally acceptable method of disposing and utilizing organic waste (Zhang and Sun, 2015). Since SMS contains 45% moisture and bulky with 55% solids, it is within the range for compost production (De Jin *et al.*, 2005; He *et al.*, 2001). Additionally, producing compost with SMS might be an extra income source for mushroom cultivators by allowing them to produce spent mushroom compost (SMSC) using naturally available nitrogen sources that thrive well in organic farming sector (Eudoxie *et al.*, 2011). Hence, SMSC would be a beneficial amendment which improves soil water and nutrient holding capacity, soil water infiltration, permeability, aeration, and helps to improve the soil structure (Wiafe *et al.*, 2018; Fidanza *et al.*, 2010).

However, the high carbon percentage of saw dust in SMS and depleted nutritional condition due to rapid utilization by the mushroom mycelium hinder the bio degradation of SMS into compost (Mallick *et al.*, 2023). To enhance the composting process, the Carbon-to-Nitrogen (C/N) ratio needs to be maintained between 25:1 to 30:1 (Ashrafi *et al.*, 2014b). With the high carbon content of SMS, the C/N ratio rises to 70-80, necessitating the amending nitrogen source to lower the C/N ratio.

Urea is an excellent N source which can be integrated into the composting process (Wever *et al.*, 2004). However, rather than relying on inorganic fertilizers, using natural N sources in composting would be advantageous from both economic and environmental perspectives. High N containing plants such as *Gliricidia sepium*, *Tithonia diversifolia* and *Pueraria phaseoloides* have been proven to be good sources of N while *G. sepium* and *P. phaseoloides* host N-fixing symbiotic bacteria that colonize in their roots, where bacteria capable of fixing atmospheric nitrogen and transferring it to the plant in an available form (Omari *et al.*, 2016; Quansah *et al.*, 2001). Moreover, *T. diversifolia* accumulates high biomass nutrient density (N, P, K) from soil uptake (Jama *et al.* 2000; George *et al.*, 2001). Therefore, use of these N sources in SMS composting will increase the N content and thereby reducing the C/N ratio facilitating the biological decomposition.

On the other hand, as SMS being a fungus-cultivated media, it lacks the diversity of microorganisms which help in biodegradation (Xu *et al.*, 2022). Inoculating SMS with a microbial inoculum could alter the microbiota structure of SMS (Xu *et al.*, 2022) and enhance the biodegradation rate, thereby increasing the rate of compost process. For instance, *Bacillus* inoculation has been shown that kitchen waste can be composted rapidly by increasing bacterial diversity (Zhang *et al.*, 2021). Therefore, the present study hypothesizes that adding N sources and microbial inoculum enhances the degradation and quality of produced compost using SMS. The objective was to characterize the chemical properties of the final compost samples treated with *G. sepium*, *T. diversifolia*, *P. phaseoloides*, Urea and bio-dynamic inoculum and to find the optimal compost mixtures which resemble the standard compost quality measures.

## 2.0 Methodology

Spent Mushroom Substrate (SMS) from oyster mushroom (*Pleurotus ostreatus*) cultivation was obtained from mushroom production unit of Faculty of Agriculture, University of Ruhuna,

Sri Lanka. It was used as the bulking agent for compost production. Green leaves of *Pueraria phaseoloides*, *Gliricidia sepium*, and *Tithonia diversifolia* which were collected from the farm of Faculty of Agriculture, University of Ruhuna, Sri Lanka and commercially available urea were used as N sources for this experiment. The SMS was amended and mixed thoroughly with each of the above-mentioned N sources separately, according to the equation provided by Rynk *et al.* (1992).

## **2.1 Preparation of biodynamic formulation**

The biodynamic formulation was prepared following the method by Kulkarni and Gargelwar., (2019). In a bucket, 500 g of cattle manure, and 250 ml of cattle urine, were mixed thoroughly with 10 L water. Then, 100 g of brown sugar and 100 g of mung bean flour were added and mixed well. Finally, a handful of topsoil was added to the mixture. The mixture was stirred twice a day to aerate and kept for 15 days. The optimal growth period for the microorganisms in this solution is noted to be 15 days.

## **2.2 Treatment preparation**

Ten treatments were prepared: five mixtures without applying the inoculum and five mixtures with the inoculum (Table 1). The inoculum was applied at a rate of 5 ml per 1 kg of the substrate, accounting for the bulk density of the substrate (Amarasinghe and Jayaweera, 2022) which was  $340 \text{ kg m}^{-3}$ . Each mixture weighing 2 kg, was separately placed into a black polythene bag of gauge 150 and labelled accordingly. Each treatment was replicated three times. The bags were arranged under ambient temperature ( $\sim 30^\circ\text{C}$ ) and relative humidity ( $\sim 78\%$ ) conditions following a completely randomized design (CRD). Every compost mixture was mixed manually every other day for five minutes to speed the decomposition process. The moisture level was monitored and adjusted accordingly by field method, around 50-60% at the

initial stage of composting and later it was kept for about 25-30% (SLS 1635, 2019). Mixtures were allowed for aerobic decomposition for 90 days.

**Table 1: Treatments used for the experiment.**

<b>Treatment</b>	<b>Description</b>	<b>Abbreviation</b>
<b>T1</b>	SMS + Urea	U
<b>T2</b>	SMS + Urea + Inoculum	UI
<b>T3</b>	SMS + <i>Gliricidia sepium</i> leaves	G
<b>T4</b>	SMS + <i>Gliricidia sepium</i> leaves + Inoculum	GI
<b>T5</b>	SMS + <i>Tithonia diversifolia</i> leaves	W
<b>T6</b>	SMS + <i>Tithonia diversifolia</i> leaves + Inoculum	WI
<b>T7</b>	SMS + <i>Pueraria phaseoloides</i> leaves	P
<b>T8</b>	SMS + <i>Pueraria phaseoloides</i> leaves + Inoculum	PI
<b>T9</b>	Only SMS	C
<b>T10</b>	SMS + Inoculum	CI

### **2.3 Data Collection and statistical analysis**

The sampling was done after 90 days from each experimental unit and air dried. These were analyzed for pH, EC, total organic matter (%), total Nitrogen (N%) content, total Potassium content (K%) and total Phosphorus (P%) content. pH and EC were analyzed using pH and EC meter by following the standard method with 1:2.5 w/v and 1:5 w/v, respectively. At the end of composting, samples were collected and analyzed for the total organic matter %, N%, K% and P%. Total organic matter was analyzed using the loss on ignition method and N% was analyzed following the Kjeldahl method (Bremner, 1960). Both Total K% and total P were extracted using the wet ash method. Potassium (K%) was analyzed using a flame photometer (Page *et al.*, 1982) and P% was analyzed using a UV visible spectrophotometer (Self-Davis *et al.*, 2000). Total Organic C% was calculated using the conversion factor 1.724 (Amarasinghe *et al.*, 2024). Based on the results of organic C% and N%, C/N ratios of each compost sample were calculated. Normality of the data was tested using Shapiro-Wilk Test. Statistical analysis was done using ANOVA using SAS statistical software (Version 9.1.3) and post-hoc tests were done using Duncan's Multiple Range Test (DMRT).

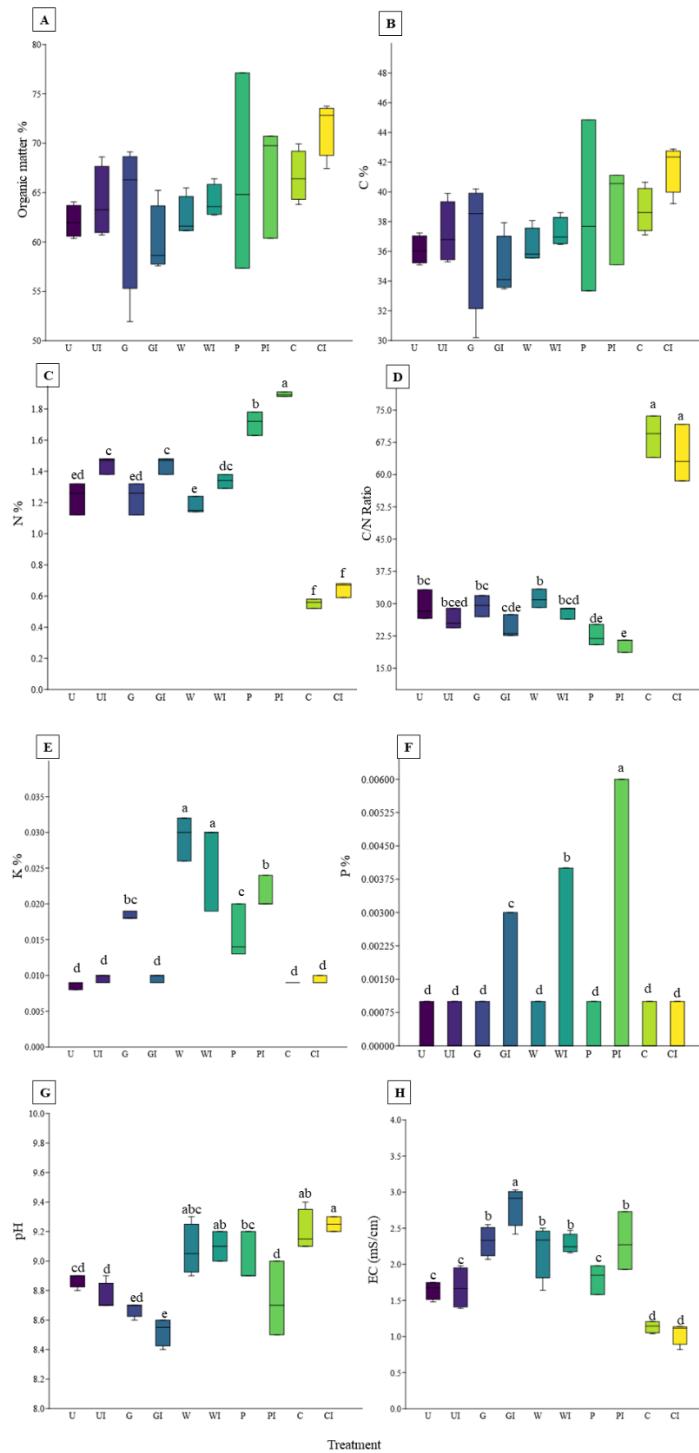
### 3.0 Results and Discussion

The following results were obtained after analyzing the final compost samples. According to Table 2, the pooled results from the ANOVA for the chemical properties of the prepared compost samples: N%, C/N ratio, K%, P%, pH and EC were significant at  $p \leq 0.05$ .

**Table 2:** ANOVA Results for compost parameters

Parameter	Degrees of freedom	F Value	p Value
Organic C%	9	0.95	0.503
Organic Matter%	9	1.99	0.079
N%	9	125.31	<b>&lt;0.0001</b>
C/N ratio	9	79.12	<b>&lt;0.0001</b>
K%	9	25.60	<b>&lt;0.0001</b>
P%	9	658.03	<b>&lt;0.0001</b>
pH	9	14.21	<b>&lt;0.0001</b>
EC	9	21.44	<b>&lt;0.0001</b>

Statistically significant results at  $p \leq 0.05$  are in bold.



**Figure 1.** Analyzed chemical properties in the final sample of prepared compost. A- Total organic matter %, B: Total Organic Carbon%; C: N%; D: C/N ratio; E: K%; F: P%; G: Electrical Conductivity (mS/cm) Values followed by the same letter are not significantly different as determined by the DMRT ( $p<0.05$ ). Abbreviations are presented in Table 1.

### 3.1 Total Nitrogen (%)

Nitrogen, the outstanding major nutrient for crop growth and development, is essential to analyze when producing compost. The Carbon content (C%) of SMS was significantly high (58.33%) compared to its N level (0.76%), resulting in a C/N ratio of 76.78 which is not appropriate for efficient biodegradation. Therefore, amending the SMS with N sources is crucial to optimize the C:N ratio for rapid composting process (Table 3).

**Table 03:** Initial N% of the N sources used

N source	N%
<i>Urea</i>	46.00
<i>G. sepium</i>	8.81
<i>P. phaseoloides</i>	9.60
<i>T. diversifolia</i>	10.44

Compost samples collected at the end of composting were analyzed for N% (Figure 1C). Treatment 7 and 8 (SMS + *P. phasioloides* leaves with and without inoculum) showed significantly higher values compared to all the other treatments. However, T5 has shown only 1.17% N, despite having the highest initial N content in *T. diversifolia* leaves. This may happen due to the alkalinity developed when composting with *T. diversifolia*, which accelerates the N loss through ammonia volatilization (Rochette *et al.*, 2013). When comparing the N content of the compost samples prepared with and without inoculum, treatments with the inoculum have resulted in higher N%. Cattle manure and urine contain diverse microbial communities including cellulotic bacteria, N-transforming bacteria, actinomycetes and fungi (Adegunloye *et al.*, 2007; Li *et al.*, 2020 and Nguyen & Nguyen, 2018). These microorganisms accelerate organic matter decomposition, enhance N mineralization and reduce N losses during composting, resulting in higher total N content in inoculated compost. In addition, cattle manure and cattle urine-based inoculum contains higher amount of N, which may contribute

to the increment in N% (Nguyen & Nguyen, 2018 and Raj *et al.*, 2014). Consequently, the present study found that inoculated SMS treatments (T2, T4, T6, T8 and T10) had higher N% than non-inoculated treatments.

Using *G. sepium*, *P. phaseoloides*, and *T. diversifolia* as green manures and nitrogen sources in organic agriculture can improve soil fertility, physical and chemical properties, as well as increase total soil nitrogen and NO<sub>3</sub>-N (Setyowati *et. al.*, 2018). *G. sepium* is an effective soil nitrogen source, with studies showing that it enhances the activities of decomposing microflora and results in higher soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> compared to other plant material treatments (Omari *et al.*, 2016). *P. phaseoloides*, a common cover crop found in Sri Lanka, has also shown potential as a nitrogen source. Experiments have indicated that its compost result in higher levels of nitrogen and decomposition rate compared to other legumes (Quansah *et al.*, 2001). These findings confirm that these plant leaves effectively increased N% in compost production.

### **3.2 Total Organic Carbon**

Soil organic carbon is a major factor prompting plant growth and development. The highest C% was recorded in samples without nitrogen sources (Figure 1B). However, compared to the initial high C% of SMS (58.33%) final SMS compost (SMSC) amended with N sources showed a reduction in C% during the composting process. During the process of composting, microbes start decomposing the organic material utilizing C as the energy source and N for the protein synthesis (Insam *et al.*, 2023). Thus, effective decomposition needs a balance between the C and N. With low N content, microbes may immobilize any available N from inoculum into their biomass to meet their needs, rather than building enzymes that degrade carbon compounds (Ayilara *et al.*, 2020 and Nguyen & Nguyen, 2018). Therefore, total N required to sustain robust decomposition of SMS is insufficient and overall carbon breakdown remains limited

increasing the total C content. The study conducted by Zeng *et al.* (2022) showed that the total organic C of the initial SMS was 44.89%.

### **3.3 C/N Ratio**

When comparing the C/N ratio of all treatments, the optimum C/N ratio (10-25) according to the SLS 1635:2019 was observed in T8 (*P. phaseoloides* and inoculum incorporated) and T7 (without inoculum) (Figure 1D). The control (T9) has a significantly higher value for the C/N ratio which did not achieve the standards of compost. SMS is rich in C and lacks in N because of developing fungi mycelium has utilized most of the N in the substrate during mushroom production.

As mentioned by Owaïd *et al.* (2017), C/N ratio plays a very important role in crop growth and development. The quality assessment done by Ashrafi *et al.* (2014b) compost made of SMS revealed that the initial N level of SMS was very low N (0.78%), and it reached a level of 1.11% at the end of 12 weeks of composting. Further, according to the above study, the organic carbon level of the SMS has reduced from 32.9% to 25.3% at the end of 12<sup>th</sup> week. Thereby, they observed a C/N ratio of 23 at the end of the composting process. A research study showed that the SMS amended with cattle manure is rich in TOC % whereas low in TN and the C:N ratio was changed from 29 to 19 after composting (Zeng *et al.*, 2022). In the present study, the initial organic carbon level of SMS was 58.33% and N was 0.76%, with initial C/N ratio as 76. However, with the application of the treatments, the N level of the final samples have increased to a level of 1.2% - 1.8%, reducing the C/N ratio of the final compost samples.

### **3.4 Total Potassium (%)**

Potassium is an important nutrient in demining compost quality which should meet a minimum of 1% in compost according to the SLS (1635:2019). However, all the treatments showed K%

lower than the standard level (Figure 1E). The K% of T5 and T6 were significantly higher than the other samples.

The higher K content in compost treatments containing *Tithonia diversifolia* can be attributed to the inherent high K content of the *Tithonia* biomass (Kaboneka *et al.*, 2021). These leaves typically contain 3.4% – 4.1% of K on dry weight basis where reported K% of *Gliricidia* and *Pueraria* is around 1.3% – 2.7% on dry weight basis (Gabhane *et al.*, 2023; Zurhalena *et al.*, 2023). Unlike N, K in plant tissues is mostly in readily mineralizable forms and is nonvolatile during decomposition, input of *Tithonia* biomass can result in greater K pool that persist through the composting process to the final product.

### **3.5 Total Phosphorus (%)**

All the treatments showed very low P% in final compost, which did not meet the minimum requirement of 0.5% P according to the SLS 1635: 2019. However, according to the results obtained, the P% of treatments T4, T6 and T8 were significantly higher than the other treatments (Figure 1F). SMS, used as the common substrate in all treatments inherently contain notable P residues which have a possibility to be bound with other amendments in initial mushroom mixture such as  $\text{CaCO}_3$  and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  as calcium and magnesium phosphates (Aiduang *et al.*, 2025; Matar *et al.*, 1992). When SMS is amended with *Gliricidia*, *Pueraria* and *Tithonia*, total P inputs are increased due to the higher P content in these species (Gabhane *et al.*, 2023 and Kaboneka *et al.*, 2021). The addition of inoculum likely stimulated microbial activity and phosphatase-mediate P mineralization, increasing available P in mature compost (Nguyen & Nguyen, 2018). As the P is not viable for volatilization losses, final compost product can result in increased P contents than non-amended treatments.

### 3.6 pH and Electrical Conductivity (EC)

Measured values of pH in final samples (Figure 1G) had an average pH range from 8.5 – 9.5. According to the compost standards by SLS 1635: 2019, an ideal compost sample should have a pH of 6.5 – 8.5. The higher pH values observed in the present study may be due to the residual  $\text{Ca}^{2+}$  ions which were added as lime ( $\text{CaCO}_3$ ) and Epsom salt ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) utilized during the mushroom production process. Based on the results, the lowest pH was recorded in T4 (8.5) while the highest was in T10 (9.25).

In Contrast to the pH results, the EC of T10 (1.05 mS/cm) was the lowest, T4 had the highest EC (2.82 mS/cm) (Figure 1F). According to SLS 1635 (2019), an ideal compost sample should have EC <4 mS/cm. Thus, within a specific range, EC would indicate good nutrient availability for plants, with the low end indicating nutrient poor soil that is structurally unstable and disperses readily and the high-end salinity problems (Smith *et al.*, 1997). Accordingly, all the SMS were within the acceptable range for the EC, showing less salinity in final compost mixtures.

EC of compost is mainly determined by the cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{NH}_4^+$ ) and the anions ( $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$  and  $\text{HCO}_3^-$ ) (Bhardwaj *et al.*, 2021). The EC of all the treatments amended with N sources increased compared to the non-amended SMS which may be due to the increment in  $\text{NO}_3^-$  and  $\text{NH}_4^+$  compared to the control treatment. Further, SMS pre-treated with lime and Epsom salt has a higher affinity for  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions which can result for higher EC. Over time, decomposition by microorganisms accelerates the extraction of ions from organic matter (Zeng *et al.*, 2022). Khater (2015) has highlighted that an average EC of 2.0 – 4.0 mS/cm as the optimum EC level for the plant growing media.

#### **4.0 Conclusions**

Based on the results obtained, it is concluded that SMS compost which is incorporated with *Pueraria phaseoloides* leaves and bio-dynamic inoculum is more suitable for compost production compared to other utilized nitrogen sources, when considering the SL standard for quality measurements of compost. It revealed that incorporating N sources and inoculum have led to higher N and appropriate C/N ratios than utilizing SMS directly as a soil amendment or compost. The addition of the inoculum acted as a beneficial microbial enhancer to accelerate the composting process and significantly increases the N level of the final compost. This shows that microbial inoculum can be an effective strategy to upgrade the compost by adding N and expedite the compost process.

However, in this study the P and K levels in final composts were below the standard levels. Therefore, supplementation of P and K sources in SMS should be considered in future research to meet the compost quality.

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