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Utilization of Spent Mushroom Compost as Carrier for Bio-Fertilizer Production using Pseudomonas Fluorescens and Azotobacter Chroococcum

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Abstract

Background: Spent Mushroom Compost (SMC), is a residual by-product of organic waste made up of plants, animals and fungi mycelia from a harvested mushroom fruits. Piled up of this waste create pollution and makes environment unhygienic for human activities. Research had it that, input of chemical fertilizers to increase soil fertility by farmers is detrimental to the environment and our health. Thou, SMC, the carrier contain fungi mycelia and trace of carbon source which serve as soil amender and organic fertilizer to improve plant growth. The aim of this study is to test SMC whether is a suitable and effective carrier for soil amendment and bio fertilizer production using *Azotobacter* and *Pseudomonas* strain. The organisms are best known as Plant growth promoting rhizobacteria (PGPRB) which lives in our soil with great potential. Inoculation of these with SMC has help improve the nutrient recovery of the carrier material. Materials and Methods: Spent Mushroom sample was collected from FIIRO botanical garden Langtang North-Plateau state, and was transported to Microbiology lab. Nasarawa State University, Keffi, for further experiment. Samples were sterilized, allowed to cool before inoculation with mixed consortium of *Azotobacter chroococcum* and *Pseudomonas fluorescens*.

Results: The following were recorded: Ca 31.437 %, K 12.36 %, P 11.201 %, Fe 3.000 %, Zn 2.211 %, Mg 2.102%, Mn1.311 %, Cu has 1.002 %, N 0.98% and the least was Mo having 0.137 % respectively. Similarly, Maximum water holding capacity was 94.77, organic matter 59.13 %, organic carbon 46.37 %, Carbon-Nitrogen ratio 46:1, moisture content 43.86%, pH.7.4 and the least Ash content having 6.01% respectively. Mean and standard deviation method was employed to find the average mean for the entire triplicate sample measured. Statistically, there was a significant difference in the value recorded because the P>0.05

Conclusion: Thus, SMC is therefore considered a suitable carrier for bio-fertilizer production.

Keywords: *Spent Mushroom Compost (SMC), Carrier, Plant growth promoting Rhizobacteria, Inoculation, Bio fertilizer.*

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I. INTRODUCTION

Spent mushroom compost (SMC) is a residual by-product of organic waste made up of plants, animals and fungi mycelia in which mushroom fruits is harvested from (Davis *et al.,* 2006). The residual biomass generated after ending fruiting mushroom cultivation, lignocellulose-based substrates (Martín *et al.* 2023). Substantial quantities of this waste in large tone are often discarded on the refuse dumpsite. Piled up of this waste create environmental pollution and makes it unhygienic for human activities (Ochijenu, 2021). Studies have shown that SMC can serve as soil amender and organic fertilizer to improve plant growth (Klausen *et al*. 2025).

Due to high organic matter and little presence of essential macro and micronutrients, SMC is considered a suitable carrier for soil amendment and bio fertilization (Alves *et al*. 2022). Particles size of SMC are tiny with a sticky surface for adhering surfaces of diverse microbial communities especially rhizospheric bacterial to improve soil nutrients and support plant growth. Potential of SMC in bioremediation has also been explored; particularly its capacity to remediate pollutants and contaminants from soils (Malik *et al*. 2021). SMC have proven effective to enhanced soil physical properties, like the water retention and filtration capacity (Courtney and Mullen 2009, Priadi and Saskiawan 2018). Research has it that input of chemical fertilizers by farmers to increase soil fertility is highly detrimental to the environment and our health (Makut *et al*., 2019). The aim of this study is to test SMC whether it is a suitable and effective carrier material for bio fertilizer production using *Azotobacter* and *Pseudomonas* strain. These are Plant growth promoting rhizobacteria (PGPRB) that freely lives in the soil. *Azotobacter* and *Pseudomonas* strain was inoculated directly on the SMC to stimulate it nutrient availability and recovery by the root plants.

II. MATERIAL AND METHODS

Sample Collection
Spent Mushroom sample was collected from FIIRO botanical garden Langtang-North, Plateau state and was transported to Microbiology lab. Nasarawa State University, Keffi, for further experiment. The SMC was sterilized and allowed to cool before inoculation.

Determination of Nutrients and Chemical Properties of the Spent Mushroom Compost (SMC)
Macro and micronutrients of the SMC like the Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn), Iron (Fe), Copper (Cu), Manganese (Mn), Molybdenum (Mo) were determined using Atomic Absorption spectrophotometer (AAS) (Muller *et al*., 1981). Using this method, 5g of SMC was treated with 2ml 33% HNO_3 and 0.1g CuSO4 catalyst before digested at 105^0 C inside a fume cupboard. The particles where then dissolved in 5ml distilled water and then filtered. Filtrate volume where then adds up to 10 ml with sterile distilled water before analyzed with Atomic absorption spectrophotometer. All values were expressed as percentage mean ± standard deviation (%).

Percentage pH value
The pH was measured using pH meter equipped with electrode as described by Row, (2009). 2g of the SMC was dissolved in 10 ml sterile distilled water, thoroughly shaken before dipping the pH electrode on the medium.

Percentage Moisture content
Percentage moisture content (d/w) was determined using oven-dry method as described by Neboh *et.al*., (2013). An initial weight of the crucible (W_1), then weight of crucible + sample (W_2) before oven-dry. Sample oven-dried at 105^0C for 2hours, and allow to cool in a dessiccator. The weight of the crucible + sample (W_3) was determined after cooling accordingly.

$$\%\text{Moisture content} = \frac{W2 - W3 \ X\ 100}{W1}$$

Where

W_1= Weight of the crucible

W_2= Weight of crucible + sample before oven-dry

W_3= Weight of crucible + sample after oven-dry

Percentage Ash content
Percentage ash content of the SMC was determined using furnace incineration method (AOAC 2016).
2g of the crucible + sample (W1) was heated at a high temperature of 450^0C for total combustion of the particles into ashes, allows to cold and re-weighed (W2).

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$$\%Ash = \frac{W2 \times 10}{W1}$$

Where,

W_1 = Weight of the crucible + sample before ash (2.0715)

W_2= Weight of the crucible + Ash (0.1152)

Maximum water holding Capacity (MWHC)
The maximum water holding capacity was determined using method described by Association of Analytical Chemist (OAC) (2016).

10g of the sample dissolved in water and after complete saturation was then decanted.

$$MWHC = \frac{100 - \{W3\} X100}{W_2}$$

Where,

W_1 = Initial weight of the sample before saturation with water

W_2 = Weight of the sample saturated with water

W_3 = Mass of water contain in the saturated sample ($W_2 - W_1$)

Percentage Nitrogen content
Percentage nitrogen content of the SMC was determined using kjedahl procedure as described by AOAC (2016).
5g of SMC (d/w) was mixed with 10ml concentrated H_2SO_4, 0.12g of CuSO4 and 1.0g of Na_2SO4 catalyst before the mixture was digested at a very high temperature of 550^0C for total digestion. Digested particle was then dissolved with a sterile distilled water and filter with whatman filter paper. Supernatant of the digest was mixed with equal volume of 45% 5N NaOH solution and placed on a kjeldahl distillation chamber for distillating process. The evaporated Ammonia gas at the completion of the process is then condensed to the cover lid of the distillation flask. This vapour was then received into a flask containing 4% boric acid solution. 3 drops of mixed indicator (methyl red and methylene blue) was added to the mixture and titrated against hydrochloric acid solution (0.001N).Nitrogen content of the sample is thus determine.

$$\%\ TN = \frac{TS \times Nx\ M \times Vd \times 100}{Vb \times W1}$$

Where,

%TN= Percentage total Nitrogen content

T_S= Titre value (0.7)

N= Normality of Hydrochloric acid used (0.01N)

M= Mass of Nitrogen (14)

Vd= Volume of the digest (250ml)

Vb= Volume of the base (50ml)

W_1=Weight of the sample (5g)

Percentage organic carbon (%OC)
Organic carbon was determined using wet oxidation method (Jackson, 1973). Using this method, 2.0g of the SMC was treated with excess amount of dipotassium dichromate solution ($K_2Cr_20_7$) in the presence of 50ml concentrated H_2SO_4solution. Available organic carbon in the sample was oxidized to CO_2. The excess potassium dichromate unused in the sample after filtration was back titrated against 0.5 N ferrous ammonium sulphates with 5drops of concentrated orthophosphoric acid and diphenyl amine indicator.

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$$\% OC = \frac{T \times 0.2 \times 0.3}{W1}$$

Where,

T = Titre value (163.1, 167.9

N= Normality of acid used (0.2)

W_1= Weight of the sample (2g)

0.3= Constant

Percentage organic matter (dry weight).
This was carried out using di acid digestion methods as described by AOAC, (2016).The organic matter content of the sample was determine using the formula below.

% Organic matter= 100 – [%moisture -- %Ash].

$$\% Ash = \frac{W2X\ 10}{W1}$$

Where

W_1= weight of sample before ashes

W_2= weight of sample after ashes.

Carbon-Nitrogen ratio was determined by finding the ratio of the % carbon to % Nitrogen obtained as described by AOAC, (2016).

Preparation of the carrier material
Sample carrier material was supplemented with Calcium carbonate and rice bran before bagged and sterilized. On cooling, samples were inoculated with mixed culture of *Azotobacter* and *Pseudomonas*. This was incubated at ambient temperature (28^0C) for 21days before analyzed using standard method described by AOAC, 2016.

Data analysis
Data obtained were analyzed using the mean and standard deviation equation to find the average mean of the triplicate samples used.

III. RESULTS

Table 1: Percentage Nutrients and Chemical Properties of the SMC.

Parameter measured		Content Before inoculation ($\dot{x} \pm SD$)	Content after inoculations ($\dot{x} \pm SD$)	χ^2	df	P value
Macronutrients content (%)						
	Phosphorus (P)	7.001±0.010	11.201±0.01	0.96912	1	0.3249
	Nitrogen (N)	1.40±0.001	0.980±0.001	0.074118	1	0.7854
	Potassium (K)	13.0617±0.20	12.3611±0.10	0.019307	1	0.8895
	Calcium (Ca)	24.3048±0.02	31.4366±0.02	0.91247	1	0.3395

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	Magnesium (Mg)	0.1426±0.02	2.1016±0.010	1.1108	1	0.2919
Micronutrients content (%)						
	Zinc (Zn)	0.0634±0.01	2.211±0.02	2.0279	1	0.1544
	Copper (Cu)	0.00174±0.02	1.0021±0.10	0.99689	1	0.3181
	Iron (Fe)	1.8355±0.02	3.0003±0.02	0.28057	1	0.5963
	Manganese (Mn)	0.1451±0.02	1.311±0.01	0.93354	1	0.3339
	Molybdenum (Mo)	0.0610±0.10	0.137±0.10	0.029172	1	0.8644
pH		7.04±0.389	7.4±0.041	0.008975	1	0.9245
Moisture content		28.20±0.163	43.86±0.153	3.4032	1	0.06507
Ash content		5.56±0.0082	6.01±0.0051	0.017502	1	0.8948
Organic matter		66.24±0.194	59.13±0.165	0.40322	1	0.5254
Organic carbon		48.94±0.017	46.37±0.019	0.069299	1	0.7924
Maximum water holding capacity cm/hour		96.45±0.082	94.77±0.071	0.01476	1	0.9033
Carbon-Nitrogen ratio		35:01:00	47:01:00	1.7561	1	0.1851

All values measured are the average mean (ẋ) data of the triplicate sample obtained, SD = standard deviation, % = percentage, d/w = dry weight, χ = Chi square, df = degree of freedom.

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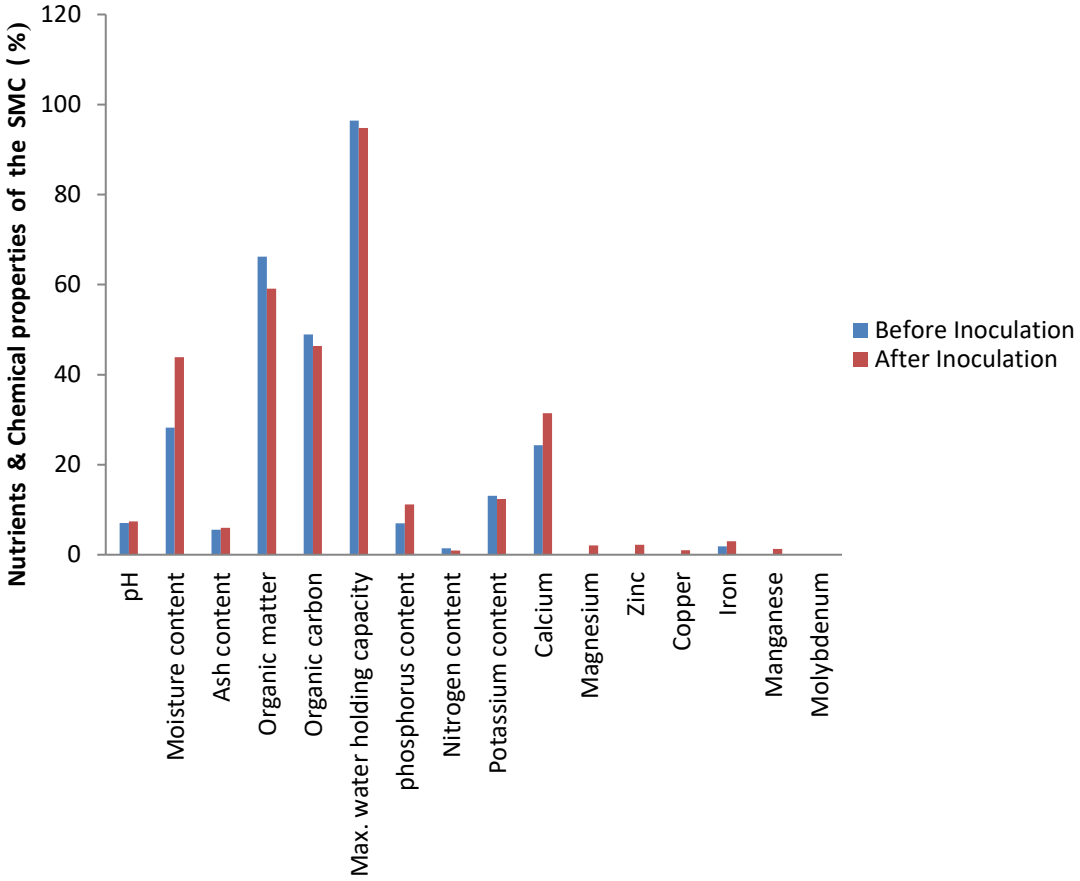


Figure 1.
Percentage Nutrients & other Chemical properties Of the SMC.

IV. DISCUSSION

Before inoculations of organism, analyzed data shows Ca= 24.30%, K= 13.06%, P= 7.00%, N= 1.40%, Mg = 0.14%, Zn= 0.06%, Mo = 0.06%, = 0.14%, and the least was Cu = 0.00%. Similarly, the MWHC = 96.45%, Organic matter = 66.24%, Organic carbon = 84.94%, and the least was Ash content=5.56% respectively.

After inoculations, the analyzed results is thus, Ca = 31.437 %, K = 12.36 %, P = 11.201 %, Fe = 3.000 %, Zn = 2.211 %, Mg = 2.102%, Mn = 1.311 %, Cu = 1.002 %, N = 0.98% and the least was Mo having 0.137 % respectively. Similarly, Maximum water holding capacity was 94.77, organic matter 59.13 %, organic carbon 46.37 %, Carbon-Nitrogen ratio 46:1, moisture content 43.86%, pH.7.4 and the least Ash content having 6.01% respectively.

In comparing results, the percentage N = 1.40 % and 0.98 % before and after inoculations shows that the presence of ammonium on the SMC suppresses the efficiency of the bacteria to fix elemental nitrogen but instead convert the excess ammonium into nitrate for plant use. This agreed with findings by Polat *et al*., (2009) that SMC has low nitrogen content ranging between 1% to 3% due to the presence of ammonium which suppresses capacities of free-living diazotrophic bacteria in fixing atmospheric nitrogen directly but instead fix useable nitrate which root plants absorb. So also, Fidanza *et al*., (2010), in his previous research stated that Spent Mushroom Compost do not have extremely low or highs percentage values. Very low percentage value of micronutrients element such as Zn, Fe, Cu, Mn, and Mo of the SMC before and after inoculations agreed with findings by Medina *et al*., (2009) who reported that the percentage of micronutrient elements is low.

In this study, the Maximum Water holding capacity (MWHC) before and after shows 96.45 cm/hour and 94.77 cm/hour respectively. This figure correlated with findings by Fidanza *et al*., (2010) that water holding capacity of SMC is usually high due to the uniformity in the particles size; this makes it easier to retain much water. And also the pH value was 7.04 % and 7.40 % respectively. This figure also agreed with the findings by Kumbhar (2012), who reported that percentage average pH value of SMC is 7.30. This pH value greatly supports growth of saprophytic bacteria.

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Statistically, data analyzed showed, there is no significant difference in the percentage of nutrients and others chemical properties of the SMC (P>0.05).

V. CONCLUSION

There was increased in the overall percentage nutrients content and chemical properties of SMC after inoculations. Thus, we conclude that SMC is a suitable carrier for bio-fertilizer production because is sticky and contain carbon and energy source that support growth of saprophytic bacteria.

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