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Full Length Research Article

Influence of Spent Oyster Mushroom Substrates on Growth of Waterleaf (*Talinum triangulare*) and Microbial Population

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ABSTRACT

Agricultural waste poses a significant environmental pollution problem annually. Repurposing waste, like spent mushroom substrate, can address economic challenges. This study examined microbial populations in spent oyster mushroom substrate and their impact on the growth of water leaf (*Talinum triangulare*). Microbial analysis of the spent mushroom substrate (SMS) was conducted before and after planting, measuring total microbial counts. Planting substrates comprised three ratios (9:1, 8:2, and 7:3 SMS to soil mix), with a control group using only soil. Six-centimetre water leaf stems were sown in sixteen polythene nursery bags, with each group of three bags containing 2 kg of a planting substrate. Growth parameters were assessed four weeks after planting. Microbial analysis revealed various bacterial species (e.g., *Pseudomonas* sp., *Micrococcus leuteus, Shigella sp., Salmonella sp., Bacillus subtilis, Klebsiella sp., Staphylococcus sp.*) and fungal species (e.g., *Aspergillus sp., Fusarium sp., Penicillium sp., Alternaria sp., Rhizopus sp.*) in SMS. The control group exhibited superior growth compared to SMS-amended soil. The study concluded that while microbes in spent mushroom substrate facilitate organic nutrient decomposition, improving nutrient availability and moisture retention, they adversely affect water leaf growth due to excessive moisture, making it unsuitable for water leaf cultivation.

Keywords: Spent mushroom substrate, Fungi, Bacteria, Plant height, Root height.

INTRODUCTION

Waterleaf (*Talinum triangulare),* a crucial leafy vegetable native to tropical Africa, is primarily cultivated in West Africa, Asia, and South Africa for its culinary significance within the Portulacea family (Schippers, 2000). The past two decades have witnessed a significant surge in vegetable production and consumption, emphasizing the pivotal role of growth mediums and ecological conditions in vegetable cultivation. Consequently, searching for superior crop substrates has become paramount in vegetable production (Sterrett, 2001). Traditionally, farmers have relied on inorganic fertilizers and manures to enhance vegetable growth, including waterleaf. However, there is a growing interest in organic, environmentally friendly alternatives. Spent mushroom substrate (SMS), the residual material following mushroom cultivation emerges as a cost-effective and ecologically sound option for organic manure in crop production systems. Rich in organic waste and essential nutrients for field crops, mushroom mycelia, and a diverse population of heterotrophic microbes, SMS has garnered attention for its potential benefits.

Substrates used in edible mushroom cultivation primarily consist of agricultural waste such as sawdust, rice bran, dried plantain or banana leaves, cereal straw, manure (poultry and horse), calcium sulfate, soil, and residual inorganic nutrients and pesticides (Medina *et al*., 2009; Markson *et al*., 2012; Agba *et al*., 2021; Oni *et al*., 2021). Incorporating spent mushroom substrates into soil enriches nutrient levels, fosters faster crop growth, improves crop density and yields, enhances rooting, and reduces the need for external fertilizers and irrigation (Williams *et al*., 2001; Muchena *et al*., 2021).

Research has demonstrated the efficacy of spent mushroom substrates in enhancing crop production, as Kadiri and Mustapha (2010) observed in the case of *Lentinus subnudus* SMS, which improved cowpeas' and tomatoes' vegetative growth and yield. Similarly, Muchena *et al*. (2021) found that higher SMS application rates significantly increased baby spinach's fresh yield and quality, establishing SMS as a viable organic fertilizer. Numerous studies have underscored the positive impacts of spent mushroom substrates in crop production (Polat *et al*., 2009; Jonathan *et al*., 2012; Roy *et al*., 2015).

Furthermore, the presence of microorganisms in SMS renders it a bio-fertilizer. These microbes play a vital role in safeguarding plants against soil-borne pathogens and nematodes (De Moraes *et al*., 2020), including species like *Azospirillum, Enterobacter, Klebsiella, Bacillus, Pseudomonas, Fusarium,* and others (Hayat *et al*., 2010). Identifying the microbial composition of SMS before use is crucial, as it can help determine the beneficial microbes for plant growth. For instance, De Moraes *et al*. (2020) identified numerous bacterial and fungal pathogens in SMS from *Agaricus bisporus* and *A. brassicae*. This study evaluated the influence of spent mushroom substrates on the growth of waterleaf and microbial populations present in the spent mushroom substrates obtained from *Pleurotus ostreatus* cultivation.

MATERIALS AND METHODS

Study Area

This research was carried out in the Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, Nigeria. The laboratory investigations were carried out in the same department's laboratory, while the growth study was carried out in a field under natural light intensity and daily temperatures.

Collection and Preparation of Experimental Materials

The Spent mushroom substrate, composed of sawdust, rice bran, and lime, was obtained from a Johncollinsmushroom farm in Akpabuyo, Cross River State while the waterleaf stems were purchased from Goldie market in Calabar, Cross River State, Nigeria. Topsoil and a mixture of SMS-topsoil were used as the growth medium. The SMS-soil mix was in the ratios 9:1 (T2), 8:2 (T3), and 7:3 (T4), while soil alone served as the control (T1); 2 kg of each dried substrate was weighed into four polythene nursery bags (39 cm diameter and 49 cm deep) and labelled appropriately. Three waterleaf stems, measuring 6 cm, were sown in each bag; these were watered at an interval of three days. Watering was stopped at three weeks when water logging was observed in the substrate.

Total Bacterial Counts

Serial dilutions of the spent mushroom substrate (SMS) were made following the methods of Atlas and Bartha (1992); and Eja *et al.* (2006). One gram (1 g) of the spent mushroom substrate was dissolved in 1 ml of sterile distilled water, and 10-fold serial dilutions in the range of 10^{-1}-10^{-9} were prepared. One Milliliter (1 ml) aliquot of the sample dilution from 10^{-3}-10^{-6} was then seeded into sterile Petri dishes. The pour plate technique used nutrient agar to determine the total bacterial count. Aerobes were recovered by supplementing the nutrient agar with 1 % (w/v) cysteine hydrochloride and nystatin and then incubated for 48 hours at ambient temperature, after which bacterial colony counting was carried out by multiplying the reciprocal of the dilution factors with visible colonies of 30 and 300 recorded as colony-forming units per gram (cfu/g) of SMS.

Total Fungal Count

Sabouraud dextrose agar was used, and the medium was supplemented with 100 µg of streptomycin and 15 µg of penicillin to inhibit bacterial growth. Petri dishes containing fungi pathogens were incubated at room temperature for 72 hours. Discrete colonies were sub-cultured on malt extract agar (Oxoid) and acidified to a pH of 4.8 to obtain pure cultures. Colonies were enumerated as colony-forming units (cfu/g) per gram substrates.

Microbial identification

Microorganisms from both SMS and SMS-soil mix were identified by collecting samples from three points each on the Petri dishes. Microbial colonies were identified using conventional methods based on morphological, biochemical, and physiological characteristics (Houpikian and Roult, 2002).

Harvesting and Data collection

After four (4) weeks of growth, the plants were harvested carefully, and the mean values of growth parameters such as stem height, root height, number of leaves, number of branches, number of flower buds, wet weight, and dry weight were obtained.

Statistical Analysis

The data was presented as mean ± SE and statistically analysed using SPSS v20. One-way ANOVA followed by LSD to compare means.

RESULTS

Growth performance of Waterleaf Grown on Soil Supplemented with SMS

Supplementing soil with SMS did not impact the growth performance of waterleaf (Table 1). The height, root length, number of leaves and branches, and wet and dry weights of waterleaf grown in soil alone (control) were significantly higher than those grown SMS supplemented soil. Also, there was a total absence of flower buds in waterleaf grown in SMS supplemented soil, while the control had fully developed flower buds

Distribution of Bacterial Species in the Spent Mushroom Substrate Before and After Supplementing into the Soil

The bacterial population of the SMS before planting (Figure 1) included *Pseudomonas sp., Micrococcus leuteus, Shigella sp., Salmonella sp*., *Bacillus subtilis,* and *Klebsiella sp*. At point 1, *Mirococus leuteus* was the highest at 19%, while *Klebsiella* sp. was the least at 14%. At Point 2, *Mirococusleuteus* was still the highest at 19%, *Klebsiella sp*. was the least at 13%, and at Point 3, *Pseudomonas sp.* showed the most growth at 22% while *Klebsiella* sp. showed the lowest at 12%.

The frequency of bacterial colonies obtained from the mixed substrate is shown in Figure 2. The colonies isolated included *Pseudomonas sp., Micrococcus leuteus, Salmonella sp., and Staphylococcus sp.* At point 1, *Pseudomonas sp.* was the highest with 31% frequency, while *Staphylococcus sp.* was the least with 21%. Point 2 also showed *Pseudomonas sp.* As the highest with 42% frequency while *Staphylococcus sp.* was the least with 7%. Also, at point 3, *Pseudomonas sp.* had the highest frequency of 22%, while *Staphylococcus sp.* had the lowest with 13%.

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Table 1: Effect of SMS on morphological characteristics of waterleaf (Mean ± SE)

GROWTH PARAMETERS	TREATMENTS (CONCENTRATIONS)				
	T1	T2	T3	T4	LSD (5%)
Stem height	11.80^{b}±1.18	7.30^{a}±0.66	6.80^{a}±0.72	6.60^{a}±0.34	2.49
Root height	13.18^{b}±4.45	6.68^{a}±0.46	6.15^{a}±0.34	7.08^{a}b±0.19	6.92
No. of leaves	23.88^{b}±4.78	8.83^{a}±0.64	6.00^{a}±0.58	6.38^{a}±0.72	7.56
No. of branches	3.13^{b}±0.66	1.83^{a}±0.18	1.43^{a}±0.17	1.55^{a}±0.21	1.13
No. of flower buds	9.25^{b}±3.79	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	4.86
Wet weight	4.10^{b}±1.33	0.58^{a}±0.06	0.50^{a}±0.10	0.56^{a}±0.04	2.05
Dry weight	1.33^{b}±0.56	0.13^{a}±0.07	0.13^{a}±0.06	0.19^{a}±0.04	0.31

Value across the groups with similar case letter superscripts indicates no significant effect at 5% probability. In comparison, value across the groups with different case letter superscripts indicates a significant effect at 5% probability. T1=0% sms+100% soil, T2=10% sms + 90% soil, T3=20% sms+80% soil, T4= 30%sms+70% soil.

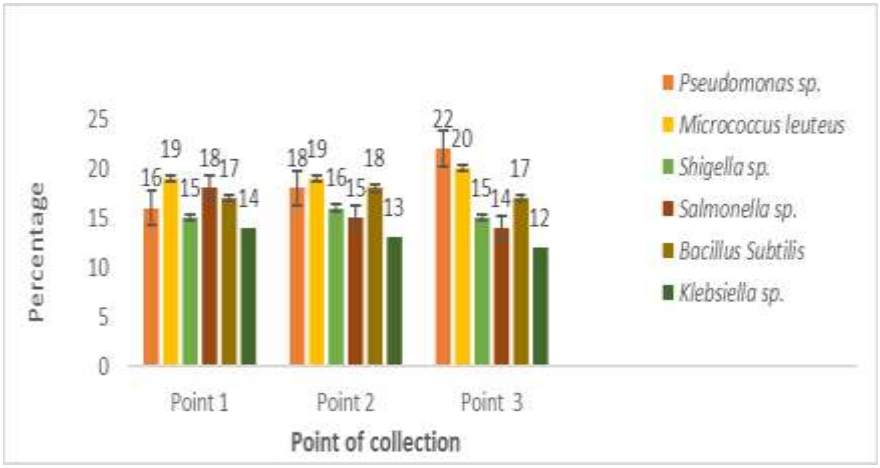

Figure 1: Percentage of isolated bacterial species from the spent mushroom substrate before planting.

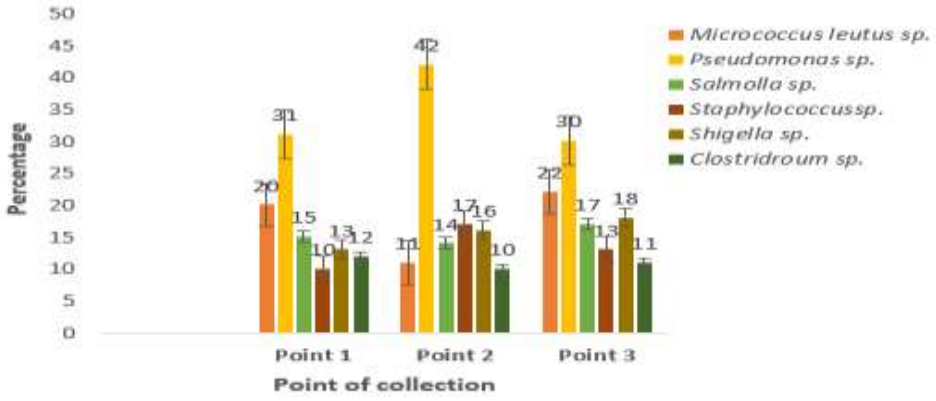

Figure. 2: Bacterial species isolated from spent mushroom substrate mixed with soil

Distribution of Fungal Colonies from the Spent Mushroom Substrate Before and After Supplementing into the Soil

Fungal species, including Aspergillus *sp. Fusarium sp. Penicillium sp* and *Alternaria sp.* were isolated from the SMS (Figure 3). From the distribution at Point 1, *Aspergillus sp*. had the highest frequency (27%). *Penicillium* sp. (23%) was the minor distribution. *Aspergillus sp*. and *Fusarium sp*. had the highest frequency of 27% at point 2, while *Alternariasp.* had the least at 22%. At point 3, *Aspergillus sp*. and *Penicillium sp*. had the highest frequency of 26%, while *Fusarium sp. and Alternaria sp.* had the lowest frequency of 24%.

The fungi species isolated in the mixed substrate included *Aspergillus sp., Fusarium sp., Penicillium sp, Alternaria sp*, *Mucor sp*., and *Rhizopus* sp. (Figure 4). At point 1, *Aspergillus sp.* had the highest occurrence with 23% frequency, while *Alternaria sp.* and *Rhizopus sp* were the least with 14% frequency. At point 2, *Penicillium sp.* was the highest with 21% frequency, while *Rhizopus sp.* was the lowest with 12%. Finally, at point 3, *Aspergillus sp.* had the highest frequency of 24%, while *Rhizopus sp.* remained at least 12%

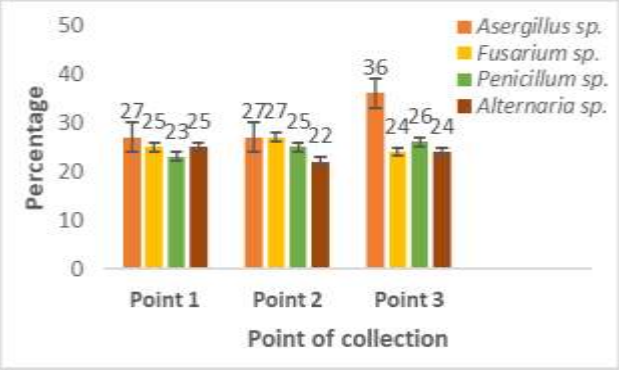

Figure 3: Isolated fungal species from SMS before planting

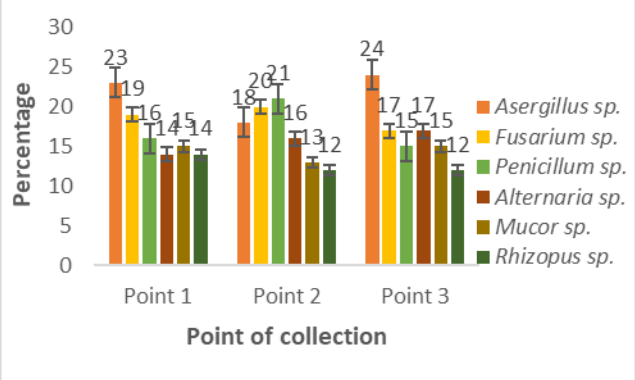

Figure 4: Isolated Fungal species from SMS mixed with soil for planting

DISCUSSION

The growth performance of waterleaf grown on soil supplemented with SMS showed that the SMS had a negative effect on the growth of waterleaf as observed in all measured parameters. However, some researchers have reported that adding spent mushroom substrate in nutrient-poor soil improved health by improving the texture, water-holding capacity, and nutrient status (Ahlawat *et al*., 2009; Jonathan *et al*., 2012; Velusam *et al*., 2021). Jonathan *et al*. (2012) reported a higher number of flowers and fruits of four Nigerian vegetables when planted on soil supplemented with SMS. Further reports have also confirmed that SMS had 0.87% nitrogen, 0.26% phosphorus content, 0.19% potassium, and a maximum water holding capacity of 95%, all of which help improve soil fertility (Kambhar *et al*., 2014; Shanmugavelu and Sevugaperumal, 2020).

Waterlogged soils result in a reduction of oxygen, thereby making plants absorb less oxygen. Limited oxygen also causes microorganisms to create metabolic pathways that affect nutrient uptake (Suat *et al*., 2014). Therefore, the negative effect of SMS supplementation on the growth of waterleaf recorded in this study could result from the inability of the test plant to thrive in the presence of excess water (Surukite *et al.,* 2018).

The microbial analysis confirmed that the spent mushroom substrate contains many indigenous beneficial microbes capable of breaking down organic matter in the soil, thereby making the nutrients more available for plant uptake and improving water-holding capacity. Kambhar *et al.* (2014) recommended SMS for soil amendment to avoid and reduce nutrient leaching levels and hold the nutrient at the surface level for plant uptake and usage. However, knowledge of a plant's moisture requirement is essential when using SMS due to its high water retention capacity. Although supplementing with SMS did not improve growth in waterleaf, it could help improve growth in other crop species, especially those having high moisture requirements.

CONCLUSION

The spent mushroom substrate is a suitable substrate used in amending soil. However, due to the presence of microbes that help in decomposing the substrates, its use in growing waterleaf must be closely monitored due to the low tolerance of waterleaf for excessive moisture and the high water holding capacity of the SMS.

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