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Maximizing production of *Pleurotus ostreatus* (Oyster) in Tanzania

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Abstract: *Pleurotus ostreatum* is a nutritive and medicinal mushroom. A study to maximize production of the previous results from the previous study was conducted. A four-treatment setup with four replicates was established to maximize previous results of *Pleurotus ostreatum*. In this setup, substrate ratios and greenhouse materials used as control (T0) were kept constant across all treatments, while growth conditions (T1-T4) varied from one treatment to another Sterilized substrates were packaged in 150 sterilized plastic bags of 2 kg each. *Pleurotus ostreatum* spores were inoculated and lightly watered for 30 days for mycelia sprouting. The number and percentage of mycelia sprouting were recorded over three months. Data were analyzed using SPSS, where ANOVA and post hoc tests were conducted. The results indicated the highest mycelial sprouting percentage and sprouting number in T4 (92% and 351, respectively), while T1 exhibited the highest Biological Efficiency Index (BEI) of 7.5. Significant differences between treatments were observed for both mycelia sprouting percentage and sprouting number ($p<0.001$, df=3, and $p<0.001$, df=3, respectively). Maximization was successful as sprouting increased from 17% in previous research to 92% and the sprouting number from 173 to 351. Despite T4 showing absolute sprouting metrics, it suggests that T1 is more efficient in the sprouting process, while T4 conditions are more effective for mushroom production. Optimized growth condition in T4 (80% humidity, 21^{o}C and pH 7.5), substrate ratios (20:20:5:5:5) and greenhouse materials (palm fronds) contributed to this maximization and are recommended for use in Songea.

Keywords: Mushrooms, maximization, greenhouse, palm fronds, substrates, germination.
Abbreviations: ANOVA_Analysis of Variance, BEI_Biological Efficiency, T_Treatment, T0_Control treatment.

Introduction

Various studies on mushrooms and fungi have focused on classification, uses, ethnobotany, habitat, medicinal uses, nutritional values, and to a lesser extent, cultivation techniques (Tibuhwa and Kivaisi 2010; Venturella et al., 2015; Sadhukhan, 2023; Washa 2022; Washa 2023). Most previous research, particularly in Tanzania, has been conducted using mushrooms from their natural environments (Washa 2022), with limited studies on controlled environments, especially in regions like Dar es Salaam and Iringa (Washa 2023). Despite this, trials in Tanzania used local technological facilities, and the Songea region, known for its abundant forest mushrooms, was not included in these trials (Washa 2024). Reports by Tibuhwa and Kivaisi (2010), Crosby (2016) and Washa (2025) indicate that *Pleurotus ostreatus* which is a nutritive and medicinal food can grow in areas where mushrooms grow locally in the forest.

The discovery of edible, non-edible, and poisonous mushrooms has spurred research into their nutritional value, medicinal uses, toxic chemical production, and pharmacological properties (Manimozhi, 2013). Collecting enough edible mushrooms from natural sources for nutritional use or commercialization has proven challenging (Tibuhwa and Kivaisi 2010; Washa 2022). Consequently, efforts to establish cultivation techniques have advanced earlier in developed countries and have more recently begun in lower-tech African countries (Grivetti and Ogle, 2000, Venturella et al. 2015; Washa 2023).

Cultivation techniques in controlled environments, such as greenhouses, face several challenges that hinder high mushroom production (Sharma et al, 2023, Sadhukhan, 2023). These include improper substrate combinations and ratios, unsuitable greenhouse materials, suboptimal growth conditions (humidity, temperature, and pH), and severe fungal and bacterial infections in the growth media (Crosby, 2016, Sadhukhan, 2023). These issues can lead to bacterial or fungal contamination rather than successful mushroom growth (Manimozhi, 2013; Washa 2023). Although Washa (2023, 2024) reported a low germination percentage (17%), the study managed to control fungal infections in the growth media and

established an effective substrate ratio (20 kg sawdust, 20 kg grain chaff, 5 kg banana leaves, 5 kg lime, and 5 kg sugar). This substrate combination, along with the favourable greenhouse material (palm fronds), created optimal mushroom growth conditions (75% humidity, 25°C, and a pH of 7.5). This study aimed to build on previous research to maximize the production of edible mushrooms using *Pleurotus ostreatus*, which can be easily cultivated (Tibuhwa and Kivaisi 2010). The optimization process involved increasing the number of trials and replicates from 3 to 4 and raising the humidity from 70% to 80%, while maintaining the same substrate ratios and greenhouse materials. The goal was to explore optimal growth conditions and substrates for maximum mushroom production, facilitating the commercialization of mushroom products in both local and international markets (Washa 2024).

Results

Mycelia sprouting percentage and sprouting number

The analysis revealed a significant increase in mycelial sprouting percentage from 17% (Washa 2023) to 92% in Treatment 4 (Table 2 and Table 3). Treatment 4 recorded the highest mycelial sprouting number, 351, while Treatment 1 had the highest Biological Efficiency Index of 7.5. The data indicated significant differences between treatments, with both mycelia sprouting percentage and sprouting number varying significantly ($p<0.001$, $df=3$). The optimized conditions in Treatment 4 contributed to the maximum production, whereas Treatment 1 demonstrated higher efficiency in the sprouting process (Table 2).

Results from Table 2 indicated that increasing humidity and decreasing temperature generally improve both sprouting percentage and sprouting number (Sadhukhan, 2023). The optimal conditions in this set of treatments appear to be 80% humidity, 21°C temperature, and pH 7.5, leading to the highest sprouting percentages and sprouting numbers Table 2). The effect of growth conditions in this experiment correlates to effects revealed in experiment by Crosby (2016) and Washa (2024).

Index (BEI) calculation

The BEI is generally a Biological Efficiency measure that relates mycelia sprouting number to sprouting percentage, essentially reflecting how effectively the sprouting process is (Sadhukhan, 2023). Although the exact formula for BEI isn't given, a common formula used in such cases is: BEI= Sprouting Number/ Sprouting Percentage (Crosby, 2016)

This formula assumes that a higher BEI indicates more efficient sprouting, where a higher sprouting number relative to percentage reflects better efficiency.

Treatments comparison

Treatments 1 and 4 have a higher BEI than treatment 3 and 2. BEI of all treatments were recorded in Table 2.

Sprouting Percentage Impact:

Treatment 4 has a much higher average sprouting percentage (68%) compared to Treatment 1 (3.5%). Higher sprouting percentages typically indicate a more favorable growing condition or process, leading to more successful sprouting (Table 2 and 3).

Sprouting number impact

Treatment 4 also has a significantly higher average sprouting number (277.5) compared to Treatment 1 (26.25). This suggests that while Treatment 4 is more successful in absolute terms (higher sprouting number and percentage), the BEI calculation shows that its efficiency, relative to its percentage, is lower than Treatment 1 (Table 2 and 3).

Efficiency Interpretation:

Treatment 1 has a higher BEI (7.5) because it has a relatively high sprouting number for its lower sprouting percentage. In other words, Treatment 1 is more efficient in converting its lower sprouting into a higher number of sprouting. Treatment 4 has a lower BEI (4.05) despite having higher absolute values because the efficiency of converting a high percentage into a high number is lower when you compare the two. The higher BEI of Treatment 1 compared to Treatment 4 (Table 2), despite Treatment 4's better absolute sprouting metrics, suggests that Treatment 1 is more efficient in its sprouting process relative to its percentage. Treatment 4's higher sprouting numbers and percentages are impressive, but the BEI indicates that the efficiency of these conversions isn't as high as in Treatment 1.

Results from (Table 3) indicated that, in both sprouting percentage and sprouting number, Treatment 4 (D) consistently shows significant differences when compared to Treatment 1 (A), 2 (B), and 3 (C). For sprouting number, significant differences are found specifically between D and the other treatments. For sprouting percentage, significant differences are observed between D and the other treatments, as well as between A and C. Overall, Treatment 4 (D) exhibits notable differences in both sprouting number and sprouting percentage compared to the other treatments. The results show a significant impact of varying growth conditions on both sprouting percentage and sprouting number. As the growth conditions become more favorable (higher humidity, low temperature, higher pH), there is a notable increase in both sprouting percentage and sprouting number. These effects of growth conditions to sprouting number and percentage have also been reported by Venturella et al. (2015), Washa (2023), and Washa (2024). Treatment 4 with the highest growth conditions (80% humidity, 21^0C, and 7.5 pH) exhibited the highest sprouting percentage (92%) and sprouting number (351). This suggests a positive correlation between optimal growth conditions and mushroom production (Venturella et al. 2015) which produced very healthy mushrooms as indicated on Figure 2.

The constant use of palm fronds and a specific substrate ratio (20:20:5:5:5) across all treatments allowed for a focused evaluation of the impact of growth conditions which also correlates with results reported by Washa (2024). The uniformity in substrate ratios minimises the variability attributed to substrate composition, enabling a clearer assessment of the impact of growth conditions on germination (Washa 2024). The ANOVA results for both sprouting percentage and sprouting number indicate statistically significant differences among the treatments.

Table 1. Summary of experimental design (Treatments 1-4 and control treatment, T0).

S/N	Control (main plot) or T0	Treatments (T1-T4) or sub-plots
1	Palm fronds (greenhouse material), substrate ratio (20:20;5:5:5)	T1 = 65% humidity, 30^{o}C temperature, pH 8
2	Palm fronds (greenhouse material), substrate ratio (20:20;5:5:5)	T2 = 70% humidity, 27 ^{o}C temperature, p 7.7
3	Palm fronds (greenhouse material), substrate ratio (20:20;5:5:5)	T3 = 75% humidity, 25 ^{o}C temperature, pH 7.5
4	Palm fronds (greenhouse material), substrate ratio (20:20;5:5:5)	T4 = 80% humidity, 21 ^{o}C temperature, pH 7.5

Table 2. Summary of mycelia sprouting percentage and sprouting number across treatments.

T0-T4	GM	Sb ratio	Gc	Rs %	Rs number	Mn of sn	Cp	P-value	BEI value
1	Pf	20:20;5;5::5	65%, 30^{o}C, 8 pH	3, 2, 5, 4	25, 27, 24, 29	26.25	1 vs 2	p>0.05	7.5
2	Pf	20:20;5;5::5	70%, 27 ^{o}C, 7.7 pH	15, 17, 23, 21	48, 50, 43, 46	46.75	1 vs 3	p>0.05	2.46
3	Pf	20:20;5;5::5	75%, 25 ^{o}C, 7.5 pH	23, 34, 42, 42	89, 75, 91, 170	106.25	1 vs 4	(p<0.001	3.01
4	Pf	20:20;5;5::5	80%, 21 ^{o}C, 7.5 pH	53, 57, 72, 92	156, 292, 311, 351	277.50	2 vs 4	(p<0.001	4.05

NB: T0-T4 = Treatments T0-T4, Gm = Greenhouse material, Pf = Palm fronds, Sb ratio = substrate ratio, Gc = Growth conditions, Rs % = Replicates of sprouting %, Rs number = replicates of sprouting number, Mn of Sn = Mean of sprouting number. Cp = Comparison, BEI = Biological efficiency Index,

The post hoc tests reveal specific pairwise comparisons with significant differences, particularly between Treatment 4 and others. This reinforces the idea that the extreme growth conditions applied in Treatment 4 had a substantial positive effect on both sprouting percentage and sprouting number despite that Treatment 1 has highest BEI but is submissive to the higher humidity of Treatment 4. The consistency in the number of replicates (4) for each treatment enhances the reliability of the results (Washa 2024). The standard deviation and standard error values (Table 3) provide insights into the variability within each treatment, contributing to the robustness of the findings.

Discussion

The study demonstrates that *Pleurotus ostreatus* can be successfully cultivated in Songea under optimized conditions, achieving a significant increase in mycelial sprouting percentage and sprouting number. This research has confirmed results of various research which concluded that above 80% humidity, below 20^{o}C temperature and acidity above 7 pH give maximum production of mushrooms especially *Pleurotus ostreatum* and *Agaricus bisporus* (Mirah et al, 2022, EI-Ramady et al, 2022 and Washa, 2024). This is because the mushrooms body is mostly made of water so maintaining humidity above 80% is enabling mycelia cell expand and remain turgid for proper growth (Mirah et al, 2022). Temperature above 20^{o}C can stress the mushroom mycelia causing drying and death of the mushroom (EI-Ramady et al, 2022). Too acidic or too alkaline level in a substrate causes mycelia growth to stunt and reduce yields (Mirah et al, 2022). While Treatment 4 showed superior absolute production metrics, Treatment 1 proved to be more efficient in the sprouting process. These results underline the effectiveness of optimizing environmental conditions to enhance mushroom production. Optimization of growth conditions to maximize growth of *Pleurotus* ostreatus has been also demonstrated in the study by Sadhukhan (2023), Crosby, (2016) and Washa (2024).

The experiment successfully maximized mushroom production, achieving a remarkable increase from 17% to 92% in sprouting percentage and from 173 to 351 in sprouting number. The extreme growth conditions in Treatment 4 played a pivotal role in achieving these high levels of sprouting. Mycelia sprouting percentage of 17% and sprouting number of 173 are the results from the previous study by Washa (2024) while 92% and 351 respectively are results from the present and follow-up study maximized.

The use of a split plot design allowed for the controlled evaluation of growth conditions while keeping substrate ratios constant. The consistent substrate preparation and inoculation procedures contributed to the reliability of results the situation which was also revealed in the study by Crosby, (2016); and Sadhukhan (2023).

The results emphasize the critical role of growth conditions, particularly humidity, temperature, and pH, in mushroom production. The conditions used in Treatment 4 (80% humidity, 21 ^{0}C, 7.5 pH) emerged as optimal for maximising both sprouting percentage and sprouting number. The experiment successfully demonstrated the influence of growth conditions on mushroom production, providing valuable insights for optimising mushroom production. The systematic approach to experimentation and data analysis enhances the credibility of the findings as also can be supported by experimental set up in the research by Simerjit and Priyanka, (2022).

Status of mushroom cultivation in Tanzania including Songea a study area in Ruvuma region compared to global practices, the study presents an in-depth analysis of mushroom cultivation, focusing on *Pleurotus ostreatus* in Tanzania and comparing the results with global practices. This discussion has highlighted the current status of mushroom cultivation in Tanzania, examining the progress and challenges in the context of global advancements.

As a current status, mushroom cultivation in Tanzania has historically been limited, with much of the research focusing on wild mushrooms from natural environments (Tibuhwa and Kivaisi, 2010). Despite the potential for cultivating mushrooms like *Pleurotus ostreatus* in controlled environments, there has been relatively little advancement in this area within Tanzania compared to global standards. Trials have predominantly been conducted in specific regions such as Dar es Salaam and Iringa, while areas like Songea with abundant forest mushrooms have been overlooked (Washa 2024). Globally, mushroom cultivation has seen significant advancements, particularly in developed countries (EIN presswire, 2024, Market data forecast, 2024, The business research company, 2025 and 6Wresearch 2024). Techniques in controlled environments, such as greenhouses, have been refined to optimize growth conditions and maximize yield. However, similar

Table 3. Sprouting percentage and sprouting number statistical results.

Sprouting Percentage Statistical Results				Sprouting Number Statistical Results			
ANOVA (One way)				ANOVA (One way)			
Comparison	Diff.	q	P value	Comparison	Diff.	q	P value
A vs B	-15.400	3.767	p > 0.05	A vs B	21.000	0.9492	p > 0.05
A vs C	-31.800	7.779	p < 0.001	A vs C	76.800	3.471	p > 0.05
A vs D	63.000	15.412	p < 0.001	A vs D	-227.20	10.269	p < 0.001
B vs C	-16.400	4.012	p > 0.05	B vs C	-55.800	2.522	p > 0.05
B vs D	-47.600	11.644	p < 0.001	B vs D	-206.20	9.320	p < 0.001
C vs D	-31.200	7.632	p < 0.001	C vs D	-150.40	6.798	p < 0.01

Note: A = treatment 1, B = treatment 2, C = treatment 3, D = treatment 4

advancements in Tanzania are still in nascent stages (Temise Tanzania, 2024).

The study indicates that challenges such as improper substrate combinations, unsuitable greenhouse materials, and suboptimal growth conditions are prevalent in Tanzania (Wong and Chye 2009; Washa 2023). These issues are not uncommon and have been reported globally; however, developed countries have typically implemented more sophisticated technologies and practices to overcome these challenges. For instance, developed nations employ advanced monitoring systems and automated controls to maintain optimal growth conditions, which are less prevalent in Tanzanian cultivation practices (Temise Tanzania, 2024).

Despite the challenges, recent studies, including those by Washa (2023) and Washa (2024), demonstrate progress in optimizing mushroom cultivation techniques in Tanzania. The research has highlighted the successful establishment of effective substrate ratios and control over fungal infections, which are critical for improving mushroom yields. The trials showed that using a specific substrate combination and maintaining favourable growth conditions (75% humidity, 25°C, and pH 7.5) significantly enhanced mushroom production (Simerjit and Priyanka, 2022, Washa 2024).

The findings align with global research, which suggests that optimizing environmental conditions such as temperature, humidity, and pH is crucial for successful mushroom cultivation (Venturella et al. 2015, EI-Ramady et al, 2022, Gonzalez, 2011, Mirah et al 2022; Mukherjee et al, 2023; Royse and Beelman, 2023; Simerjit and Priyanka, 2022). Treatment 4, with the highest growth conditions, exhibited the best results in terms of sprouting percentage and number, demonstrating the effectiveness of optimized conditions (Washa 2024). This is consistent with global trends where enhanced environmental control leads to increased mushroom yields (Ahmed et al. (2013).

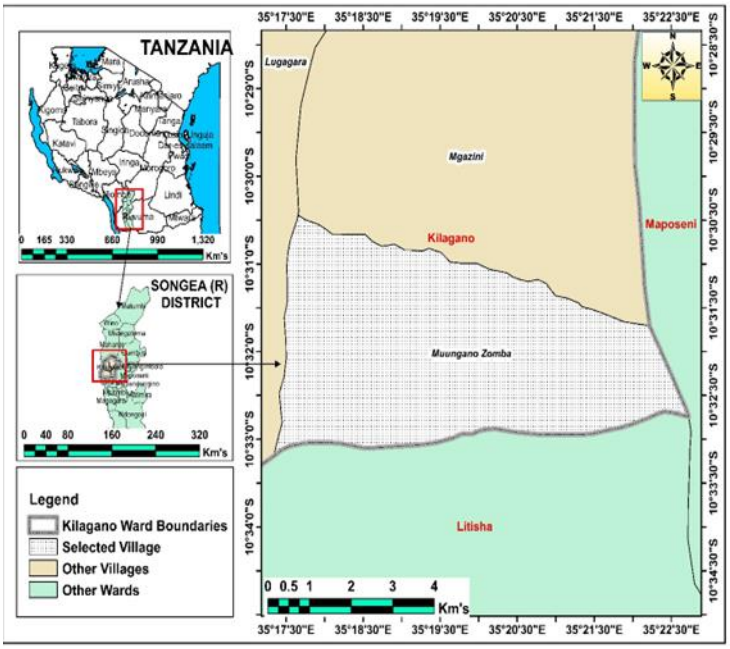

Figure 1. Map showing the study area.

Figure 2. *Pleurotus ostreatus* produced in the maximization treatment 4 of the research.

Materials and Methods

Description of the study area

The study was conducted from December 2023 to February 2024 in Muungano Zomba Village, Kilagano Ward, Songea Rural District, Ruvuma Region (10°31'15.13337"S, 35°19'43.24969"E), as shown in Figure 1. This village was selected due to the easy availability of greenhouse materials, such as reject timber and stock wood, as well as substrate materials like grain chaff, sawdust, and banana leaves, all at an affordable cost. *Pleurotus ostreatus* was chosen for cultivation because it thrives in areas where various local mushrooms grow (Tibuhwa and Kivaisi, 2010). Although local residents prefer mushrooms collected from the forest for their daily meals, no controlled cultivation of mushrooms has been conducted in the region. Previous cultivation trials have been limited to Dar es Salaam and Iringa, Tanzania (Washa, 2024).

Research and experimental design

A split plot design was used to design the experiment, whereby constant greenhouse materials (a control treatment or (T0) and substrate ratios were used as main plots, while the replicated growth conditions (Treatments 1-4 or T1, T2, T3, T4 were used as sub-plots (Washa 2024) and (Table 1). This setup was intended to measure the effect of varying growth conditions on mycelia sprouting in the substrates and greenhouse set (Sadhukhan, 2023).

This is a setup in which four different growth conditions were set but each condition contained the same substrate combination and ratio and the same greenhouse materials (Table 1). This was to make easily understandable a growth condition which can produce mushrooms at a maximum level and what is the optimal growth condition (Sadhukhan, 2023, Washa, 2024). Maximum results in this study mean higher yielding compared to previous results of the previous study by Washa (2024). It means this is a follow-up study to maximize results of the previous study. Spores as seed type were obtained from mature mushroom cap for sterility (Ahmed, et al. (2013). Each 100 ml bottle of spore (used as pots) was used to inoculate 10 sterilized plastic bags of substrates kept in a sterile condition. One teaspoon of spores was used to inoculate both sides of a single plastic bag. Palm fronds loaf and palm fronds walls were able to allow moderate light to maintain higher humidity and the plastic bags were sparingly watered twice a day for 30 days to maintain moisture (Washa, 2024).

Four replicates were used for each treatment with standard-sized plastic bags and spores to ensure reliability and uniformity. A thermometer, hygrometer, and pH meter were used as measurement tools. By adhering to this experimental design and setup, it was systematically evaluated how varying growth conditions impact mycelia sprouting, leading to meaningful conclusions from the data (Crosby, 2016).

Replicates of the experimental design

This research employed 4 treatments and 4 replicates of data recorded for mycelia sprouting number and sprouting percentage. The importance of 4 replicates in this research was to increase reliability and accuracy of results, to control variability and improve statistical power (Zar, 2010).

Sample Preparation and methods (Substrates, sterilization, spores and packaging)

Substrates were sterilized by boiling at a constant temperature for 4 hours, followed by a 24-hour cooling period. The substrates were then soaked for 4 days to ensure adequate moisture content for mycelium growth, and subsequently dried for 4 days (Crosby, 2016, Sadhukhan, 2023). In a sterile environment, different ratios of sugar and lime were mixed with the cooled combination of sawdust, grain chaff, and dry sliced banana leaves. This mixture was then packaged into 150 plastic bags, each weighing 2 kg. Mushroom spores collected from mature mushroom cap were inoculated on both sides of each 2 kg growth media bag (Ahmed, et al. (2013). The bags were sealed and placed in a dark area for 30 days to allow the spores to colonize the growth media (Crosby, 2016, Sadhukhan, 2023). After the colonization period, the growth media were opened and sparingly watered for another 30 days to facilitate mushroom sprouting. Mushrooms were harvested twice a week for three months to record the sprouting percentage and sprouting number of mushrooms (Crosby, 2016 and Washa, 2023, 2024).

Substrate combination and its importance

Substrate is a growth media which is needed for the supply of various requirements to the mushroom spore sprouting and growth (Crosby, 2016). In this study, substrate combination included: Sawdust which is a carbon source for mycelia growth and area for moisture retention. Grain chaff which is a nutritional supplement to mycelia and enhance air exchange. Dry sliced banana leaves which provide organic materials to mycelia and enhance moisture retention. Sugar which provides energy and stimulate mycelia growth and finally the lime which adjusts the media pH and supply calcium to the growing mycelia (Crosby, 2016).

Mushroom spores

Mushroom spores are microscopic cells produced by mushrooms to reproduce (Sharma et al, 2023). They serve as the seeds of mushroom, though they aren't seeds in the botanical sense. When they land in a suitable environment, they germinate and grow into mycelia, the vegetative part of the mushroom that eventually produces fruiting bodies (the mushroom) (Sharma et al, 2023).

Preparation for spores inoculation

Collecting spores

Place a mature mushroom cap (the top of the mushroom) gills down on a sterile surface, such as aclean glass or piece of paper, to allow it to release spores. Cover the cap with a jar or container to prevent air drafts (Sharma et al, 2023). Leave it foe 24-48 hours to collect the spores, which will fall into the surface below (Crosby, 2016). Scrape the spores into a sterile container, such as a small glass or test tube. Add sterile water to the container and swirl to dissolve the spores. Draw the spore-water mixture into a sterile syringe for inoculation (Crosby, 20160.

Inoculation process

Sterize the substrates by pressure cooking to kill unwanted microorganism (Crosby, 2016). Inject the spore solution into the substrate using the prepared syringe (Crosby, 2016). Ensure the environment is sterile during the entire process to prevent contamination. Work in a clean space (Washa, 2024). Once inoculated, the spore will grow into mycelia, which will colonize the substrate. When fully colonized, the substrate can be moved to a fruiting chamber to initiate mushroom grow (Washa, 2022).

Growth conditions of the greenhouse and the mushroom

The favorable conditions in a greenhouse for mushroom growth include: Temperature: Mushrooms thrive in a temperature range of 13^{o}C to 18^{o}C depending on the type of mushroom (Crosby, 2016). Humidity: High humidity

around 80% to 95% is essential for optimal growth (Sadhukhan, 2023). Ventilation: Proper airflow is needed to prevent excessive heat and maintain oxygen level while avoiding direct drafts that can dry out the mushrooms. Darkness: Mushrooms grow well in low light or total darkness as they do not require sunlight for photosynthesis (Sadhukhan, 2023). Substrates: A nutrient-rich sterilized substrate like sawdust, straw or compost supports mushroom mycelia grow (Sadhukhan, 2023, Simerjit and Priyanka, 2022). Acidic and alkaline level: Mushrooms generally prefer a slightly acidic environment with a pH of around 6.0 to 7.0 (Crosby, 2016). By maintaining these conditions in a greenhouse, the growth process can be maximized. Materials for constructing the greenhouse should be poor heat conductor such as wood, palm fronds which can maintain temperature below 20oC (Washa, 2024)

Measurements and recording of data

Measuring and recording of humidity, temperature, degree of acidity (pH), mycelia sprouting percentage and mycelia sprouting number were done for three months consecutively. Humidity was measured using a hygrometer in percentages (%), temperature was measured in celsius (^oC) and degree of acidity was measured in pH (power of hydrogen). The sprouting number of mycelia was measured by counting the number of germinated mycelia and converted to a percent to get sprouting percentage. Greenhouse materials which are trees (palm fronds) are not measurable and therefore were not measured or counted. Substrates were measured in kilograms (kg) before being processed. These included sawdust, grain chaff, dry sliced banana leaves, sugar and lime.

Split-plot design

A split-plot design is a type of experimental setup used when there are two types of plot experimental factors: one that is difficult to change (called the whole plot factor or main plot) and another that is easier to manipulate (called the sub plot factor) (Crosbt, 2016). In this study, the whole-plot factor was a control plot which was substrate ratios (20:20:5:5:5, and greenhouse materials (palm fronds) which were constant across the experiment (Sadhukhan, 2023). The substrate ratios of this sudy can be elaborated as sawdust 20 kg, grain chaff 20 kg, dry sliced banana leaves 5 kg, sugar 5 kg and lime 5 kg. The sub-plot factor in this study included the varying growth conditions from treatment 1 to 4 (T1 = 65% humidity, 30°C temperature, pH 8, T2 = 70% humidity, 27°C temperature, pH 7.7, T3 = 75% humidity, 25°C temperature, pH 7.5 and T4 = 80% humidity. 21°C temperature. pH 7.5. This was done to enable easier studying of interaction between the whole-plot and subplot factors

Data analysis

The data obtained were analyzed using SPSS software, employing ANOVA and post hoc tests. The results for mycelia sprouting percentage, sprouting number, Biological Efficiency Index and P-values were presented in Tables 2 while other statistical results were presented in table 3, additionally, the tested material arrangements, substrate ratios, and recorded optimal conditions were compared to previous studies to understand their effectiveness in maximizing mushroom production and results presented on table 2 and 3.

Conclusion

Findings of this research highlight the potential for improving mushrooms cultivation in Tanzania and the world where controlled cultivation has been underexplored. Aithough challenges such as improper substrate combination and suboptimal greenhouse materials persist in Tanzania, the study provide valuable insights into overcoming these obstacles. Optimizing growth conditions, as demonstrated in this study, could significantly enhance mushroom yields in Tanzania and help align its practices with global advancement.

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Authors contribution

The author will contribute to the world of science, to AJCS, to Tanzania as well as the world the new, simple and affordable techniques for mushrooms cultivation especially *Pleurotus ostreatum* and make this nutritive and medicinal food easily available to communities.

Declaration of conflict of interest

The researcher declares to have no financial and non-financial conflict of interest for publication of this work.

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