Investigation on the effective substrate for high yields of *Pleurotus ostreatus*: A case study of Kinyerezi Tanzania

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ABSTRACT
Investigation on what substrate combination is more effective in producing high yields of Pleurotus ostreatus in local greenhouses was conducted at Kinyerezi (6°50'27.2"S, 39°10'26.5"E) in Dar es Salaam in 2020 aiming on producing a substrate protocol which will maximize yielding of mushrooms in local greenhouses. Sugar, grain chaff and lime were added in specified ratios to the compost of sliced banana leaves and saw dust and followed by sterilization for 4 hours and cooling for 24 hours before being packaged in 1 kg bags for fungal inoculation. 450 plastic bags of compost were inoculated with mushroom spores and left for 28 days for colonization. The compost was sparingly watered to maintain the optimal humidity, temperature and pH for germination of mushrooms. Mushroom germination in 100 bags with additional sugar, 100 bags with additional grain chaff and 100 bags with additional lime were monitored for four months where germination percentage and germination number were monthly recorded and used for statistical analysis. Bags with additional sugar indicated a significant difference in the monthly germination percentage (P=0.002248, df = 2). Additional sugar descriptively increased germination number from 30 to 173 than other substrates which increased from 0.5 to 16.5. However, the increase was statistically not significant P=0.25246, df = 2. Mushroom growers are advised to use the compost formula as indicated in this investigation as a new knowledge contributed by the research. The use of added sugar as a substrate in the compost is mandatory.

Keywords: *Substrates, compost, mushrooms, inoculation, local greenhouse*

INTRODUCTION
Mushrooms have a broad range of uses including medicinal use and as nutritive food materials. They are a good source of vitamins, protein and minerals. They have a saprophytic type of life inhabiting soil, farm lands,

open fields, roadsides and woods by saprophytic mode of life. Mushrooms belong to the class Basidiomycetes and order Agaricales. Fresh mushrooms contain 80-95% moisture, 3% protein, 0.3-0.4% fats and 1% vitamin and minerals (Venturella et al., 2015).

In recent years, there has been a rapid increase in mushrooms cultivation by local growers and interprenuers aiming at gaining money but also domestic consumption. Among preferably cultivated mushrooms includes Oyster mushrooms (*Pleurotus spp*), *Agarics spp* and others (Okigbo et al, 2021). To date there are no standard protocol agreed upon as to what compost substrates should be used at what ratio to grow successfully what mushroom species. Different mushroom growers have been using different substrates to grow different mushrooms species in different shelters and plastic bags and most of them do not report their substrates used (Moonmoon et at, 2011). Few researches have conducted research based on the experiences of local mushroom farmers leading to absence of standard protocol which can be maximized to improve yields in mushrooms (Venturella et al., 2015).

Pleurotus ostreatus are commonly edible, nutritive and worldwide cultivated for commercial and home consumption. Is mostly cultivated by local farmers and entrepreneurs (Venturella et al., 2015). *Pleurotus ostreatus* can be easily identified by the presence of an oyster-shaped cap, decurrent gills, gills are white running down the cap and stem, caps are white to brown, smooth with no scales, a flesh stem is white with no rings, spores are white to lilac-grey. This species is saprophytic growing on logs and dying trees and found on deciduous trees, they smell sweet like liquorice; they grow during summer or winter and in warmer areas (Moonmoon et al, 2011).

Due to the higher demand for Oyster mushroom from the family level for consumption to the national level for commercial, it was found significant to investigate and obtain a standard substrate protocol which can be used by local as well as big farmers and researcher to obtain maximum yields of mushrooms for livelihood consumption and commercial. This research was conducted to initiate a beginning of researches towards standard methods for higher production of Oyster mushrooms in local and technological green houses. This is therefore; the objective of the study was intended to investigate the effective substrate for high yields of

Pleurotus ostreatus in local greenhouses by testing the effects of additional of sugar, grain chaff and lime to the substrate.

MATERIAL AND METHODS

The study area

A research was conducted at Kinyerezi Mnembwe, (6°50'27.2"S, 39°10'26.5"E) Dar es Salaam where materials for green house construction, material for substrates formation and mushroom spores were easily purchased. A local greenhouse was constructed from January to February 2020.

Collection of materials

Purchased materials included: timber reject, nails, boxes, iron sheets, stock blocks of woods, bags of cement, trips of concrete stones, trip of clay, sacks of sliced banana leaves, sacks of saw dust, lime, teen of grain chaff, sugar, plastic bags, and bottles containing mushroom spores. The mushroom spore bottles were 100ml each. This was self funded research.

Preparation of a local green house

A local greenhouse of 12m x 7m x 3m (12m lengths, 7m widths and 3m heights) was constructed using timber reject for walls and covered by iron sheets but with boxes under the sheets. Rolls and cages were constructed using stock blocks of wood as indicated in Photograph 1a&b below.

(a) (b)

Photography 1a&b: a=Outer part of green house, b=Inner part of green house with colonized bags in cages

Timber rejects and boxes materials were used to reduce temperature and retain darkness as a mushroom growth requirement. A higher temperature above 30ºC (85ºF) will kill mycelia, first growth of spore and reduce yield. Darkness is a requirement for Oyster to grow. Sliced banana leaves, saw dust and grain chaff in compost are used to initiate mycelia formation. Cement, concrete stones and clays were used to construct a flow where nails were used to strengthen timber reject, sheets and stock blocks of woods.

Preparation of the compost (growth media) and sterilization

Sliced banana leaves (10 sacks), saw dust (10 sacks) and grain chaff (50 kg) were soaked in water for 4 days then left to dry for 4 days. Then, sliced banana leaves, saw dust and grain chaff were mixed with lime (50 kg) as minerals and sugar (50 kg) as an additional source of energy. The mixture was to be used as a growth media to initiate fungi colonization. A total of 450 plastic bags, 1 kg each were filled with the made growth media. Sugar was used as a source of energy, grain chaff as a source of food and lime as mineral rising pH to 7.5 and preventing the hardening of the compost. Additional of extra 2 kg sugar, 2 kg lime and 2 kg grain to compost were done to test their effect to germination percent and germination number of mushrooms in the green house.

The 450 plastic bags of growth media were tightly closed in both sides and boiled for 4 hours at boiling temperature to sterilize the media and kill the ammonia producing bacteria then were left to cool for 24 hours to dissipate ammonia before being transferred to cages for inoculation (photograph 1b). Both sides of the bags were inoculated in a sterile condition and closed with papers on 1 March 2020 and kept in cages.

Mushroom spore inoculation for mushroom germination initiation
One bottle of mushroom spores was used to inoculate two sides of 9 bags. Bags were monitored on cages for 1 month to allow fungi colonization (Photograph 1b). Every day the covering papers were removed prior to water spraying on the bags and the bags were covered again. Recording of germinated mushrooms was done daily to obtain germination number and germination percentage. Harvesting was conducted once a week for 4 months to allow recording of observations.

Experimental design:
A Split plot design was used to handle the experiment where substrate additional rows were used as the main plot and the monthly observation column was used as a sub plot (statistical able 1&2 below). Monthly observations on data were summed to obtain monthly germination numbers and monthly germination percentages. Observations were first transformed into arcsine before computation (Zar, 2020). Results from the experiment were analysed. Means and Least Significant Difference (LSD) for germination number and germination percentage for all treatments were computed to get an inference.

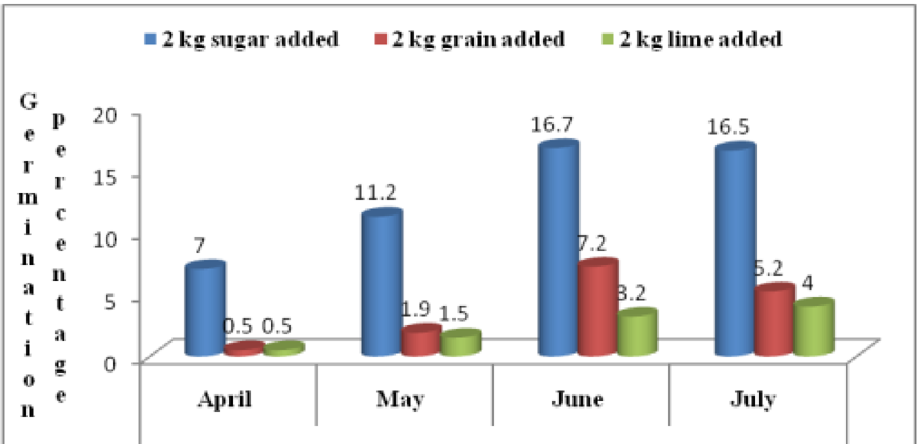
Data analysis

Data analysis was accomplished according to (Zar, 2020), using Statistical Packages for Social Sciences (SPSS version 14) software for analysing germination percent and germination number. Descriptive statistics were first performed to observe the characteristics of the study variables. The characteristics of the study variables were presented by using frequency tables and charts. Since the study constitute of only one dependent variable with three independent categories, the one factor Analysis of Variance (ANOVA) were suitable in observing the presence of significant effect among the categories. The effect of groups was considered significant if the P-value were less than 5%.

RESULTS AND DISCUSSION

Effect of additional sugar to germination percentage of *pleurotus ostreatus*

The findings in graph 1 shown germination percentage ranged from 7 to 17 between April 2020 and July 2020 in the compost with an additional of 2 kg sugar while the compost with an additional of 2 kg grain chaff and 2 kg lime, germination percent ranged from 0.5 to 7 and 0.5 to 4 respectively in the same duration, the same greenhouse and the same environmental conditions of temperature, humidity, pH and precipitation. Moreover, the inferential statistics in table 1 revealed the statistically significant difference in mean germination percentage due to the additional sugar P-values = 0.002248 and df = 2 as it came from (N-1 = 2) in table 1 where N is the number between the groups which were 3 groups (sugar, lime and grain chaff) and df is the degree of freedom.. Other scholars have been reporting that Mushroom growers have been using different compost and substrate ratios as a probability game. The same probabilities have been conducted on the type of materials used for the construction of the greenhouse (Moonmoon et al, 2011). Photograph 2 and 3 are examples of germinated mushrooms in bags and cages in the greenhouse of the study.



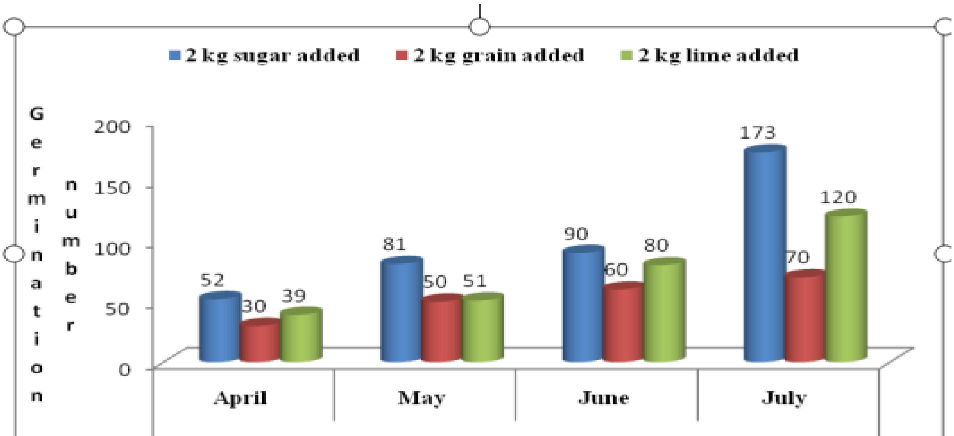
Graph 1: Monthly observations on germination percentage

Table 1: Analysis of means

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	245.7622	2	122.8811	19.90157	0.002248	5.143253
Within Groups	37.04667	6	6.174444			
Total	282.8089	8				

Effect of additional sugar to germination number of *pleurotus ostreatus*

As table 2 and graph 2 indicated, the highest germination number was observed in compost with additional of 2 kg sugar which ranged from 52 to 173. The lowest germination number was observed in compost treated with 2 kg grain chaff which ranged from 30 to 70. Germination number in compost treated with lime was moderate (39-120) next to sugar treatment. Descriptively, it is evident that additional of sugar was associated with higher number of Mushroom germination than other treatments (graph 2). However, statistical results indicated that there was no significant difference of germination number between treatments ($P>0.05$) with P-value = 0.25246 and df = 2 as it came from (N-1 = 2) in table 2 where N is the number between the groups which were 3 groups (sugar, lime and grain chaff) and df is the degree of freedom.



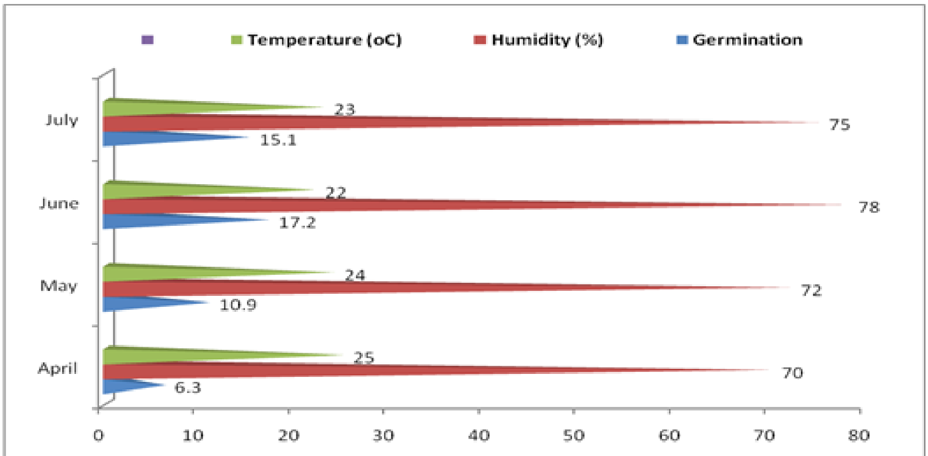
Graph 2: Monthly observations on germination number

Table 2: Analysis of mean

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4509.556	2	2254.778	1.746686	0.25246	5.143253
Within Groups	7745.333	6	1290.889			
Total	12254.89	8				

Average monthly germination

Results in graph 3 indicated high average germination in June (78%) followed by July and May (75% and 72) respectively while the lowest average germination was 70% in April (graph 3). Rise and fall in the average monthly germination reflected the effect of humidity and temperature as determining conditions for mushrooms germination in the greenhouse (Oh et al, 2003). A higher temperature above 30°C (85°F) kill mycelia, first growth of spore and reduce yield. At such temperature the levels of humidity for a greenhouse which do not have humidify is below 70% (Oh et al, 2003). The month of June during the research had higher humidity (78) which was attributed by high rainfall which influenced higher average monthly germination (17.2). At such higher humidity the temperature is always around 20^0C. The high temperature in April and May (25^0C and 24^0C) respectively affected the average monthly germination (6.3 and 10.9) respectively compared to the moderate temperature in July (23) in which average monthly germination was 15.1%



Graph 3: Average Monthly Germination

Potography 2: Mushroom germination arising from bags

Photography 3. Mushrooms grown out of the bags.

Successful production of mushroom as observed in this investigation depends on growth media and growth condition as influenced by atmospheric condition and maintained optimal in the local greenhouse. Observation also realized that, substrate and growth condition are all specific in such that substrate will support sprouting and growth of mushroom at specific growth conditions. Specified growth condition which was maintained in this local greenhouse was as follows: humidity above 75% was more favourable, humidity below 70% dried the mycelia and brought death of Mushrooms, temperature below 30°C but above 20°C was more favourable. These conditions influenced high average monthly germination of 17.2 in June at 78% humidity and 22^0C compared to low average monthly germination of 6.8 in April at 70% humidity and 25^0C. The effect and influence of atmospheric conditions in this research to mushrooms germination and growth agree with many other researchers including Moonmoon et al (2011) and Washa (2015).

Temperature above 25°C killed the mycelia, first growth of spore and reduced yield. Higher precipitation above 200 mm a month kept humidity optimal provided materials constructed in the local green house maintain the humidity optimally.

Sugar, grain chaff and lime as substrates used to add in the compost in this research differ in supporting mushroom germination and growth; they are all good and proper for growing Oyster mushroom. Content differences from their structural composition is obviously the source of different performance in supporting mushroom in the compost provided the growth condition of the atmosphere and that of local greenhouse are optima. The effect of substrate used in this research and results reviled in the research are in agreement with the findings by (Gerrits, 1981) and (Oh et al., 2004b and Washa, 2015).

Sugar in the growth media

.Sugar is a carbohydrate yielding 3.94 calories per gram as do all carbohydrates. This stored energy in form of calories was used for initiating mushroom germination and growth through all months of the research and brought significant growth in additional of 2 kg in the growth media as indicated in (Table 1&2, figure 1&2). Sugar as a substrate is cheaper for local mushroom growers to buy than Agar and Murashige and Skoog used in technological laboratories.

Grain chaff in the growth media
Grain chaff used as a substrate in this research is composed of cellulose, lignin, hemicelluloses, and a protein matrix. These compositions are nutritive materials to be absorbed by the mushroom through mycelia parasitically as mushroom have parasitic mode of life. The composition also prevents solidification of the media (compost) to allow parasitic penetration of the mycelia. Grain chaff are the cheapest substrate to be bought by the local mushroom growers compared to Agar and Murashige and Skoog used in technological laboratories. The composition of grain chaff presented in this research agree with the findings by (Oh et al., 2004b).

Lime in the growth media
Lime used as a substrate in this research is a calcium-containing inorganic mineral composed primarily of oxides, and hydroxide, usually calcium oxide and/ or calcium hydroxide. Lime in the compost of this research was of two functions: One was to sticky the mycelia but also the media. This means solidified the growth media to the required level to support standing of the germinated mycelia and sticky mycelia is when the flegile mycelia which is not able to stand by its own tissue, the tissues to some extend harden and mycelia can stand in the media. The other function of lime was to balance and maintain the pH of the media at optimal level as a growth requirement. This is why the average pH in the months of mushroom cultivation of the research was 7.45. Recommended growth media pH range is (7-8), Gerrits (1981). As it is, lime used in this research as a substrate is cheaper than the Agar and Murashige and Skoog. The composition and functions of the lime substrate as elaborated in this research is in agreement with findings by (Chang and Miles, 1989).

Wood rejects in the local greenhouse
Woody materials used to construct local green house in this research as poor conductor of heat were able to minimize the destructive temperature especially in June 2020 to optimal level (22^{o}C). In additional to reduction of temperature, timber reject in this research was used to retain darkness as a growth requirement (Moore and Chiu, 2001, Washa, 2015). Darkness is a requirement for biosporous to grow (Oh et al., 2004b).

The growth media (compost)
Compost used in this research which comprised of sliced banana leaves and saw dust were able to create good parasitic environment for rising,

feeding and growth of mycelia, biosporous and mushrooms as well. This is because they easily decompose and its decomposition is a good food for mycelia but also in presence of lime and grain chaff substrate they do not solidify easily.

CONCLUSION AND RECOMMENDATIONS

As reflected from results of this research, the optimal mushroom growth condition are (moisture > 75%, temperature < 25^{o}C and pH ranges 7-8). The relevant compost is sliced banana leaves (50 kg), saw dust (50 kg), sugar (10 kg), grain chaff (10 kg) and lime (10 kg). Additional of extra 2 kg sugar to the compost increases mushroom germination. Woody materials succeeded to maintain growth conditions at optimal.

Mushroom growers are advised to use woody materials in constructing a local green house, use sliced banana leaves and saw dust to form compost and add sugar as a substrate in the compost. More research is encouraged to improve the compost, substrate and shelter but also the possibility of constructing a local humidifier. Mushroom growers are advised to use the compost formula as indicated in this investigation as a new knowledge contributed by the research.

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