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Comparative Analysis of *Agaricus bisporus* Under Different Substrates And *Agaricus bitorquis* initiation, Incubation, And Casing Period.

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Abstract: *Research was conducted to compare the growth of Agaricus bisporus under different substrates. The four substrates used in the experiment were: wheat straw, rice straw, sugarcane bagasse and maize straw. Chicken manure was used as a source of nitrogen. Dry weight, diameter of the basidiocarp, protein content, ash content and different minerals in the mushroom under different substrates were analyzed using, drying oven, microkjeldah method and atomic absorption spectrometry method. The mushroom yield was also calculated under different substrates. Incubation, spawning, casing and initiation were compared between A. bisporus and A. bitorquis under the same substrates. The obtained data was analyzed statistically using SPSS one-way ANOVA to reveal the slight changes in the tested parameters. Agaricus bisporus had a significant difference in protein content, basidiocarp dimeter, and yield as shown in tables 2,6 and 8 respectively while parameters such as dry weight, ash content, sodium and potassium content did not have a significant difference as shown on tables 1,3,4, and 5 respectively. A. bisporus had a shorter incubation, casing, initiation and mycelium colonization period compared to A. bitorquis as shown in figure 1-4. The findings in this study provide a better understanding and useful information for mushroom cultivation.*

Keywords: *Agaricus bisporus, maize straw, wheat straw, sugarcane bagasse, rice straw, temperature, compost, pinning, yield*

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I. Introduction

Agaricus bisporus (Button mushroom) is one of the most cultivated mushrooms globally[1-3]. Commercially, it is grown on a compost that provides it with the necessary nutrients for growth. The ratio of nutrients may differ depending on the type of substrate used for cultivation. Some of the substrates used for cultivation include wheat straw and rice straw while the most common sources of sources of nitrogen include chicken and horse manure. The method used for *A. bisporus* cultivation involves two composing steps since it provides a better substrate[4, 5]. The first composting phase takes around six to fourteen days depending on the choice of substrate[6]. This stage softens the substrate and breaks down the soluble sugars while lowering the carbon to nitrogen ratio. A very strong odor is experienced at this stage due to the high chemical and bacterial activity[6, 7]. The second stage is a pasteurization process that reduces the growth of other microbiota thus allowing the cultivated mushroom to grow. Before the mycelium is introduced into the substrate, pH and temperature are applied to ensure the compost is specific for *A. bisporus* growth[8]. The substrate should contain approximately 27% (w/w) carbohydrates based on the whole substrate dry matter[9]. A mixture of lime and peat (casing layer) is used to cover the substrate to induce the formation of fruiting body.

Agaricus bisporus is a secondary decomposer and its grown mostly on composed substrates however, the mushroom can also be grown on non-decomposted substrates[10]Due to its ligninolytic activity, it is also regarded as a basidiomycete degrading lignin hence able to grow on non-composted agricultural and industrial wastes[11]. Various methods have been proposed for the cultivation of *A. bisporus* such as non-composted pasteurized agricultural wastes to reduce the cultivation time by avoiding the composting process [5]. Another study proposes mixing of calcium hydroxide 2% with raw materials under a controlled condition ensuring self-pasteurization. This technology has proved helpful in the cultivation of strains such as *P. citrinopileatus, P. djamor, P. eryngii, Agrocybe* and *Auricular* since it takes two days to complete the entire sterilization process [5].Substrates are important for the cultivation of mushrooms. Recently, various agricultural and industrial wastes have been used for the cultivation of mushroom[12-17]. Wheat and rice straw are the most common substrates for mushroom cultivation in Europe and Asia respectively[18]. In Africa, other substrates such as sugarcane bagasse can also be used for mushroom cultivation however the mushroom yield has not been evaluated[19]. Mushroom

farming in developing countries has become a challenge to the farmers because of the expensive substrates used in the cultivation of mushroom.
Mushrooms are a cheap source of protein and vital nutrients that can be made affordable and available to individuals who cannot afford other expensive sources of protein[20-22]. Farmers need to minimize the cost of the substrates used for cultivation and maximize the cultivation of mushroom. Due to the challenges in mushroom cultivation, it has necessitated the need to find cheap and easily available substrates for mushroom cultivation. This study will enhance the cultivation of mushroom at a cheap cost using easily available substrates hence making it a cheap source of protein and vital nutrients. The yield from each substrate will also be evaluated.

II. Materials And Methodology

The experiment was carried out in Jomo Kenyatta University of Agriculture and Technology (JKUAT), Horticulture Department Kenya-Juja. *A. bisporus* and *A. birtoquis* strains used in the experiment were obtained from the Horticulture department, JKUAT.

Substrate preparation and supplement

The substrates and chicken manure used in this experiment were obtained locally. The experiment was conducted to compare the growth of *Agaricus bisporus* under different substrates. The substrates used in this research included wheat straw compost, maize straw compost, rice straw compost and sugarcane bagasse compost. Twenty kilograms of each substrate was placed on a clean polythene paper (300-millimeter gauge). The substrates were watered to maintain the right moisture content. After 24 hours, 400 grams of diluted molasses, 20 kilograms of chicken manure and 250 kilograms of urea was added to each pile followed by turning to allow even mixing. On the fifth day, the following were added to the each of the substrates: 300 grams cotton seed cake, one kilogram of gypsum and a half kilogram of wheat bran were added and the pile turned to allow even mixing. Each substrate pile was turned after 2-3days to facilitate aeration. Twenty kilograms of each of the above substrates was composted separately and pasteurized to remove pests.

Cultivation

Mushroom spawn was prepared from spores. The spores were blended with nutrients from organic material to obtain the spawn. The spawn was then inoculated in the composted substrates for growth. 30 ml of the spawn was used for each of the 5 kilograms of the substrates. The incubation period lasted for 15 days at 22-23 ˚C and after mycelial growth, casing was done with an overlay of 4 cm. The substrate was further incubated for 15 days at 23 ˚C at 90 % relative humidity. The mushrooms were harvested when the pileus opened and the veil broke.

Variables analyzed

The time taken for the mushroom to grow under different substrates was recorded. Dry matter was determined gravimetrically as the residue remaining after drying at 105 degrees in a ventilated oven. % DRY MATTER = (W3-W1) *100/W2-W1. Ten mushrooms from each substrate were picked randomly from each of the substrates. The diameter of the basidiocarp was recorded and the average diameter for each lawn was calculated and recorded. The protein content was determined using micro-kjeldahl method[23]. The ash content was determined through dry ashing and mineral content was determined using atomic absorption spectrophotometer. Incubation, initiation, and casing time was also compared between *A. bisporus* and *A. birtoquis.* The data obtained during the experiment was analyzed using Analysis of Variance (ANOVA).

III. Results And Discussion

Period taken to fruit under different substrates

Wheat straw, sugarcane bagasse and rice straw took almost the same time to fruit. Mushroom pinning was observed after eighteen days. Wheat and rice straw formed fruiting bodies on the 18nth day while sugarcane bagasse lagged behind by one day and hence mushrooms under the substrate formed basidiocarp on the 19nth day. Mushroom in wheat straw pinned earlier followed by rice straw and lastly sugarcane bagasse. Through observation, fruiting bodies on sugarcane bagasse were the largest followed by wheat straw and lastly rice straw. Under the maize substrate, a very slow rate of colonization was observed during the first flush but pinning was not observed. All the observations above were made as per the first flush.
After inoculation of the mushroom spawn i.e. 30ml in each substrate, growth was observed 18 to 22 days after casing, on three types of substrates namely: sugacane bagasse, wheat straw, rice straw. Maize showed very little signs of colonization. The photos taken for each substrate are shown below in plate1,2,3 and 4.

Plate 1: maize straw.

Plate 2: Rice straw.

Plate 3: Sugarcane bagasse.

Plate 4: Wheat straw.

Percentage dry weight

The comparison for dry weights was done on different substrates as shown in table 1. Mushrooms grown under wheat straw and rice straw had the highest dry weight was followed by sugarcane bagasse.

Type of substrate	**% Dry weight**
Sugarcane Bagasse	7.62
Wheat straw	9.75
Rice straw	9.73

Table 1: Average dry weight.

Nutrients of mushrooms under different substrates

The protein content of *A. bisporus* was calculated under different substrates. Table 2 indicates the average protein percentage under different substrates. There is a slight difference in the protein content under different substrates.

Type of substrate	**% protein content 100grams**
Sugarcane Bagasse	2.85
Wheat straw	3.12
Rice straw	2.87

Table 2: Average protein percentage per 100 grams.

Ash content

Ash content was calculated under each substrate as indicated in table 3. Below is a summary of the average ash percentage under different types of substrates.

Type of substrate	**% ash in mushroom**
Sugarcane bagasse	0.95
Wheat straw	1.05
Rice straw	1.07

Table 3: Average % ash content.

The minerals analyzed in this study included sodium and potassium. The tables below show the average concentration of sodium and potassium.

Type of substrate	**Na mg/100 grams**
Sugarcane bagasse	1105.38
Wheat straw	1071.87
Rice straw	4041.24

Table 4: Concentrations of sodium.

Type of substrate	Potassium mg/100grams
Sugarcane bagasse	47.37
Wheat straw	52.10
Rice straw	51.38

Table 5: concentration of potassium.

Diameter of the basidiocarp under different substrates

Table 6 shows the average diameter of the mushroom obtained from the three types of substrates indicate sugarcane bagasse having biggest average diameter of 3.78 cm followed by wheat straw with an average diameter of 3.5 cm and lastly rice straw with an average diameter of 3.27 cm. Below is an average of the diameter of mushrooms obtained from each substrate.

Type Of Substrate	Sugarcane Bagasse	Wheat Straw	Rice Straw
Average	**3.78 Cm**	**3.50 Cm**	**3.27 Cm**

Table 6: Diameter of mushroom under different substrates.

Comparison between *A. bisporus* and *A. bitorquis*

The species had a different incubation period, casing period, and initiation period. In table 7, incubation period for *A. bisporus* was 15 days while *A. bitorquis* had 18 - 22 days, casing was done 15 days after incubation in *A. bisporus* while in *A. bitorquis* it was done on the 19nth day. Initiation period was 3 days for *A. bisporus* while *A. bitorquis* took 6 days. Lastly, harvesting was done 18 -22 days after casing while for *A. bitorquis*, the harvesting period took longer than expected hence making it impossible to compare it with *A. bisporus*. The table below shows the comparison in various growth aspects between *Agaricus bisporus* and *Agaricus bitorquis.*

Growth Aspects	*Agaricus bisporus*	*Agaricus bitorquis*
Incubation	15 Days	20 To 22 Days
Casing	15 Days	18 Days
Initiation	3 Days	6 Days
Harvesting Time	18 To 22 Days After Casing	It Was Not Possible To Harvest The Mushroom Within The Required Time Since It Took Longer Than *A.bisporus*

Table 7: Comparison between *A. bisporus* and *A. bitorquis*.

Mushroom yield

The data obtained through weighing of the mushroom in table 7was summarized as shown below.
Totals grams per substrate (15kg)

Mushroom yield = kilograms/Ton of substrate

Yield for each substrate Sugarcane bagasse= 47 Kg/Ton
wheat straw = 45.3 kg/Ton
Rice straw = 32.5 kg/Ton

The table below shows the yield in different substrates i.e. first batch, second batch and the third batch.

BATCH	TYPE OF SUBSTRATE (5 Kgs)	MUSHROOM HARVESTED (grams)
1st BATCH	Wheat straw	250 grams
	Rice straw	200grams
	Sugarcane bagasse	350 grams
2nd BATCH	Wheat straw	220 grams
	Rice straw	175 grams
	Sugarcane bagasse	180 grams
3rd BATCH	Wheat straw	210 grams
	Rice straw	112 grams
	Sugarcane bagasse	175 grams

Table 8: Mushroom yield under different substrates.

Substrates

Wheat straw had the best composting temperature and the composting process took the required time frame. Colonization and mushroom pinning was observed within the required period. Sugarcane bagasse composting time was similar to wheat since it has a lot carbon source which is utilized by microorganisms during the composting process. Colonization and pinning was also observed within the required time. Rice straw broke down easily compared to other substrates during composting however, colonization and pinning took place at the required time. Maize substrate showed a very slow rate of colonization because the maize pith retains a lot of water resulting to a high moisture content. This slows down the rate of composting and deters the mycelia growth. Maize substrate had the lowest temperature during the composting periods.

Comparison of the results obtained with the USDA, Human Nutrition Research Center, USDA-ARS 2006
Dry matter values were within the required range when compared to USDA nutrient data[24]. There is a good correlation between the values noted in different types of mushrooms namely; oyster, maitake, white and enoki. Values obtained for moisture content had a good correlation between the values observed in different mushrooms. The percentage protein (g/100g) content similar values of with the protein content in the mushroom USDA table[24].
Sodium levels and Potassium levels (mg/100g) in *A. bisporus* were compared to the USDA table[24] There is a slight deviation observed in the experiment compared to the USDA data. The ash content had a slight difference while there was a significant difference in the diameter of the mushrooms under different substrates.

IV. Conclusion

Agaricus bisporus grown under different substrates showed a significant difference in protein content and basidiocarp diameter. The dry weight, the ash content and mineral content did not have any significant difference. Sugarcane bagasse had the highest protein content and the largest diameter followed by wheat straw and rice straw respectively. Dry weight for mushrooms grown under wheat straw and maize straw had similar content however, sugarcane bagasse had a slightly reduced dry weight. Ash content was higher in wheat straw, rice straw and sugarcane straw respectively. There was no significant difference in mineral content for sodium and potassium under the three substrates. The overall yield was higher in sugarcane bagasse, wheat straw and rice straw respectively. From this study, it may be concluded that sugarcane bagasse, wheat straw, and rice straw are suitable for the cultivation of mushrooms however, maize straw may not be appropriate for the cultivation of mushrooms. The result obtained from this experiment is useful for mushroom cultivation. However, further research needs to be done to evaluate the use of other agricultural and industrial wastes for mushroom production.

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