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Efficacy of Selected Sorghum Genotypes as Cereal Base Medium for the Growth of White Button Mushroom Mycelium (*Agaricus bisporus* (L))

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Abstract: *The feasibility of standardizing a sorghum grain basal media as an alternative to different culture media such as potato dextrose agar and wheat basal media was assessed using different local sorghum genotypes. The relative mycelia growth over time using different genotypes, preparation methods and concentrations was investigated along with standard potato dextrose and wheat grain medium as standard set-up. The use malting method proved to be beneficial for producing visually prominent and dense cultures of mushroom. The malt method provides readily available nutrition that favours early-growth mushroom mycelia The study also recommended sorghum genotypes; Tabat, Tetron, Faterita White and Faterita, Red, respectively. The concentration of sorghum preparation remains a minor factor during the early stage of mycelium growth. It starts showing a significant effect at a later stage of mycelium extension, particularly at 21,24 and 27 days after inoculation.*

Keywords: *Cereal grain, Sorghum, Media, Mycelial growth, Agaricus bisporus*

Introduction

Food gap, malnutrition and poverty are increasing rapidly in many parts of the developing world. Mushroom is one of the promising vegetables that can contribute to the alleviation of poverty and the mitigation of the food gap, (Badr,2005). The introduction of mushroom farming and production technology to Sudan is one of the interventions proposed by a team of researchers in AAU-Sudan in early 2011. The value of consumption of wild mushrooms is well documented in the traditional knowledge of local Sudanese. People tend to eat mushrooms with traditional local receipt cooked using okra or after drying it as porridge, other uses include consuming it as tonic, medicine or cosmetics. The farming of commercially grown mushrooms is relatively new in Sudan.The growth of microorganisms in cereal is old in history even before scientific principles are understood. Cereals are an important source of nutrients such as carbohydrates, protein, lipids, vitamins and minerals as well as phytochemicals. They typically contain around, 70-75% carbohydrates, 8-15% protein and a substantial amount of fibre, fat, vitamins and minerals depending on the type of grain (Erkmen & Bozoglu, 2016). the Nutrients even vary within the same species of grain and to some extent to growing environments and cultural management. Their nutrient composition makes them ideal substrates for the sustenance of micro-organisms especially moulds, provided appropriate environmental conditions exist (Rico-Munoz et al., 2019). Cereal can provide a suitable media that is equal to or exceed standard medium such as potato dextrose medium for raising healthy and vigorous mycelia. Reports also showed that wheat and sorghum had been used to produce media for mycelium growth. Sorghum is an important crop and staple food in semi-arid zones of Africa including Sudan. It is cultivated in many parts of Sudan mainly in Gezira and Gadarief regions. It is produced mainly in Eastern Sudan in rain-fed areas, with a limited amount being produced in Western Sudan and the irrigated areas at the Gezira Scheme (Vogel and Graham, 1979). The most popular varieties grown in Sudan are Mayo (Milo in the USA), Dabar, Safra, Gasabi Wad Ahmed and Feterita. Sorghum has great genetic diversity in Sudan and varies in composition due to genetics and environment (Waniska and Rooney 2000). In Sudan, for all practical purposes, all malt (zirreea) is prepared from the sorghum variety (faterita) and only a small amount of malt is prepared from millets such as pearl millet and finger millet. Sorghum malt is prepared and used throughout the country. Standardizing sorghum media out of Sudanese sorghum genotypes will be part of localizing production technology and replace the high cost of microbial cultural media, utilizing the advantage of Sudan sorghum-wide genetic diversity.

Materials and Methods

Different local genotypes of sorghum had been used namely; Faterita White, Faterita Red, Tetron and Tabat. Each Sorghum genotype was prepared in two preparation methods, grain and malt meal. The grains of Sorghum were prepared as described by Upadhyay, *et al*. 2004, in the case of wheat basal media. Different concentrations of grains were prepared (15,20,25,30 and 35gm)and boiled in water for 1 and 1/2 hours and filtered through muslin cheesecloth. The residues were discarded and the volume of the sieved extracted was completed to one litre using double distilled water. Agar- Agar powder was added by stirring continuously and pH was adjusted

to 6.5 -7 before autoclaving at 121ºC for 15 minutes. Malt meal was prepared following steeping, germination and Kilning as described by DeWet, (1978) and Lyumugabe, (2010). The malt was then dried and finely grinded to a meal. The meal was prepared following the same concentration as the procedure. Standard potato dextrose agar (PDA) and wheat agar medium (WA) were prepared as a standard setup as described by (Upadhyay, *et al*., 2004). Cultures were inoculated with a disc-like cut of diameter (15mm) from a fresh culture of *Agaricus bisporus* in each treatment. Treatments were arranged in a completely randomized design CRD with four replicates. Data were analyzed using analysis of variance and the interaction between methods (Boiled and Malt); varieties (Faterita White, Faterita Red, Tetron and Tabat) and concentrations (15, 20, 25, 30, and 35g/l) with 2×4×5 level was subjected to three-way factorial (CRD) analysis model as described by Gomez and Gomez 1984. Parameters measured included linear mycelia extension (cm) and relative mycelium growth.

Results and Discussion

malt seed preparation methods were consistently significant at 5% with time .seed meal of Malting grain was superior providing early pick-up growth over boiled grain basal media. The malting method promotes the development of hydrolytic enzymes for the degeneration of starch and proteins in cereal grains and thus provided mycelia with a readily available source of nutrition and energy as compared to standard sorghum basal media. Sorghum genotypes for the experiment were chosen from the popular varieties in the Sudanese markets. Tabat, Faterita Red and Faterita White genotypes are rich in protein, starch and tannin, (Roony et al., 1984; Salunkhe et al., 1977; Gassem and Osman 2003). This was clear in their differential performance as basal medium .

Sorghum genotypes recorded significant differences in 3,6, 12, 15, 21, 24 and 27 days after inoculation. Tabat, Faterita Red and Faterita white Genotypes respectively outperform the Tetron genotype, producing a significant higher mycelium extension. The concentration chosen in this experiment ranged around the recommended concentration of 32 g/liter for the standard wheat medium as described by Sainos, E. (2006) and Upadhyay, et al.,(2004). The concentration of sorghum preparation remains a minor factor during the early stage of mycelium growth. It starts showing a significant effect at 5% at a later stage of mycelium extension, particularly at 21,24 and 27 days after inoculation. Fifteen and twenty grams of malt sorghum per litre recorded significant performance at 5% and remained the best economically feasible concentration with equal performance as compared to a concentration of 35 grams per litre at 27 days after inoculation .

The interaction between methods and varieties was consistently significant at 5% across the culture growth over time. The highest interaction was recorded between Malt preparation with Tabat, Faterita white and Faterita Red genotypes respectively. The interaction between methods and concentration was consistently significant at 5% across the culture growth over time. The highest interaction was recorded between Malt preparation with concentrations of 15,20 and 35 grams per litre respectively. The above two interactions reinforce the merit of malt seed preparation as a determining factor for superior cereal basal media.The interaction between varieties and concentration remains significant across the 27 days of incubation at 5%. The combination of Tabat and Faterita White with 15,20,30 and 35 grams per litre gives predominantly significant interaction across the 27 days of readings.

Three-way interaction remained significant across the 27 days at 5%. The malt preparation method ranked all their combinations at the top of the performance list. The top performance combinations were further analysed and compared to standard PDA and wheat basal medium at twenty-four days as per table 2. Mycelia radial growth and growth rate are affected by the type of medium and proportion of additives supplemented. Growth mycelium was observed to be better in the formulated media relative to those on the standard set-up, PDA and wheat basal medium. The formulated media is more suitable for early mycelium growth. This is supported by studies demonstrating variation in mycelium proliferation among different cereal basal mediums (Adesemoye and Adedire 2005). . Fully colonization of Petri dishes ranged between 12-27 days. The number of days from inoculation to the total colonization is related to the mycelial growth rate on the substrate. A faster growth rate results in a corresponding reduction in the days required for the complete colonization of the petri dish as per Table 2.

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Method	Variety	Conc.	3 DAI	6 DAI	9 DAI	12 DAI	15 DAI	18 DAI	21 DAI	24 DAI	27 DAI
Malt	Faterita Red	15 g	25.00	31.25	42.50	56.25	65.00	71.25	80.00	83.75	85.00
		20g	22.50	31.25	41.25	55.00	67.50	73.75	80.50	83.75	85.00
		25g	22.50	31.25	42.50	56.25	67.50	72.50	78.75	83.75	86.25
		30g	25.00	37.50	43.75	55.00	66.25	71.25	78.75	86.25	86.25
		35g	23.75	35.00	48.75	56.25	66.25	70.00	78.75	80.00	82.50
	Faterita white	15 g	20.00	35.00	51.25	66.25	72.50	74.25	83.50	87.00	87.50
		20g	30.00	40.00	50.00	59.25	65.00	73.75	80.00	85.00	85.00
		25g	26.25	32.50	46.25	60.00	70.00	76.25	80.00	82.50	83.75
		30g	25.00	33.75	40.00	47.50	57.50	63.75	67.50	70.00	75.00
		35g	22.50	37.50	50.00	66.25	73.75	75.00	78.75	85.00	86.25
	Tetron	15 g	25.00	33.75	38.75	47.50	60.00	73.75	77.50	81.25	85.00
		20g	22.50	35.00	47.50	65.00	71.25	78.75	82.50	86.25	86.25
		25g	23.75	31.25	37.00	43.25	50.00	61.25	65.00	66.25	67.50
		30g	22.50	31.25	39.50	47.50	53.75	58.00	61.75	66.25	67.50
		35g	21.25	58.75	62.50	67.50	73.75	73.75	79.25	83.75	85.00
	Tabat	15 g	30.00	37.50	46.25	62.50	66.25	72.50	78.00	82.50	83.75
		20g	31.25	38.75	51.25	70.00	71.25	76.25	78.25	82.50	85.00
		25g	31.25	38.75	42.50	60.00	72.50	77.50	80.00	82.50	87.50
		30g	25.00	38.75	51.25	66.75	75.00	80.00	80.75	85.00	87.50
		35g	26.25	32.50	41.25	61.25	66.25	75.00	81.25	82.50	86.25
Boiled	Faterita Red	15 g	15.00	22.50	27.50	38.75	45.00	56.75	71.25	72.50	78.75
		20g	15.00	21.25	28.75	41.25	45.00	53.75	68.75	72.50	76.25
		25g	15.00	20.00	23.75	33.75	40.75	52.50	62.00	66.25	75.00
		30g	15.00	22.50	28.75	37.50	44.50	56.25	65.00	73.75	78.75
		35g	15.00	21.25	26.25	38.75	46.00	61.25	68.75	71.25	76.25
	Faterita white	15 g	15.00	20.00	27.50	37.50	44.25	57.50	70.75	75.00	78.75
		20g	15.00	23.75	28.75	36.25	58.75	68.75	72.50	74.25	76.25
		25g	15.00	21.25	27.50	32.50	48.75	53.75	66.25	71.25	76.25
		30g	15.00	20.00	29.50	37.50	57.00	62.50	66.25	76.25	76.25
		35g	15.00	20.00	31.25	37.50	47.50	57.50	66.25	66.25	78.75
	Tetron	15 g	15.00	22.50	30.00	38.75	43.75	52.50	65.00	71.25	72.50
		20g	15.00	23.75	30.00	37.50	48.75	55.00	73.75	78.75	81.25
		25g	15.00	23.75	30.00	37.50	45.00	51.25	61.25	71.25	75.00
		30g	15.00	20.00	25.00	36.25	45.00	45.00	60.00	67.50	70.00

		35g	15.00	17.50	22.50	32.50	40.00	51.25	56.25	60.00	62.50
	Tabat	15 g	15.00	22.50	33.75	38.75	46.75	56.25	68.75	73.00	77.50
		20g	15.00	25.00	32.50	40.00	45.00	57.00	67.50	72.50	73.75
		25g	15.00	22.50	30.00	36.25	41.25	51.25	65.00	73.75	73.75
		30g	15.00	25.00	30.00	42.50	53.00	61.25	68.75	75.50	78.75
		35g	15.00	20.00	27.50	36.25	41.25	52.50	61.25	73.75	77.50
Mean (mm)			19.79	28.45	36.69	47.45	55.86	67.94	71.39	76.07	78.99
CD 5%			**5.64**	**9.53**	**11.32**	**12.73**	**13.70**	**11.51**	**11.44**	**10.99**	**9.63**

- "DAI" Days after inoculation

Table1. Mean performance of different preparation, sorghum genotype and concentration combinations for mycelium radial growth of *Agaricus bisporus*

Table 2. Growth rate, total colonization and mycelia density at twenty four days after inoculation

Treatment	Mean radial mycelium growth (mm)	Growth rate mm/day	Total colonization (days)	Mycelium density
MFr15	83.75 [a]	2.86[a]	15	+++
MFr20	83.75 [a]	2.86[a]	15	+++
MFr25	83.75 [a]	2.86[a]	15	+++
MFr30	86.25 [a]	2.97[a]	12	+++
MFr35	80.00 [a]	2.71[a]	21	+++
MFw15	87.00 [a]	3.00[a]	12	+++
MFw20	85.00 [a]	2.92[a]	12	+++
MFw25	82.50 [a]	2.81[a]	18	+++
MFw35	85.00 [a]	2.92[a]	12	+++
MTa15	82.50 [a]	2.81[a]	18	+++
MTa20	82.50 [a]	2.81[a]	18	+++
MTa25	82.50 [a]	2.81[a]	18	+++
MTa30	85.00 [a]	2.92[a]	12	+++
MTa35	82.50 [a]	2.81[a]	18	+++
MTe15	81.25 [a]	2.76[a]	21	+++
MTe20	86.25 [a]	2.97[a]	12	+++
MTe35	83.75[a]	2.86[a]	15	+++
PDA - Control	70.00 [b]	2.29[b]	24	++
Wheat-Control	62.50 [b]	1.98[b]	27	+
CD 5%	**11.80**	**0.492**		

"M" for Malt preparation ; "Ta","Te", "Fw" for genotype Tabat, Tetron and Fetarita white respectively; the number donate the concentration in grams/litre.
\+ Cottony texture, low density, regular growth and white mycelium
++ Cottony texture, regular density, regular growth and white mycelium
+++ Cottony texture, high density, abundant growth and white mycelium

Figure 1. Radial mycelial growth on different medium combinations along with standard PDA and wheat basal medium as control