

Biofortification of mushroom (*Pleurotus floridanus*) using calcium based supplements

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ABSTRACT: The nutritional value and yield of mushrooms depend on the substrate on which it is grown. This study sought to biofortify *Pleurotus floridanus* with calcium supplements and assess its effect on the yield and calcium levels. The experiment was set up in a 2 × 5 factorial and replicated thrice in a completely randomized design. Two calcium supplements, OML and OMW, were added to two growth media. The examination of total dry weight yield showed that calcium supplements OML and OMW in the sawdust medium containing wheatbran in the ratio 1:10 had a mean value of 4.37 g, which was significantly higher ($P < 0.05$) than that in the control (1.29 g). However, in the sawdust-only medium, there was no significant difference ($p > 0.05$) in the application of treatments. No significant difference ($p > 0.05$) was observed between the calcium types in both growth media. The mineral analysis showed that calcium levels were increased in harvested mushrooms with the addition of calcium OML and OMW to the growth media.

KEYWORDS: Calcium supplements, Crop improvement, *P. floridanus*

INTRODUCTION

Mushrooms are invaluable foodstuffs virtually in all nook and cranny of the earth largely due to their nutritional value and medicinal use especially with increased interest in recent decades.

According to World Health Organization (WHO, 2019) biofortification is the process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology. Biofortification differs from conventional

fortification in that biofortification aims to increase nutrient levels in crops during plant growth rather than through manual means during processing of the crops. Biofortification may therefore present a way to reach populations where supplementation and conventional fortification activities may be difficult to implement and/or limited.

Agriculture is the most sensitive sector to climate change, impacting negatively on food security and sustainability. Crop improvement is important to increase agricultural productivity and to contribute to food and nutrition security. The need for improved crops is exacerbated by climate change. Farmers need to replace crop varieties with better adapted ones to match rapidly evolving climate conditions to ensure climate change adaptation (Porter *et al.*, 2014)

Furthermore, it is estimated that atmospheric carbondioxide (CO₂) concentration should not go over 450ppm to meet the 2°C goal of global warming or 430ppm for 1.5°C (IPCC, 2007). Governments, organisations and individuals have a role to play to reduce Carbon emissions and thus, mitigating climate change. It is reported that 1 kg of beef meat generates 19 kg of CO₂/e. Crop based alternatives however, generates less than 1kg, helping consumers reduce their Carbon foot print (Jan *et al.*, 1999)

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To increase mushroom yield and boost production, supplements are added to bulk substrate. The mostly used supplements are Wheat and Rice bran which are rich in protein. Literature about element based supplements are however limited. Additionally, based on mineral analysis of common Oyster mushrooms, Calcium level is insufficient when compared to other minerals (Akindahunsi and Oyetayo, 2006).

Calcium is the most abundant mineral in the human body, accounting for 1–2% of an adult's body mass. Over 99% of Ca stored in the teeth and bones, where it plays an important structural role (WHO/FAO, 1998). Ca, like Zn, is also an enzyme cofactor and an important signaling molecule (a secondary messenger). It plays a pivotal role in the blood clotting cascade. Calcium deficiency has a profound impact on bone health, resulting in rickets if deficiency occurs in the young and osteoporosis if it persists into old age (Singh *et al.*, 2016).

The main purpose of this research was to determine the effect of calcium supplements on *Pleurotus florida* yield and Calcium levels, as well as compare its yield on substrate with and without wheat bran.

MATERIALS AND METHODS

Study environment

This study was carried out at the mushroom unit of the University of Port-Harcourt Demonstration Farm, Choba Port-Harcourt in Rivers State, Nigeria. It lies at latitude 4°53N and longitude 6°57E.

Substrate source

Sawdust, a mixture of hard wood of *Khaya anthotheca*, *Milicia excels* and *Gmelina arborea* was obtained from a mill at Rumuosi, nearby the University. The calcium supplements were product from Nutrigain Limited, Macclesfield, United Kingdom. The product names are Organic Mycroliquid (OML) and Organic Mycrowhitener (OMW). The calcium supplement act as nutrient sources as well as stimulating the metabolism of the growing mushroom and are based on patented calcium salts of carboxylic acids. The pure culture of the mushroom used for this study was that of PF001UPHNIG of the mushroom farm from the mushroom Bank in the faculty of Agriculture, University of Port-Harcourt.

Mushroom cultivation

Modified methods of Ofomana and Adedokun (2020)

were used. The pure culture of the mushroom was used to prepare the mushroom spawn on guinea corn (*Sorghum bicolor*) using standard methods (Stamets, 2000). The grain spawn was kept at room temperature $28\pm 2^{\circ}\text{C}$ until use.

Preparation of substrate

Two growth media were prepared as bulk substrate – Sawdust supplemented with wheat bran (SD + WB) and sawdust only (SD). Sawdust, wheat bran, calcium carbonate (CaCO_3) and water were thoroughly mixed together in ratio 12:1:0.13:5 for substrate with wheat bran supplement. The substrate with no wheat bran supplement had a ratio of 12:0.13:5 for mixture of sawdust, lime and water respectively. Water was added till a moisture content of about 60% was attained. 1kg of the mixture was weighed into a transparent polyethylene bag with dimensions $43 \times 21 \times 43 \text{ cm}^3$ replicated three times.

Sterilization of Substrates Bags

Sterilization of substrates bags was by using a fabricated sterilization drum. Heat was applied for 4 hrs. at a temperature of 100°C .

Inoculation and Incubation

Sterilized bags were inoculated with already prepared grain spawns in an inoculation chamber. Fully ramified grain spawn (2.5%, w/w) was used for inoculating sterilized bags. Inoculated bags were incubated at $28\pm 2^{\circ}\text{C}$, for 4 weeks, after which they were transferred to the fruiting room.

Application of Calcium supplement

After one month of incubation, when the bags were fully ramified with the mycelia of the mushroom, the calcium supplements were applied prior to fruiting using four different ratios of Ca:water (v/v). The ratios were 1:1, 1:5, 1:10, and 2:1, prepared with sterile water. The mixture was applied into the ramified substrate bags using a 10 mL syringe. The center was bored with a sterile syringe and 20 mL of each prepared ratio was applied to the bored center of the substrate bags. Experiment was set up as 2×5 factorial replicated 3 times in Completely Randomized Design.

Data collection

Yield data collected was the fresh weight and dry weight.

Fruiting and harvesting

Substrate bags, after about a week of calcium application

were transferred to the fruiting room and opened to initiate fruiting, through sprinkling of water on the bags. Primordia started forming from about 5 ± 2 days; sporophores (fruiting bodies) were harvested by hand-twisting, weighed with electronic digital balance and dried in a fabricated solar dryer of temperature $48 \pm 2^\circ\text{C}$ for 4 days. When constant weight was observed, the dried samples were kept in air-tight envelopes and taken to the laboratory.

Calcium analysis

Mineral (calcium) analysis was determined using the standard AOAC (2005) procedure.

Data analysis

Data obtained was run on GENSTAT 12th Edition software using Two-way Analysis of variance (ANOVA) program. The means were separated using Least Significant Difference (LSD) at 5% probability level.

Results

Full ramification of substrate bags was observed within 28 ± 2 days. Substrate bags which were treated with calcium supplements started showing primordial formation 4 ± 2 days after application.

Effect of calcium supplements and ratios on mushroom total yield in SD growth medium

Presented in Table 1 is the total yield, fresh weight of *P. floridanus* when supplemented with calcium OML and OMW in SD growth medium at different ratios. For Ca OML, highest yield was for ratio 1:10 while for Ca OMW, ratio 2:1 had the highest yield. There was no significant difference ($p > 0.05$) between the means of the calcium types, neither the different ratios used nor any interaction amongst the calcium types.

The trend is similar in the total yield for dry weight in the SD growth media (Table 2), with calcium OMW at ratio 2: 1, having the highest yield with mean, 4.05 g while the control had the lowest with mean, 1.47 g.

Effect of calcium supplements and ratios on mushroom total yield in SD+WB growth medium

Tables 3 and 4 show the total fresh and dry weight yield respectively of *P. floridanus* in Sawdust supplemented with wheat bran growth medium. For OML, ratio 1:1 had the highest value while for OMW the highest value (5.56 g) was recorded for ratio 1:10, with a significant difference

Table 1. Total yield of fresh weight of mushroom for Ca OML and OMW in SD medium

Ca Types (CaT)	Ratios of Ca Supplements (RCaS)					Means of CaT (g)
	0	1:1	1:5	1:10	2:1	
OML (g)	14.0	16.8	17.7	25.0	12.4	17.2
OMW (g)	14.0	17.6	26.7	22.9	38.2	23.9
Means of RCaS (g)	14.0	17.2	22.2	23.9	25.3	

LSD ($P = 0.05$; SE (standard error of difference of means)

CaT = 20.1 (SE = 9.6)

RCaS = 31.8 (SE = 15.2)

Interaction (CaT x RCaS) = 45.0 (SE = 21.5)

Table 2. Total yield of dry weight of mushroom for Ca OML and OMW in SD medium

Ca Types (CaT)	Ratios of Ca Supplements (RCaS)					Means of CaT (g)
	0	1:1	1:5	1:10	2:1	
OML (g)	1.47	2.00	1.96	2.75	1.42	1.92
OMW (g)	1.47	2.13	3.26	2.12	4.05	2.60
Means of RCaS (g)	1.47	2.06	2.61	2.43	2.74	

LSD ($P = 0.05$; SE (Standard error of difference of means)

CaT = 1.97 (SE = 0.94)

RCaS = 3.12. (SE = 1.49)

Interaction (CaT x RCaS) = 4.42 (SE = 2.12)

Table 3. Total yield of fresh weight of mushroom for Ca OML and OMW in SD+WB medium

Ca Types (CaT)	Ratios of Ca supplement (RCaS)					Means of CaT (g)
	0	1:1	1:5	1:10	2:1	
OML	17.0	44.7	29.2	35.7	25.9	30.5
OMW	17.0	35.1	35.8	52.8	43.7	36.9
Means of RCaS	17.0	39.9	32.5	44.2	34.8	

LSD ($P = 0.05$; SE (Standard error of difference of means)

CaT = 12.7 (SE = 6.1)

RCaS = 20.1 (SE = 9.6)

Interaction (CaT x RCaS) = 28.5 (SE = 13.6)

over control with mean value 1.29 g. However, there was no significant difference ($p > 0.05$) between the two calcium types. The yield in substrates with wheat bran was higher than substrates that only had sawdust as growth medium. Table 5 indicated calcium analysis of the fruiting bodies for ratio 2:1 treatment. Calcium contents were higher for treatments with calcium (OML or OMW) addition. For both substrates used, calcium content increased more with addition of calcium OML (Table 5).

Table 4. Total yield of dry weight of mushroom for Ca OML and OMW in SD+WB medium

Ca Types (CaT)	Ratios of Ca Supplements (RCaS)					Means of CaT (g)
	0	1:1	1:5	1:10	2:1	
OML (g)	1.29	4.79	2.73	3.19	2.64	2.93
OMW (g)	1.29	2.75	3.30	5.56	3.43	3.27
Means of RCaS (g)	1.29	3.77	3.02	4.37	3.04	

LSD (P = 0.05); SE (Standard error of difference of means)

CaT = 1.30 (SE = 0.62)

RCaS = 2.06 (SE = 0.99)

Interaction (CaT x RCaS) = 2.92 (SE = 1.40)

Table 5. Calcium analysis of *Pleurotus floridanus*

Sample Identity	Ca (Mg/Kg)
SD Control	135.31
WB Control	93.81
SD + OML (2:1)	194.02
SD + OMW (2:1)	179.22
SD + WB + OML (2:1)	358.72
SD + WB + OMW (2:1)	325.49

DISCUSSION

The two calcium types improved mushroom yield and this could explain why there was no significant difference observed in their applications. The lower yield in sawdust only substrates compared with yield in sawdust supplemented with wheat bran, could be explained by the presence of the wheat bran supplement. This underscores the importance of supplementation in mushroom growing and agrees with the reports of Carrasco *et al.*, (2018) and Ofomana and Adedokun (2020). The higher yield in sawdust supplemented with wheat bran substrates when calcium supplements were applied indicated the importance of the calcium supplementation in yield increase. This agrees with the findings of Salami and Bankole (2017) who reported that substrate supplementation with calcium increases yield in mushroom production.

In addition, the fact that the yield for Sawdust supplemented with wheat bran growth medium was significantly higher at ratios 1:10 whereas, for the sawdust only medium, there was no significant difference in all the ratios used, indicated the combined supplements effect. This agrees with the work of Carrasco *et al.*, (2018) who explained that a combination of supplements in mushroom can improve yield and quality. Thus, a combination of

sawdust supplemented with wheat bran and any of the calcium types OML or OMW at ratio 1:10 can improve mushroom production.

Even without the supplementation with calcium, the control substrate contained some amount of calcium. However, calcium levels were increased when calcium (OML) and (OMW) were applied to SD medium, and was further increased when added to the SD and WB growth medium. This concurs with the work of Chiu *et al.*, (1998) who stated that calcium increases yield and nutritional values of edible mushroom. They also explained that the general effects of increasing the calcium content in edible mushrooms is favored as calcium is a necessary macronutrient for humans. Daily consumption of 2–3 g (D.W) of the oyster mushroom fruited in calcium-supplemented compost will meet the recommended dietary allowances for calcium (0.8–1.3 gd^{-1}) by the Food and Nutrition Board, U.S.A. (Chiu *et al.*, 1998). This is approximately between 25–35g (F.W). The use of calcium supplement being an organic source is safer to use for increasing yield and less susceptible to feed other competitor moulds, making it a helpful tool to growers.

Calcium supplements increased yield in the sawdust/sawdust supplemented with wheat bran growth medium. Calcium OML and OMW, at ratio 1:10 yielded better in the wheat bran growth medium. Calcium levels were also increased in the mushroom fruit as indicated in the mineral analysis.

To that end, a combination of Sawdust supplemented with wheat bran and Calcium supplements is recommended in the biofortification of mushroom. This is good news for mushroom producers as they are interested in increased yield so as to maximize profit. The use of these supplements serves dual purpose – increased mushroom yield and increased Calcium levels in the mushroom. Hence, the findings of this work can be harnessed to produce high nutritional value mushrooms as well as ensure higher yields.

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