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Phosphorus biofortification of *Pleurotus floridanus* Singer and its effect on fruit body yield and phosphorus bioavailability

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ABSTRACT

Phosphorus is an essential mineral needed for the growth, maintenance, and repair of all tissues and cells. This study was designed to biofortify *Pleurotus floridanus* with phosphorus supplements and assessed its effect on the yield and phosphorus contents. Phosphorus supplement was added to mushroom growth media. The experiment was set in Completely Randomized Design with five replicates. The result showed a positive effect of phosphorus supplementation on the number of mushroom fruit bodies, duration to fruiting and harvesting, and wet & dry weight of fruit bodies. Proximate analysis of treated samples had lower carbohydrate, higher fibre, lower lipid compared with the normal control. Phosphorus content was higher in control compared with treated samples. Conclusively, P is an essential element and it may be important to use the appropriate type of phosphate sources to ensure its availability.

Keywords: Mushroom, *Pleurotus floridanus*, phosphorus, biofortification, bioavailability

Phosphorus is a non-metal chemical element that belongs to the nitrogen group in the periodic table. Phosphorus is a highly reactive element and therefore does not occur in nature as a free element but as inorganic phosphatic (PO_4) rocks and as part of many minerals (Anderson, 2018). Most of the phosphorus in the human body is found in the form of hydroxyapatite [$Ca_{10}(OH)_2(PO_4)_6$] in bone and teeth, the rest is found in organs and extracellular fluid (EFSA, 2015; Kalantar-Zadeh *et al.,* 2010). Recommended intakes of minerals for optimal health often include a lower and safe upper intake limits (Anderson, 2018). Phosphorus serves a variety of functions in living system. Cell membranes are composed of phospholipid molecules, which due to its negative charge contribute to the repulsive forces that organize the membrane into lipid bilayers (Elser, 2012). Phosphorus also helps in cell energy cycle by acting as part of the adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) molecules (Abrahamsson *et al.,* 2013). Anderson, (2018) reported that the phosphorylation of glucose and proteins is crucial for various transport mechanisms and metabolic processes such as glycolysis, oxidative phosphorylation and urea cycle. Several vitamins such as thiamine (B1) and pyridoxine (B6) are activated through phosphorylation.

Phosphorus deficiency known as hypophosphatemia can lead to mineral and bone metabolism disorder (Chang and Anderson, 2017) and

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is strongly associated with increased cardiovascular disease and mortality. Phosphorus deficiency in the body can manifests through poor appetite, anemia, muscle weakness, bone pain, bone disease (osteomalaia and rickets) and increased susceptibility to infections.

In fungal metabolism also, phosphorus play a key role in general and mushroom in particular as it is a part of cytoskeleton, phospholipids, energy transfer molecules, intermediary metabolism, nucleic acid and factors or part of co-enzymes in enzyme system (Griffin, 1981; Treschow, 1944). Phosphorus deficiency in liquid cultures caused greater inhibition of mushroom mycelium growth than any other mineral deficiency (Styer, 1928). Watson (1973) showed that a large proportion of nitrogen in liquid culture medium could not be taken up by the mycelium as long as phosphorus was limiting. Addition of inorganic phosphate invariably increased the mycelial growth rate, extracellular enzymes and protein production in different mushrooms (Kamal *et al.*, 2012).

Biofortification is an enrichment process to enhance nutritional quality of food crops using agronomic practices, conventional plant breeding, or modern biotechnology (WHO, 2019). According to Odiketa *et al.*, (2020), it presents a way to reach populations where supplementation and conventional fortification activities may be difficult to implement and/ or limited.

The aim of this study was to examine the impact of phosphorus supplementation on the yield of *P. floridanus* as well as investigate its effect on the phosphorus content of the fruiting bodies.

MATERIALS AND METHODS

Description of the study area

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 4’53" N and longitude 6’57" E with average temperature of 27° C, relative humidity of 78% and average rainfall that ranges from 2500- 4000 mm (Nwankwo and Ehirim, 2010).

Substrate source

Sawdust was obtained from a saw mill at Rumuosi, nearby the University. The phosphorus supplement was a product from Nutrigain Limited, Macclesfield, United Kingdom. The phosphorus supplement act as nutrient sources as well as stimulating the metabolism of the growing mushroom. The pure culture of the mushroom used for this study was obtained from the mushroom Bank in the faculty of Agriculture, University of Port-Harcourt.

Mushroom cultivation

The oyster mushroom was cultivated following a modified method of Odiketa *et al.*, (2020). 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±2^{o}C until use.

Experimental procedure

Sawdust with wheat bran and lime were mixed together in a dried form before adding water to it to obtain a moisture percent of 70%. All the ingredients were thoroughly mixed. One kg of the mixed substrate was then placed in polypropylene bags with dimensions 43 × 21 × 43 cm^3 replicated five times.

Sterilization of substrates bags

Sterilization of substrates bags was done by using a fabricated sterilization drum. Heat was applied for 4 hours at a temperature of 100^{o}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±2°C, for 4 weeks, after which they were transferred to the fruiting room.

Application of phosphorus supplement

After one month of incubation, when the bags were fully ramified with the mycelia of the mushroom, the phosphorus supplements were applied prior to fruiting using two different ratios of phosphorus:water (w/v). The ratios were 1:5 and 1:10, 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 30 mL of each prepared ratio was applied to the bored center of the substrate bags. This procedure of application was also done for positive control but with sterile water only. Experimental design was Completely Randomized Design with five replicates.

Fruiting and harvesting

Substrate bags, after about a week of phosphorus 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±2° C for 4 days. When constant weight was observed, the dried samples were kept in air-tight envelops and taken to the laboratory.

Proximate and metal content analysis of mushroom fruit bodies

Proximate and metal analysis was formed using standard procedures (AOAC, 2005).

Data collection and analysis

Yield data collected was the fresh weight and dry weight of the mushroom fruit. Data obtained was statistically analyzed using 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

Effect of treatments on duration to fruiting

Duration of fruiting was observed to be fastest in treatments supplemented with phosphorus . The longest days to fruiting was observed in the control (Table 1). In the control treatment, fruiting took 54 days for first flush while in phosphorus treatments it was in the range of 45 to 47 days. Fruiting on the treatment P 1:5 and P 1:10 in the first flush was quickest. For the first flush, days to fruiting of mushroom were not significantly different for treatments P 1:5, P 1:10 and PC 1:5 while was significantly different in control.

Effect of treatment on first harvest days

There was a similarity in the trend of results for duration to fruiting and harvesting. The control treatment had the longest duration to harvesting. Mushrooms cultivated on the treatment P 1:5, P 1:10 and PC 1:10 were harvested first on the first flush (Table 2). For the first flush, duration to harvesting of mushroom was not significantly different for treatments P 1:5, P 1:10 and PC1:5. Harvesting duration for treatment PC1:10 and control was not significantly different, but significantly different from other treatments.

Effect of treatments on number of fruit bodies

Table 3 shows the effect of treatments on the number of fruit bodies. There was a general reduction

Table 1. Effect of the treatments on mushroom duration to Fruiting

Treatment /Flushes	Flush 1	Flush 2	Flush 3
T-1	45.00±3.56^{g}	65.00±6.11^{e}	80.00±4.07^{bc}
T-2	46.00±6.20^{h}	72.00±7.16^{d}	84.00±9.90^{ab}
T-3	46.00±6.24^{h}	62.00±10.57^{fg}	82.00±10.42^{ab}
T-4	47.00±4.38^{gh}	64.00±9.05^{e}	79.00±9.13^{bc}
T-5	54.00±12.07^{f}	74.00±6.20^{cd}	87.00±7.64^{a}
LSD (P≤0.0.5)	6.46	6.46	6.46

Treatments (T1-Phosphorus 1:5; T2-Phosphorus 1:10; T3- Positive control (basal without p element 1:5); T4-Positive control (basal without p element 1:10); T5-Normal control

Table 2. Effect of the treatments on mushroom duration to harvest

Treatment /Flushes	Flush 1	Flush 2	Flush 3
T-1	49.00±3.53^{e}	69.00±5.91^{c}	82.00±5.59^{b}
T-2	49.00±6.42^{e}	66.00±11.21^{c}	84.00±12.20^{ab}
T-3	49.00±6.59^{e}	65.00±10.74^{c}	80.00±14.91^{b}
T-4	51.00±4.74^{de}	65.00±9.14^{c}	82.00±8.97^{b}
T-5	57.00±12.34^{d}	77.00±6.24^{b}	91.00±7.59^{a}
LSD (P≤0.0.5)	7.55	7.55	7.55

Treatments (T1-Phosphorus 1:5; T2-Phosphorus 1:10; T3- Positive control (basal without p element 1:5); T4-Positive control (basal without p element 1:10); T5-Normal control

Table 3. Effect of the treatment on mushroom numbers of fruit bodies

Treatment /Flushes	Flush 1	Flush 2	Flush 3
T-1	11.70±5.12^{cde}	8.70±3.74^{defg}	5.20±1.55^{fg}
T-2	18.70±12.61^{a}	8.30±5.33^{defg}	6.20±3.79^{efg}
T-3	18.40±12.05^{ab}	10.90±6.38^{def}	6.40±1.96^{efg}
T-4	12.70±8.26^{bcd}	8.80±2.35^{defg}	6.10±3.03^{efg}
T-5	17.50±8.44^{abc}	7.30±6.98^{defg}	4.20±1.87^{g}
LSD (P≤0.0.5)	5.81	5.81	5.81

Treatments (T1-Phosphorus 1:5; T2-Phosphorus 1:10; T3- Positive control (basal without p element 1:5); T4-Positive control (basal without p element 1:10); T5-Normal control

of number of fruit bodies from flush 1 to 3 for all treatments. However, the reduction of number of fruit bodies from the first to the third flush was more pronounced in the normal control treatment. During 1st flush, the number of mushrooms in treatments with phosphorus 1:10 was significantly different from number of mushrooms with treatments Phosphorus 1:5 and control. There is no significant difference between result for phosphorus 1:10 and the other treatments, however, the value for number of fruiting bodies in phosphorus 1:10 treatment was higher than other treatments. In 2nd flush, there was a general decrease

in number of mushrooms compared to flush one, also number of mushrooms in control was significantly difference from the four other treatments. In Flush 3 also, same trend was followed, however, number of fruit bodies on the control with no supplement has the lowest number of fruit bodies.

Effect of treatments on Mushrooms Fresh Weight

Effects of treatments on fresh weight is shown in Table 4. The result shows that treatment Phosphorous 1:5 and Phosphorus 1:10 has significant effect on the first flush of the mushroom fresh weight. For the second flush, no significant effect was seen on the mushroom fresh weight with the application of all the treatments. The third flush result also shows no significant effect on the application of the treatments on fresh weight of mushroom.

Effect of treatments on mushroom dry weight

There was a general reduction in dry weight of fruit bodies from 1st to 3rd flush. Although the maximum dry weight was observed to be maximum in phosphorus supplemented substrate (1:5) but statistically all the treatments were at par in flush 1. In flush 2, P1:10 was significantly different from all other treatments including the control.

Effect of treatments on Phosphorus content and Proximate Analysis

Result in Table 6 presents the result obtained for Phosphorus content and Proximate Analysis. The

Table 4. Effect of treatments on mushrooms fresh weight (g)

Treatment /Flushes	Flush 1	Flush 2	Flush 3
T-1	106.80±21.97^{a}	43.50±15.88^{cde}	30.90±10.84^{ef}
T-2	103.80±35.95^{a}	45.80±18.56^{cde}	37.40±20.86^{cdef}
T-3	88.30±21.40^{ab}	53.20±29.69^{c}	40.80±15.89^{cde}
T-4	73.60±23.59^{b}	50.50±28.36^{cd}	31.80±14.39^{def}
T-5	91.50±21.12^{ab}	36.70±18.57^{cdef}	21.50±8.61^{f}
LSD (P≤0.0.5)	18.83	18.83	18.83

Treatments (T1-Phosphorus 1:5; T2-Phosphorus 1:10; T3- Positive control (basal without p element 1:5); T4-Positive control (basal without p element 1:10); T5-Normal control

Table 5. Effect of the treatment of mushroom dry weight (g) of fruit

Treatment /Flushes	Flush 1	Flush 2	Flush 3
T-1	15.20±3.19^{a}	9.40±3.72^{cd}	5.10±2.51^{e}
T-2	11.70±6.02^{bc}	5.90±3.03^{e}	4.90±3.70^{e}
T-3	10.70±4.30^{cd}	5.80±4.48^{de}	5.80±2.97^{e}
T-4	9.70±4.69^{cd}	7.50±3.65^{de}	5.40±2.95^{e}
T-5	14.50±4.55^{ab}	5.90±3.00^{e}	4.30±2.58^{e}
LSD (P≤0.0.5)	3.46	3.46	3.46

Treatments (T1-Phosphorus 1:5; T2-Phosphorus 1:10; T3- Positive control (basal without p element 1:5); T4-Positive control (basal without p element 1:10); T5-Normal control

Table 6. Effect of treatment in Mushroom Phosphorus content and proximate analysis

Treatments	% Ash	% CHO	% Fibre	% Lipid	% Protein	% Moisture	Phosphorus Content (mg/kg)
T-1	4.75±0.00[a]	26.57±0.04[e]	24.05±0.30[a]	0.90±0.01[c]	33.03±0.22[d]	9.82±0.59[a]	133.73±0.66[d]
T-2	2.79±0.00[c]	36.35±0.32[a]	20.02±0.18[b]	0.30±0.00[e]	32.38±0.00[e]	8.26±0.14[a]	129.95±0.00[c]
T-3	1.54±0.04[d]	30.02±0.02[c]	25.05±0.83[a]	0.50±0.00[d]	34.56±0.00[c]	8.35±0.78[a]	65.44±0.04[e]
T-4	1.45±0.05[e]	28.43±0.01[d]	25.28±0.98[a]	1.20±0.00[b]	35.22±0.22[b]	8.42±0.81[a]	194.46±0.10[a]
T-5	4.56±0.03[b]	32.02±0.08[b]	16.63±1.50[c]	1.60±0.00[a]	37.19±0.00[a]	7.99±1.62[a]	142.81±0.07[b]
LSD (P≤0.0.5)	0.06	0.25	1.77	0.005	0.23	1.88	0.58

Treatments (T1-Phosphorus 1:5; T2-Phosphorus 1:10; T3- Positive control (basal without p element 1:5); T4-Positive control (basal without p element 1:10); T5-Normal control

result shows that all the parameters were significantly different from the normal control except for moisture content. Mushroom treated with the phosphorus supplement had lower CHO, higher fibre, lower lipid compared with the normal control. The ash content was highest for treatment P 1:5 and significantly different from all other treatments while protein was highest and significantly different from other treatments for the normal control. The phosphorus content was highest for PC 1:10 and significantly different from other treatments, next to it, was the content for the normal control.

DISCUSSION

The result obtained in this study showed the positive impact of phosphorus supplement on *P. floridanus.* Number of fruits wet weight and dry biomass were also observed to be the maximum in treated samples, although, statistically significant difference could not be observed in most of the results. The impact of the supplement on duration to fruiting and harvesting is noteworthy for the treated samples.

To some extent, results obtained in this study corroborates the findings of Odiketa *et al.* (2020) on biofortification of mushroom (*Pleurotus floridanus*) using calcium based supplements where it was shown that, two calcium types improved mushroom yield. The contrast in statistical differences may be as a result of the different types of supplements used in both studies.

Nevertheless, the result of Kamal *et al.* (2012) on effect of phosphate supplementation on growth and extracellular enzyme production by some edible mushrooms showed that there was significant effect on the yield of mushroom upon the supplementation of phosphorus. For this study, the non-significant effect of phosphorus supplement on the yield of mushroom could probably be due to the fact that higher P concentration i.e. more than 0.003 M was reported to have caused growth inhibition in *Leptomitus lacteous*. The reason for low yield of the mushroom on the supplementation of phosphorus could also be due to the chelation of the phosphorus into unavailable form. Using the appropriate type of phosphate sourced could have, however, ensured the availability of P for the mushroom for effective yield. This present study is however in alignment with the study of Seth and Shandilya (1975) on the effect of different quantity of superphosphate on the yield *of A. bisporus*. The probability of chelation which might have affected bioavailability of the phosphorus supplement to the mushroom could also explain the lower phosphorus contents in the treated samples.

For the proximate composition, treated samples had lower CHO, higher fibre, lower lipid compared with the normal control. These are positive impact on the treated mushroom. The highest ash content for treatment P 1:5 which was also significantly different from all other treatments showed the presence of minerals (Ismail, 2017). The low protein contents for treated samples compared with the normal control may also be connected with the earlier observation made on phosphorus non- availability. Of note is the moisture content of treated samples with P showing significant effect. This implies mushrooms are highly perishable. High moisture contents promote susceptibility to microbial growth and enzyme activity. This significant effect on the moisture content suggests that great care must be taken in the handling mushroom as high moisture contents promote susceptibility to microbial growth and enzyme.

CONCLUSION

This study examined the effect of Phosphorus supplementation on yield and content in *P.floridanus*. Phosphorus supplementation in *P. floridanus* showed promising effects in biofortification of the mushroom. Care must be taken however, to address the challenge of inappropriate concentration and phosphate type to ensure phosphorus availability to mushrooms. Further research on what phosphate type is compatible for absorption in mushroom mycelia and what element combinations are promising versus those that are not in increasing phosphorus content in biofortified mushrooms is suggested.

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