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# Amending Traditional Substrate Rice Straw with Agroforestry Tree Foliage Increases Production Cycle and Nutritional Value of *Pleurotus floridanus*

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**Abstract**

The recent heightened attention on mushrooms has not considered shortening the production time and increasing nutritional value using substrates from agroforestry trees in addition to traditionally used agro-industrial residues. This study therefore, examined harvesting time, protein and essential minerals of *Pleurotus floridanus* grown on rice straw amended with *Gliricidia sepium* foliage in Morogoro, Tanzania. Mushrooms were cultivated in 30 x 40 cm 4 kg dry weight polythene bags in randomized complete block design (RCBD) experiment. Results showed that the addition of 5% *G. sepium* into rice straw reduced total time between spawning and first harvest of *P. floridanus*. *Gliricidia sepium* increased significantly ($P < 0.05$) protein contents of the mushrooms by up to 40%. The addition of 5–10% *G. sepium* into rice straw increased significantly ($P < 0.05$) manganese, copper and zinc in the mushrooms. The decrease in production time and high increase in protein and mineral contents indicate that agroforestry trees have the potential to increase production cycles and nutritional value of *P. floridanus*. Therefore, use of foliage from agroforestry trees in combination with traditional substrates for mushroom production could help fight malnutrition, improve food security and enhance income.

**Keywords:** Food security; *Gliricidia sepium*; Income; Mushrooms; Smallholder farmers.

## Introduction

Although often not included in forest resource assessments, edible wild mushrooms (EWMs) form an important part of non-timber forest products (NTFP) and contribute to sustainable forest production and livelihoods (Imtiaj and Rahman 2008, Siwulski et al. 2019). About 60 species of EWMs occur in Tanzania (Buyck et al. 2000, Härkönen et al. 2003), miombo woodlands, forests, pasture land and agroforestry systems being the major habitats for most of the species. The EWMs make a remarkable addition to the daily diet of the rural communities since they appear at the beginning of rain season when crops from previous growing season have for the most part been used (Härkönen et al. 2003). In almost every ethnic group, people collect mushrooms for home consumption or for sale as income generating activity (Tibuhwa 2013). Unfortunately, continued degradation of natural habitats jeopardizes the continual existence of many EWM species. Moreover, wide use of EWMs in the diet is limited by seasonal occurrence, inadequate knowledge on

nutritional values, capacity to domesticate and edibility of different mushroom species.

Although the cultivation of saprophytic EWMs is a relatively new enterprise in Tanzania, small holder to medium entrepreneurs are increasingly engaged in mushroom cultivation to increase crop diversity, generate income and enhance families' nutrition. In fact, mushrooms are an emerging new high value cash crop with great potential to reduce poverty, unemployment, food insecurity and environmental degradation among sub-Saharan African countries, including Tanzania (Mshandete 2011). Because mushroom cultivation does not require large capital, agricultural inputs and intensive labour, it can be carried out by young and old, males and females and in groups and individually in rural and urban areas within the usual agroforestry settings (Kivaisi 2007). Oyster mushrooms (*Pleurotus* spp.) are the most popular cultivated saprophytic mushrooms on various agro-industrial residues using different technologies (Oei 1991, Mamiro and Mamiro 2011). Also, high value oyster mushroom species have been produced in small scales from forest residuals and fallen logs. Different types of lignocellulosic materials support growth of the oyster mushrooms but subsistence farmers commonly use agricultural crops available within their locality including rice straw, banana leaves, cotton waste, bean trash and sisal residues (Andrew et al. 2008). Being adapted to tropical conditions and also as primary decomposers, oyster mushrooms lend themselves to easy domestication as do not necessarily require composting, large area and special treatments (Oei 1991, Mshandete and Cuff 2007). Mushrooms feed by decomposing and absorbing nutrients from the surrounding environments and thus their nutritional contents and mineral composition can be improved by altering substrates in which they grow. Unfortunately, the recent increase in mushroom production has not considered increasing nutritional contents and production cycles using cheap and available substrates from agroforestry trees in addition to traditionally used agro-industrial residues in Tanzania.

Agroforestry trees are widely known to increase nitrogen (N) in the soil, and therefore, improve soil fertility and crop yields (Dollinger and Jose 2018). In the present study, N rich foliage from an agroforestry tree species *Gliricidia sepium* was used in combination with traditional growing substrate rice straw to cultivate mushrooms. The apparent high quality of *Gliricidia* foliage combined with their high and sustainable biomass production can make *Gliricidia* suitable substrates for mushroom production. Apart from N, other mineral elements such as phosphorus and potassium absorbed by roots from deep soil layers and become incorporated into the foliage can be realized when the foliage is used as a growing substrate. Therefore, the overall objective of this study was to reduce cultivation period and increase the nutritional values of *Pleurotus floridanus* mushrooms by incorporating foliage of *G. sepium* into traditionally growing substrate rice straw. Specific objectives were to (i) evaluate the effects of the amendments on the duration it takes for the spawn to colonise substrates sufficiently to start harvesting *P. floridanus*, and (ii) determine the amount of protein and zinc, copper, magnesium, potassium, manganese and calcium due to amendments of rice straw with *G. sepium* foliage. Findings from the study will help to increase production cycles and nutritional value of cultivated mushrooms to fight malnutrition, improve food security and income particularly for the rural and peri-urban farmers.

## Materials and Methods

### Study area and mushroom spawn

The study was conducted at Solomon Mahlangu Campus of Sokoine University of Agriculture, Morogoro, Tanzania situated on the western side of the Uluguru Mountains within latitudes 37°15'E and 37°42'E and longitudes 6°45'S and 7°00'S (Figure 1). The climate is sub-humid, average temperature is 25°C and the relative humidity ranges from 70

to 80%. Spawn of *P. floridanus* with origin from Mauritius was obtained from Uyole Agricultural Research Institute, Mbeya. The spawn of *P. floridanus* was prepared with sorghum grain which is abundantly available in Tanzania.

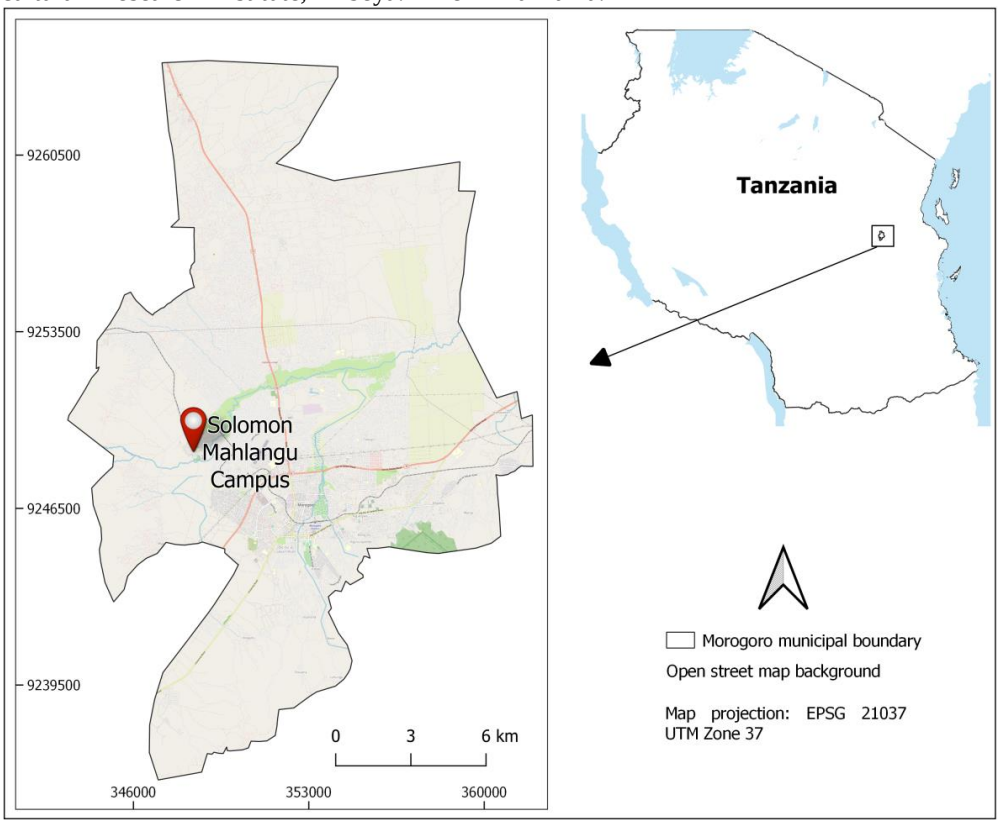


**Figure 1:** Map showing location of the study area in Morogoro, Tanzania.

**Experimental design and preparation of substrates**

Pre-test experiment was conducted to determine appropriate combinations of *Gliricidia* foliage and rice straw at which *P. floridanus* could grow. The experiment involved 25%, 50% and 75% concentrations of *G. sepium* foliage. The only level of rice straws amended with *Gliricidia* that gave out mushrooms was that which contained 25% *Gliricidia* foliage. The other levels of amended substrates did not grow mushrooms. Thereafter, the mushrooms were grown on rice straw with no foliage (as control or un-amended) and rice straw supplemented (amended) with different and low concentrations of *Gliricidia* foliage. Rice straw and foliage were obtained from the University farm and were dried before being processed. The dried rice straws and foliage were chopped into small pieces of 2-6 cm long to enhance colonization by *P. floridanus* (FAO 1990). The substrate was pasteurized by boiling for two and a half hours, excess water drained off and cooled on wire sieves. The rice straws were then mixed with different ratios of *Gliricidia* foliage (i.e. 5%, 10%, 15%, 20% and 25%), which made five treatments and a control (0%). The prepared substrates were put in polythene bags measuring 30 cm wide and 40 cm long, capable of accommodating 4 kg of substrate. Spawn of about 62 g per 4 kg of substrate was added in layers in the polythene bags. This technique reduces chances of contamination and takes shorter time for

mycelia to colonize the substrates. The bags were tied on top, and 6 holes of about 1.5 cm were made on the bags to facilitate air circulation and emergence of mushroom pins. The bags were put in dark room which was kept humid by pouring 5 litres of water daily and the temperature ranged from 25 to 30 °C. After 9–12 days, the mycelia had fully colonized the substrates. The bags with fully colonised substrates were subjected to fructification conditions (i.e. light, temperature of 25–27 °C and relative humidity of 35–75%) in well ventilated growing room and arranged on disinfected shelves. This was done to initiate pinheads formation, while at the same time lowering carbon dioxide concentration. In addition, pans filled with water were placed in the growing room and the bags were watered 2 to 3 times a day to maintain optimum conditions throughout the cropping period. In the growing room, the experiment was laid out in a randomised complete block design (RCBD). There were four blocks and each had 5 treatments and a control, which were replicated six times. One or two days after emergence, mushrooms were harvested for the laboratory analyses of protein and essential minerals. Time between spawning and full colonization and full colonization to first harvest were recorded for every treatment and replication.

**Proximate determination of protein and mineral contents**

Fresh mushroom samples from the first harvests were collected from each treatment and replication and oven dried to constant weight at 60 °C for about 48 hours in the laboratory of Food Science and Technology, Sokoine University of Agriculture, Morogoro Tanzania. The dried mushroom samples were cooled in desiccators, ground and passed through 1.5 mm sieve for further analyses. Protein and essential minerals contents analyses followed Association of Official Analytical Chemists protocol (AOAC 2002). Each analysis was replicated four times and computations were based on dry weight basis. The crude protein was computed from N as

$$N = \frac{14.01 \text{ x } (\text{Titre value mls} - \text{blank value mls}) \text{x Conc. of acid used}}{\text{Sample weight used } x\ 10}$$

and CP = N x 6.25; where N = Nitrogen (%) and CP = Crude protein (%).

Six essential minerals, i.e., zinc, copper, magnesium, potassium, manganese and calcium were analysed and their contents determined. The mineral contents (mg/l) were read from an atomic absorption spectrophotometer, subtracted the blank reading (mg/l) and multiplied by their respective dilution factor to obtain final readings in parts per million (ppm).

**Data analysis**

Data for time period (days) between spawning to full colonization and full colonization to first harvest together with data for crude protein (%) and essential mineral contents (ppm) were summarized to obtain means, standard deviations and summations. All collected data were then examined for normality and homogeneity of variances as required for parametric test. The variations between treatments means for growing time, proteins and essential mineral contents of the mushrooms were determined using One-way Analysis of Variance (ANOVA) at 95% confidence level using Statistical Package for Social Sciences (SPSS) software version 16. Least Significant Difference (LSD) Test was used to separate means where ANOVA showed significant differences. Pearson correlation coefficients were computed to check for the relationships between the duration taken from spawning to colonization and colonization to first harvest at 95% confidence level ($\alpha$ = 0.05). Final results were presented in texts, figures and tables.

## Results

### Duration from spawning to colonization and first harvest

The duration from spawning to colonization and colonization to first harvest differed among the six levels of *Gliricidia* used in this study (Figure 2).

Mushrooms grown on rice straw amended with 5% *Gliricidia* foliage took shorter time from spawning to colonization than those with 0%, 10%, 15%, 20% and 25% (Table 1). However, significant difference ($P < 0.05$) in time was evident only between 5% and 20% concentrations. The 20% concentration took 10.5 days, about a day longer than the 5% concentration. The rice straw amended with 25% *Gliricidia* took significantly ($P < 0.05$) longer time from colonization to first harvest than the other 5 levels which did not differ significantly ($P > 0.05$). On average, the 5% concentration of *Gliricidia* took shorter time in both cases, with an average of 21.3 days from spawning to first harvest against 32 days for the 25% concentration. No significant correlation ($r = –0.11$, $P > 0.05$, df = 16) was found between duration taken from spawning to colonization and colonization to first harvest.

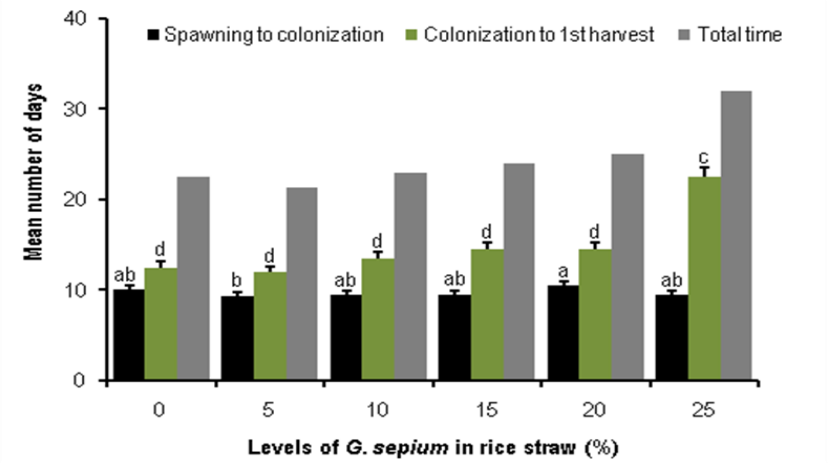


**Figure 2:** Duration taken from (i) spawning to colonization, (ii) colonization to first harvest, and (iii) total time from spawning to first harvest for rice straw amended with different levels of *Gliricidia sepium.* The values are denoted by the means ± SE. The different letters indicate that the mean values of number of days are significantly different ($P < 0.05$) among levels according to LSD test.

**Table 1:** Duration taken from spawning to colonization and first harvest for substrate amended with *Gliricidia sepium*

| Level of *Gliricidia* in rice straw | $T_1$ 0% | $T_2$ 5% | $T_3$ 10% | $T_4$ 15% | $T_5$ 20% | $T_6$ 25% | *F*-value | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Mean duration in days[1] | 10ab | 9.3b | 9.5ab | 9.5ab | 10.5a | 9.5ab | 4.57 | 0.04 |
| Mean duration in days[2] | 12.5b | 12b | 13.5b | 14.5b | 14.5b | 22.5a | 15.84 | 0.01 |
| Total duration in days[3] | 22.5 | 21.3 | 23 | 24 | 25 | 32 | | |

Means followed by a common letter in the same row are not significantly different at $P < 0.05$, Least Significant Difference test. Mean duration in days[1] = time from spawning to colonization; Mean duration in days[2] = time from colonization to first harvest; Total duration in days[3] = total time from spawning to first harvest.

**Protein contents of *Pleurotus floridanus***

The amounts of protein in mushrooms differed significantly ($P < 0.05$) as a result of applications of different concentrations of *Gliricidia* into the rice straw (Table 2). The protein contents in mushrooms grown in rice straw with addition of 5%, 10%, 15% and 20% *Gliricidia* foliage were generally high, but the means did not differ significantly ($P > 0.05$) among the levels. The control had the lowest protein content and differed significantly ($P < 0.05$) with all the other five concentrations. At higher concentrations of *Gliricidia* above 20%, the levels of protein contents declined sharply. There were significant ($P < 0.05$) increases in protein contents in the mushrooms amounting to 33%, 40%, 39% and 39% by amending rice straw with 5%, 10%, 15% and 20% *Gliricidia*, respectively (Table 2).

**Table 2:** Protein contents of *Pleurotus floridanus* mushrooms grown on rice straw amended with various levels of *Gliricidia sepium*

| Treatments<br>Levels of *Gliricidia* in rice straw | $T_1$<br>0% | $T_2$<br>5% | $T_3$<br>10% | $T_4$<br>15% | $T_5$<br>20% | $T_6$<br>25% | *F*-value | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Mean protein levels of mushroom | 22.4c (± 0.33) | 29.78a (± 1.63) | 31.32a (± 2.36) | 31.15a (± 0.24) | 31.19a (± 0.91) | 25.91b (± 0.11) | 29.37 | 0.03 |
| % increase in protein compared to $T_1$ | – | 33 | 40 | 39 | 39 | 16 | | |

Numbers in parentheses are standard deviations (SD) of the respective mean protein values. Means followed by a common letter are not significantly different at $P < 0.05$, Least Significant Difference test.

**Mineral contents of *Pleurotus floridanus***

The mineral contents of *P. floridanus* grown on rice straw amended with *Gliricidia* are presented in Table 3. Addition of 5% and 10% *Gliricidia* foliage into rice straw increased significantly ($P < 0.05$) the contents of manganese, copper and zinc in the harvested mushrooms. There were significant ($P < 0.05$) increases in contents of manganese, potassium, calcium and magnesium in the mushrooms following addition of 15% *Gliricidia* into rice straw. There were also significant ($P < 0.05$) and highest contents of zinc and copper at the concentrations of 20% and 25%, respectively. Manganese was the only mineral element that exhibited a smooth increase up to 15% addition of *Gliricidia* (Table 3). The content of zinc in mushrooms dropped sharply when the concentration of *Gliricidia* in rice straws exceeded 20%.

**Table 3:** Mineral contents of *Pleurotus floridanus* mushrooms grown on rice straw amended with various levels of *Gliricidia sepium*

| Levels of *Gliricidia* foliage in rice straw | $T_1$ 0% | $T_2$ 5% | $T_3$ 10% | $T_4$ 15% | $T_5$ 20% | $T_6$ 25% | *F*-value | *P*-value |
|---|---|---|---|---|---|---|---|---|
| Manganese (ppm) | 10.89d | 12.44c | 12.61c | 16.43a | 14.02b | 10.25d | 46.71 | <0.001 |
| Copper (ppm) | 7.81e | 10.9cd | 11.04bc | 8.59de | 13.28ab | 13.49a | 14.79 | <0.001 |
| Potassium (ppm) | 27630b | 27750b | 25750bc | 30050a | 25090c | 24490c | 11.38 | 0.001 |
| Zinc (ppm) | 43.58d | 50.14c | 47.25c | 55.46b | 61.41a | 35.61e | 88.79 | <0.001 |
| Calcium (ppm) | 29.83bc | 20.8c | 26.24bc | 73.58a | 32.35b | 29.48bc | 56.54 | <0.001 |
| Magnesium (ppm) | 144.6bc | 158.7ab | 148.7bc | 171.4a | 140.1c | 138.8c | 8.95 | <0.001 |

Means followed by a common letter in the same row are not significantly different at $P < 0.05$, Least Significant Difference test.

**Discussion**

Overall, amending rice straw with 5% *Gliricidia* shortens the duration between spawning to first harvest. The duration between spawning and first harvest is influenced largely by mushroom strain, spawn rate, substrate, sterilization method and growing conditions (Imtiaj and Rahman 2008). Amending substrates with different levels of *Gliricidia* is therefore, expected to alter the duration between spawning to first harvest. Our results which have recorded a range of 21 to 32 days corroborate well with several other studies which reported the duration of 20 to 90 days from spawning to first harvests (Mshandete 2011, Siwulski et al. 2019). However, this particular study benefits from the use of foliage from nitrogen-fixing trees as additives to the traditionally growing substrate as opposed to industrial nitrogen sources. The longest time taken to obtain first harvest was when higher concentration of *Gliricidia* was used. This was probably due to excess nitrogen from the *Gliricidia* foliage which inhibits growth of mushrooms (Bellettini et al. 2019). Imtiaj and Rahman (2008) reported that oyster mushrooms require less nitrogen source for optimum growth. The growth inhibition effect could be a reason for the mushrooms failure to grow in the pre-test experiments where 25%, 50% and 75% were used. In the pre-test experiments, the only concentration level that gave out mushrooms was 25% *Gliricidia* foliage implying that the 50% and 70% of *Gliricidia* foliage in the rice straw inhibited growth of the oyster mushrooms due to excessive nitrogen.

It is known that rice straw has high porosity and dries up very fast when used as mushroom growing substrate due to its physical nature. Therefore, addition of lignocellulosic agroforestry trees foliage increases water-holding capacity of the rice straws and decreases the mortality of young fruiting bodies due to moisture stress (Yang et al. 2013). Also, increasing levels of available nutrients has been reported to provide more energy for mycelia growth and primordial formation (Rizki and Tamai 2011), hence cut down waiting time for mushrooms to be harvested. In this case, mushroom farmers are able to carry out several production cycles within reasonable time by incorporating right concentrations of N-rich agroforestry trees foliage into the traditional substrate and hence generating more profit.

Edible mushrooms contain all essential amino acids for human nutrition and are therefore a good source of digestible proteins. The amounts of protein reported in this study for mushrooms from amended and un-amended substrates ranged from 22.4% to 31.3%. Edible mushrooms contain protein contents of 19-35% on dry weight basis (Mshandete and Cuff 2007), therefore, the range reported in this study corroborate with previous findings. The protein contents obtained in this study are higher than those of rice (7.3%), wheat (12.7%) and maize (9.4%) demonstrating that mushrooms are indeed nutritious foods and

should be consumed in addition to cereals. It is however, widely known that the protein contents of edible mushrooms vary with type of strain, species and growth substrate. Thus, amending rice straw with agroforestry tree foliage significantly increased nitrogen in the mushrooms. The highest mean protein content (i.e. 31.32 ± 2.36) was obtained when rice straw was amended with 10% *Gliricidia* foliage. Protein content of mushrooms declines when rice straw was amended with more than 20% of *Gliricidia*, indicating suppression of nitrogen uptake by mushrooms at high levels of *Gliricidia* in the substrate. The decline in protein might be caused by the excessive increase in nitrogen compounds by increasing the level of *Gliricidia* which increases acidity of the substrate. Since mushrooms are saprophytic fungi which operate under a certain range of pH (Visiteu 2004, Bellettini et al. 2019), decrease in pH by increasing substrate acidity may result into inefficient uptake of nutrients by mushrooms. It is also known that mushrooms prefer high carbon/nitrogen (C/N) ratio because they derive energy from carbon materials (Bellettini et al. 2019). Increasing the concentrations of *Gliricidia* foliage leads to further decrease of C/N ratio and thus decrease uptake of nutrients in mushrooms. The increase of 40% protein values demonstrates that *Gliricidia* highly improves the nutritional value of the mushrooms when amended with rice straw. Therefore, amending rice straw with 10% concentrations of *Gliricidia* can be used to elevate protein contents of *Pleurotus* mushrooms. Similar findings were also reported by Rajarathnam et al. (1986) and Andrew et al. (2008), that nitrogen supplementation of the substrate increased yield of *Pleurotus* mushrooms and the protein contents of the fruit bodies.

From this study, it is clear that *Gliricidia* foliage is a useful source of essential minerals when added on rice straws. The addition of both 5% and 10% *Gliricidia* into the rice straw substrate increased significantly the contents of manganese, copper and zinc in the mushrooms. Like many other food types, mushrooms are widely known to contain good amounts of minerals essential for normal functioning of the human body (FAO 1990, Valverde et al. 2015). However, mushrooms have the added advantage in that they have both macro and micro elements, and the fruiting bodies have high levels of assimilable mineral constituents (Mshandete and Cuff 2007, Valverde et al. 2015). In this study, *P. floridanus* has shown to have high levels of potassium (> 25000 ppm) as is the case for *Termitomyces* mushrooms (Munishi et al. 2008). Potassium regularizes heartbeats and improves oxygen supply to the brain and eventually relieves stress (Serunjogi 2005). The mineral contents recorded in this study fall within the reported ranges in other studies (FAO/WHO 1991, Mshandete and Cuff 2007). The high contents of minerals in mushrooms from amended substrates show that foliage from the agroforestry tree species such as the Gliricidia can be a useful source of essential minerals including copper, calcium, zinc, magnesium, manganese and potassium. However, to improve protein and many of the essential minerals in *P. floridanus* addition of 5-10% concentrations of *G. sepium* into rice straw is recommended. Therefore, improved value in cultivated mushrooms shows the importance of these agroforestry tree species in areas where protein diets are insufficient and could fight malnutrition. Most of the rural communities in sub-Saharan Africa can hardly afford balanced diets which are often based on staple crops with low protein and mineral contents (Towo et al. 2006, URT 2019). Thus, inclusion of agroforestry trees foliage in production of mushrooms could be used to address protein and mineral deficits in low income families as they offer the possibility and convert this into high value and protein rich food.

**Conclusion**

This study was designed to explore possibility of reducing production period and at the same time increasing the nutritional values of *P. floridanus* by incorporating *G. sepium* foliage into the traditional growing substrate rice

straw. Mushroom growers should utilize fully the available resources from agroforestry trees to supplement traditional growing substrates in order to obtain nutritious and more mushroom yields. To increase production cycle, protein and essential mineral contents, it is recommended that addition of 5–10% *Gliricidia* foliage into rice straw be considered during cultivation of *P. floridanus*. Thus, the techniques used in this study can be used to help fight malnutrition, improve food security and bolster income sources. Further research on more agroforestry tree species to optimize production and nutritive potentials of various substrate formulations even for small holder production of mushrooms is recommended.

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**Conflict of interest:** None.

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