Assessing the effect of sorghum spent grain (pito mash) supplementation on the growth performance and yield of *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer cultivated on cornstalks

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**ABSTRACT**

The viability of cornstalks as a substrate for *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer cultivation was studied. The effect of sorghum spent grain (pito mash), a nitrogen supplement, on growth performance and yield was also investigated. Three substrate formulae were used, viz., CSS-0 (100% cornstalks + 0% spent grains), CSS-5 (95% cornstalks + 5% spent grains), and CSS-10 (90% cornstalks + 10% spent grains). Most bags achieved complete ramification within 3 weeks after inoculation with spawns. In the second and third weeks, the mycelia growth rate of CSS-5 was significantly higher ($P < 0.05$) than CSS-0 and CSS-10. CSS-5 also produced the earliest appearance of primordia, and the lowest interval between flushes. Relative substrate nutrient distribution elicited significant differences ($P < 0.05$) in mushroom yields. The mushroom yields obtained after 6 weeks of harvesting were 118.4, 203.0 and 181.0 g for CSS-0, CSS-5, and CSS-10 respectively. The results of this study indicate that cornstalks are suitable substrates for *P. ostreatus* cultivation, and 5% sorghum spent grain supplementation is necessary for producing mushrooms with improved yields.

**Introduction**

Mushroom cultivation, one of the fastest growing biotechnological industries, provides one of the few examples of successful commercial processes based on lignocellulosic waste as substrate (Sánchez 2004). Apart from the high nutritional and medicinal values (Sadler 2003; Singh et al. 2012), mushroom fermentation also has the great advantage of converting industrial, urban and rural wood and other organic wastes into a product that is directly edible (Cohen et al. 2002).

Globally, the cultivation of *Pleurotus* spp. is second to only *Agaricus bisporus* cultivation (Sánchez 2010; Royse 2014). Compared to other edible mushrooms, *Pleurotus ostreatus* has the advantage of being able to rapidly colonise a wide range of substrates, converting a high proportion of them to fruiting bodies; and hence increasing profitability (Sánchez 2010). They are often very economical and simple to cultivate and are resistant to pest and disease attack (Patrabansh & Madan 1997). Also, *P. ostreatus* has low fat content and is rich in protein, calcium, phosphorus, iron, thiamine, riboflavin and niacin (Manzi et al. 1999).

Dundar et al. (2009) exploited *P. ostreatus* cultivation on wheat stalk, millet stalk, soybean stalk and cotton stalk. Diverse other lignocellulosic wastes have been exploited as substrates in the cultivation of *P. ostreatus* (Zhang et al. 2002; Owusu-Boateng & Dzogbebia 2005; Frimpong-Manso et al. 2011); with the choice of substrates dependent on their availability and cost (Balazs 1995; Labuschagne et al. 2000). However, despite being the world’s largest source of lignocellulosic biomass (Reddy & Yang 2005) little research has been carried out on the use of cornstalks as the main substrate for mushroom cultivation. The use of cornstalks as supplement was explored by Beltrán-García et al. (2001). In the study, it was observed that supplementing peptone glucose agar with milled cornstalks cleaned by diverse solvents doubled the mycelial growth rate of *P. ostreatus*. Hamza et al. (2002) successfully cultivated five oyster mushroom strains on cornstalks but the focus of their research was to improve cornstalk’s suitability for use as animal feed through biological...
Also, in an attempt to study the effect of various substrates on the chemical composition of mushroom, Bugarski et al. (2007) used a combination of diverse substrates including wheat straw 50% + corn stalks 50%, and observed that substrate composition has effect on the chemical composition of mushrooms. In a recent study by Adjapong et al. (2015), the viability of various maize residues supplemented with rice bran for the cultivation of P. ostreatus was explored. Cornstalks are annually renewable bio-resource of low economic value (Reddy & Yang 2005). In Ghana, corn is cultivated in all the agro-ecological zones (Duku et al. 2011) with a production of approximately 1.50 million tonnes in 2015 (IndexMundi 2016). Cornstalks, which are corn residues, are thus readily available, especially in the aftermath of corn farming seasons; coming at virtually no cost as a significant quantity of them are mostly either abandoned to rot on farms, burnt or discarded as refuse (Bernard & Prieur 2007; Otchere-Appiah & Hagan 2014).

Supplements are often added as co-substrate to serve as rich nitrogen source for mushroom mycelia. Many supplements have been studied elsewhere (Banjo et al. 2004; Mantovani et al. 2007; Alam et al. 2010). Sorghum spent grain, known locally in Ghana as “pito mash”, are by-products of low economic value produced from “pito”, a locally brewed beer from fermented sorghum grain (Asare-Bediako et al. 2014). In addition to its low price, sorghum spent grain is non-competitive and readily available (Asare-Bediako et al. 2014). It has been reported to contain between 18% and 42% crude protein (Adewusi & Ilori 1994; Adeyeye & Ajewole 1992; Holmes et al. 2013) and thus is a cheap and rich source of nitrogen. The gross chemical composition and the mineral element composition of sorghum spent grains are shown in Tables 1 and 2, respectively.

### Table 1. The gross chemical composition of sorghum spent grain (%).

| Nutrient      | Per cent composition |
|---------------|----------------------|
| Ash           | 4.46                 |
| Cellulose     | 11.4                 |
| Hemicellulose | 17.3                 |
| Lignin        | 9.35                 |
| Lipid         | 8.50                 |
| Protein       | 40.3                 |
| Starch        | 6.53                 |

Source: Holmes et al. (2013).

### Table 2. Concentration of some mineral elements in sorghum spent grains (%).

| P   | K   | Ca  | Mg  | Zn  | Fe  | Cu  | Cd  | Pb  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1.78| 0.07| 1.44| 0.58| 3.4 × 10^{-4}| 1.2 × 10^{-4}| 7.0 × 10^{-4}| 1.19 × 10^{-4}| 1.38 × 10^{-4}|

Source: Adewusi and Ilori (1994) and Ojeniyi et al. (2007).

This paper exploits the viability of cornstalks as a substrate for P. ostreatus (Jacq. ex Fr.) Kummer cultivation. The effect of sorghum spent grain supplementation on general growth performance and yield of this mushroom was also studied.

### Materials and methods

#### Preparation of spawn, substrate and compost

Sorghum grain spawns were prepared by maintaining cultures of P. ostreatus (Jacq. ex Fr.) Kummer strain EM-1, originally from Mauritius, on potato dextrose agar slants (Kortei et al. 2014). Outdoor single-phase solid-waste fermentation was used to prepare compost (Nair & Price 1991). Dried cornstalks, collected from farms in Angloga, Kumasi, Ghana, were chopped manually using cutlass and reduced to an average particle size of 0.8 cm² using grain mill. Sorghum spent grains were acquired at no cost from local pito brewers located close to the Kwame Nkrumah University of Science and Technology, Ghana. Three different formulae of cornstalks and sorghum spent grain were prepared (Table 3). Moisture content of formulated substrates was adjusted to approximately 68–70% (Obodai & Vowortor 2002) by sprinkling water on them. These were then heaped, covered with polythene bags and left for 3 days to compost.

#### Bagging and sterilisation

One kilogram each of substrate formula was delivered into heat-resistant polypropylene bags of approximate volume 650 cm³ and sterilised with moist heat in a large barrel at 95–100°C for 7 h. For each treatment, 15 replicates were used.

### Table 3. Substrate formula and codes used in the study.

| Substrate code | Substrate formula (composition by weight/g) |
|----------------|--------------------------------------------|
| CSS-0          | 100% cornstalks + 0% spent grain (1000:0)  |
| CSS-5          | 95% cornstalks + 5% spent grain (950:50)   |
| CSS-10         | 90% cornstalks + 10% spent grain (900:100) |

...
Inoculation, spawn run and cropping

After cooling, each bag was inoculated with 3 g of spawn under sterile conditions. Incubation was carried out in the spawn running room at 28–32°C until full colonisation of bags was achieved. In the cropping house, fully colonised bags were opened from the bottom of the neck by cutting with a sterile razor blade. Watering was done twice daily to ensure a humidity of 80–90% was maintained (Yildiz et al. 2002). Pores were created at the sides of each bag to permit drainage of excess water during watering. Mushrooms on appearance were pulled out gently, cutting off the base of the stalks onto which the compost adhered and removing small culls from the surfaces of the bags to prevent them from rotting and becoming contaminants.

Biological efficiency (B.E.)

This was calculated using the formula:

\[ B.E(\%) = \frac{\text{fresh weight of mushrooms}}{\text{dry weight of substrates}} \times 100 \]

Statistical analysis

One-way analyses of variance was used to analyse data obtained from the study and Duncan’s multiple range test with SPSS 23 (Chicago, IL, USA) was used to determine significant differences between variables.

Results and discussion

Reducing the size of cornstalks before composting increased the surface area for enzymatic degradation of lignocellulosic components into forms that were more easily utilised by mushroom mycelia (Hubbe et al. 2010). Growth of mycelia was observed three days after inoculation of substrates with spawns. P. ostreatus mycelia rapidly colonised the substrates with most bags achieving full colonisation within 3 weeks of inoculation with spawns. Relative substrate nutrient distribution elicited different mycelia growth responses after the first week. There was no significant difference \((P > 0.05)\) in mycelia length among the three substrate formulae in the first week. However, in the second and third weeks, mycelial growth was significantly influenced \((P < 0.05)\) by substrate formula: the mycelia length of CSS-5 (95% cornstalks + 5% spent grain) was significantly higher \((P < 0.05)\) than that of the other two formulae (Table 5). Overall, after 3 weeks of spawn run, the longest mean mycelia of 14.22 cm was recorded by CSS-5, followed by CSS-10 (90% cornstalks + 10% spent grain) with 13.16 cm mean mycelia length (Table 5). CSS-0 (100% cornstalks + 0% spent grain) recorded the shortest mycelia length of 12.66 cm. This low mycelia length recorded is however longer than the 8.1 cm reported by Kortei et al. when they cultivated P. ostreatus on 100% cassava peels after composting it for three days.

The ratio of carbon to nitrogen (C/N) in mushroom substrate is essential for optimal mycelia growth: a well-balanced C/N boosts mushroom growth and development while an imbalanced one inhibits growth (Stamets 2000). The “ideal” C/N ratio for optimal mycelial growth and fruiting body development is reported to be about 50:1 (Wang et al. 2015). The C/N ratio of CSS-5 is close to this value (Table 4), and thus accounts for the faster mycelial growth rates observed on this substrate formula. The C/N ratios of cornstalk and cassava peel are 65.67 and 44.82, respectively (Table 4; Baah et al. 2011). The difference in mycelia length recorded when P. ostreatus was grown on 100% cornstalks compared with 100% cassava peels is consistent with findings that a positive correlation exists between the C/N ratio of substrate and mycelium growth rate (Alborés et al. 2006).

Nitrogen-rich supplements are often added to optimise mycelia growth as they are required for cellular protein and enzyme synthesis. However, excess nitrogen leads to ammonia build-up which is toxic to mycelia, leading to decreased degradation of lignin and hence low mycelia growth (Rajarathnam & Bano 1989; Carlile et al. 2001). Many studies have reported the growth-limiting effect of addition of excess nitrogen sources during mushroom substrate supplementation (Mantovani et al. 2007; Moonmoon et al. 2011; Itoo & Reshi 2014). The effect of both

| Table 4. Carbon (C) and nitrogen (N) analysis of substrates used for Pleurotus ostreatus cultivation. |
|---|---|---|---|
| Substrate code | C (%) | N (%) | C/N |
| CSS-0 | 45.97 | 0.70 | 65.67 |
| CSS-5 | 46.55 | 0.93 | 50.05 |
| CSS-10 | 47.12 | 1.15 | 40.97 |
nitrogen-deficient and excess nitrogen-containing substrates on mycelia growth is evidenced in this study. CSS-5 produced higher mycelia length than both CSS-0 and CSS-10. Substrates with no supplementation, CSS-0 provided mushroom spawns with little amounts of nitrogen required for protein biosynthesis and growth. On the other hand, the excess nitrogen provided by CSS-10 inhibited growth of mushroom mycelia, resulting in no significant difference (\( P < 0.05 \)) in length between mycelia growing on this substrate and CSS-0 (Table 5).

After opening of fully colonised bags, it took only 4 days for primordia to appear on CSS-5 and CSS-10, while it took 7 days for them to appear on CSS-0. All these times are much less than the 20 days it took \( P. ostreatus \) primordia to appear on \( Ficus vasta \) leaves (Alemu & Fisseha 2015). After the first flush of mushrooms were picked, the second flush of primordia which were present as thickened mycelial knots began to develop, attaining button size within a few days. The lowest interval between flushes was observed on CSS-5 (Table 6), presumably because of its “ideal” C/N ratio of 50.05 for fruiting body development. This resulted in CSS-5 having the highest number of flushes during the 6-week harvesting period (Table 6).

Yields obtained from the three substrate formulae studied differed significantly (\( P < 0.05 \)). According to Soto-Cruz et al. (1999), the yield of mushrooms depends on mycelia development. In this study, mushroom mycelia growth rate positively correlated with yields of mushroom (Table 6). CSS-5 produced significantly higher (\( P < 0.05 \)) mushroom yields than both CSS-0 and CSS-10 (Table 6). The B.E. obtained for CSS-0 (Table 6) in this study was higher than the 11.94% reported by Alemu (2014) when he cultivated \( P. ostreatus \) on \( Grevillea robusta \) leaves without supplementation. It was also higher than the 7.6% and 14% achieved on 100% wheat straw and 100% maize cobs, respectively (Bhatti et al. 2007; Adajpong et al. 2015). However, this was lower than cultivation on 100% cassava, corn cob, barley straw, and saw dust (Kortei et al. 2014; Hoa et al. 2015; Tesfaw et al. 2015). It is also lower than the 54.27% obtained when Wang et al. (2015) cultivated \( P. ostreatus \) on a mixture of cottonseed hull, wheat bran, gypsum and lime.

Hoa et al. (2015) observed that the B.E. of \( Pleurotus \) spp. was boosted when nitrogenous supplements were added to substrates. In previous studies, the addition of chicken manure to cassava peels only increased B.E. from 26% to 27% (Kortei et al. 2014), whilst addition of 18% cow dung increased B. E. from 11.94% to 15.86% (Yildiz et al. 2002). In this study, however, addition of 5% and 10% sorghum spent grain raised B.E. from 16.9% to 29% and 25.9%, respectively. This makes sorghum spent grain a good and cheap source of nitrogen supplement for mushroom cultivation.

### Conclusion

The results of this study indicated that cornstalks are viable substrates for \( P. ostreatus \) cultivation. Sorghum spent grain, which has low economic value, can be used instead of expensive products like wheat bran as suitable supplement for the cultivation of oyster mushrooms on cornstalks. To produce mushrooms with improved yields, 5% sorghum spent grain supplementation is recommended.

#### Table 5. Mycelia growth observed on the various substrates over 3 weeks.

| Time   | CSS-0     | CSS-5     | CSS-10    |
|--------|-----------|-----------|-----------|
| Week 1 | 5.80 ± 0.23\(^a\) | 5.46 ± 0.26\(^a\) | 5.72 ± 0.16\(^a\) |
| Week 2 | 8.80 ± 0.38\(^a\) | 11.34 ± 0.39\(^b\) | 9.81 ± 0.42\(^a\) |
| Week 3 | 12.66 ± 0.27\(^a\) | 14.22 ± 0.18\(^b\) | 13.35 ± 0.22\(^a\) |

Results are mean scores of 5±SE. Values with different letters in a row are significantly different (\( P < 0.05 \)).

#### Table 6. Other growth parameters measured on the various substrate formula.

| Growth parameters                  | Substrate formula |
|------------------------------------|-------------------|
| Period for full colonisation (weeks) | CSS-0  CSS-5 CSS-10 |
| Time for primordia appearance (days) | 3.31± 0.23\(^a\) | 3.03± 0.06\(^b\) | 3.09± 0.11\(^c\) |
| Interval between flushes (days)    | 7.0± 0.63\(^a\)  | 4.0 ± 0.63\(^b\)  | 4.2± 0.40\(^c\)  |
| Number of flushes within 6 weeks   | 8.0± 0.63\(^a\)  | 5.2± 0.40\(^b\)  | 6.8±0.75\(^c\)  |
| Yield (g)                          | 118.4±1.85\(^a\) | 203±1.79\(^b\)   | 181±3.16\(^c\)  |
| Biological efficiency (%)          | 16.9 ±0.26\(^a\) | 29.0±0.26\(^b\)  | 25.9±0.45\(^c\) |

Values with different letters in the same row are significantly different (\( P < 0.05 \)).
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Disclosure statement

No potential conflict of interest was reported by the authors.

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