African Crop Science Journal, Vol. 31, No. 1, pp. 31 - 39
Printed in Uganda.
ISSN 1021-9730/2023 $4.00

DOI: https://dx.doi.org/10.4314/acsj.v31i1.3

PRODUCTION OF INDIGENOUS MUSHROOMS SPAWN USING CROP RESIDUES AS SUBSTRATES

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(*Received 17 April 2021; accepted 27 October 2022*)

ABSTRACT

Indigenous oyster mushrooms occur naturally when conditions are favourable. Achieving good quality spawn is a major challenge to small-scale farmers in Kenya, who strive to domesticate indigenous mushrooms. The use of wheat grain in mushroom growing industries for spawn production poses a threat to food security. The objective of this study was to evaluate crop residues as alternative substrates for indigenous mushrooms spawn production in Kenya. Different crop residues, including straws of wheat, barley and beans, maize cobs and sawdust were sterilised and tested for spawn production. Colonisation, pinning and mushroom yields were evaluated on bean and wheat straw substrates. There was a significant difference ($P<0.05$) in mycelia colonisation period when different spawn types were inoculated on bean and wheat straw substrates. Bean straw spawn had the shortest colonisation period (23 days) and was the best agricultural waste spawn. There was no significant difference ($P<0.05$) on pinning days and 2nd flush yields for the different types of spawn. There was a significant difference ($P<0.05$) between maize cob and wheat grain spawn in 1st flush yields. Bean straw spawn had a flush 1 yield of 125.2 g which, was not significant ($P>0.05$) compared to the wheat grain spawn (control) that gave a yield of 126.1 g. Results of this study indicate the suitability of various agricultural crop residues as alternative substrates for indigenous spawn production.

Key Words: Flush, indigenous oyster mushroom, pawn, spinning, substrate

RÉSUMÉ

Les pleurotes indigènes poussent naturellement lorsque les conditions sont favorables. L'obtention de blanc de champignon de bonne qualité est un défi majeur pour les petits agriculteurs du Kenya, qui s'efforcent de domestiquer les Les pleurotes indigènes. L'utilisation de grains de blé dans les industries de culture de champignons pour la production de blanc de champignon constitue une menace pour la sécurité alimentaire. L'objectif de cette étude était d'évaluer les résidus de cultures comme substrats alternatifs pour la production de blanc de champignons de pleurotes indigènes au Kenya. Différents résidus de culture, notamment des pailles de blé, d'orge et de haricots, des épis de maïs et de la sciure de bois ont été stérilisés et testés pour la production de blanc de champignon. Les rendements de colonisation, d'épinglage et de champignons ont été évalués sur des substrats de paille de haricot et

de blé. Il y avait une différence significative (P<0,05) dans la période de colonisation du mycélium lorsque différents types de blanc de champignons étaient inoculés dans les substrats de paille de haricot et de blé. Le blanc de champignon de la paille de haricot avait la période de colonisation la plus courte (23 jours) et était le meilleur blanc de champignon. Il n'y avait pas de différence significative (P<0,05) sur les jours d'épinglage et les rendements de la 2ème récolte pour les différents types de blanc de champignons. Il y avait une différence significative (P < 0,05) entre le blanc de champignon d'épi de maïs et le blanc de champingon de paille de blé dans les rendements de 1ère récolte. Le blanc de champignon de paille de haricot a eu un rendement de 125,2 g, ce qui n'était pas significatif (P>0,05) par rapport au blanc de champignon sur le grain de blé (témoin) qui a donné un rendement de 126,1 g. Les résultats de cette étude indiquent la pertinence de divers résidus de cultures agricoles comme substrats alternatifs pour la production de blanc de champignon indigène.

Mots Clés : Flush, pleurote indigene, blanc de champignon, paillage, substrat

INTRODUCTION

Mushrooms are a group of fungi with good sources of high-quality proteins, abundant in vitamins and minerals (Selvi *et al*., 2007). Wild mushrooms are traditionally used in many countries as both food and medicine (Sanmee *et al*., 2003; Isildak *et al*., 2004). In Kenya, indigenous mushrooms are still consumed from the wild, but mainly by the communities living around the forests. More specifically, the collection and consumption are mainly done in Kakamega and Kisumu counties; Mt Elgon and Arabuko Sokoke forests (Buyck *et al*., 2000). Wild mushrooms constitute one of the non-wood forest product (NWFP), where some mushrooms and hard wood trees or termites exist in a symbiotic relationship (Buyck *et al*., 2000).

The current production of mushrooms in Kenya is estimated at 500 metric tonnes per *annum*. From an objective point of view, however, Kenya has the potential to produce over 100,000 metric tonnes of mushroom per year (Wambua, 2004). Wild mushrooms have been a delicacy in Kenya and are often considered to be meat substitutes, especially by the rural population.

The uncontrolled harvesting of mushrooms coupled with the destruction of forest habitats to create land for settlement, agriculture and firewood, has resulted into loss of germplasm of these precious fungi (Palapala *et al*., 2006). Mushroom mycelia are important in the ecosystem because they can biodegrade the substratum and, therefore, use the wastes from agricultural production as sources of nutrition (Manzi *et al.,* 2001).

Various factors reportedly affect growth and performance of oyster mushrooms; including substrate source, substrate quality, spawn and compost type (Royse *et al*., 2004; Jafarpour *et al*., 2010). In Kenya, based on a consultative stakeholders' workshop (2007), constraints to mushroom production include high input costs, lack of quality spawn, diseases and pests, lack of proper skills in production and postharvest handling; and a lean government extension service (Gateri *et al*., 2008). The objective of this study was to evaluate different crop residues as substrates for spawn production for domestication of the indigenous oyster mushrooms in Kenya.

MATERIALS AND METHOD

This study was carried out at Egerton University in the mushroom house and Laboratory, both situated in the Department of Biological Sciences. Indigenous oyster mushroom of *Pleurotus* species obtained from Kakamega forest in Western part of Kenya, was used in this study.

Mycelial culture was produced through a tissue culture technique, whereby potato dextrose agar (PDA) was prepared by mixing

39.0 g in 1 liter of distilled water and sterilised at 121 °C in an autoclave for 15 minutes (Pandey and Tewari, 1990). The media was allowed to cool to 45 °C; after which 133 g of streptomycin sulphate was added. It was then dispensed into universal bottles to prepare agar slants by placing the media in a slanting position. The mushroom was thoroughly prewashed using distilled water. A scalpel blade was dipped in alcohol and flamed until it was red-hot and cooled for 10 seconds. The mushroom was cut lengthwise from the cap, downwards. Small piece of the internal tissue at the point where the mushroom cap joins the stipe was cut and removed with a flamed needle. The needle with the tissue attached was immediately inserted into the agar slant and the tissue laid on the agar surface. The mouth of the slant was flamed before the needle was inserted in the agar slant. The mouth was then plugged with cotton wool and the slant incubated at 25°C. When the tissue was covered with white mycelium that spread on the agar surface, sub-culturing was done to obtain pure cultures.

Substrates including sawdust, crushed maize cobs, bean straw, wheat straw and barley straw were tested for spawn production. Wheat grains were used as the control (Quimio *et al*.,1990). The substrates were obtained from farmers in Nakuru county, in the Rift Valley part of Kenya. Wheat grains were sorted to remove broken ones. They were washed thoroughly and soaked overnight in distilled water. Seeds that floated in water were discarded.

The grains were drained to remove excess water and then rinsed and boiled in water for 15-20 minutes to soften. Excess water was drained off and the grains mixed with calcium carbonate ($CaCO_3$) and calcium sulphate ($CaSO_4$) at 2% each. Grains were transferred into glass jars of 1 litre size until the jars were approximately ¾ full. This measured an average of 400 g of weight per bottle. The top of the jars were plugged with cotton wool and sterilised at 121°C for one hour for two consecutive days.

For sawdust, crushed maize cobs, bean straw, wheat straw and barley straw, sorting was done to remove foreign debris. One hundred and fifty grammes of the straw was chopped into small pieces and soaked in water overnight. Excess water was drained off and the substrates were then placed in polythene bags (300 mm x 700 mm x 2 mm thickness) and sterilised. The supplements (20% wheat bran and 5%-gram flour) were sterilised separately and mixed with the sterilised substrates. The substrates were inoculated aseptically with two mycelia agar discs of 6 mm in diameter, obtained from actively growing pure culture of the indigenous oyster mushroom. The inoculated substrates were incubated at a temperature of 25 °C and monitored for mycelia growth and colonisation rate. Each treatment was replicated three times in a Completely Randomised Design (CRD).

The best spawn in each treatment was used to spawn 500 g of dry bean straw and wheat straw substrates. Wheat and bean straw were chopped into 1-2 cm pieces and 500 g of each substrate was mixed with 100 g wheat bran and 10 g of gram flour; and sterilised using an autoclave at 121°C for 15 minutes. The layer method was used to spawn the sterilised straw, which was filled in poly bags to about four inches thick. A layer of 3% of spawn was placed on the substrate, and another layer of straw was added to cover the spawn. The spawned substrates were incubated in the mushroom house and monitored for mycelia colonisation, contamination, and fruiting. This was compared with the different spawned substrates and spawn types.

RESULTS AND DISCUSSION

Pure mushroom culture. Pure cultures of indigenous oyster mushrooms of *Pleurotus* species were successfully cultured on potato dextrose agar (PDA). Although various species were cultured, only one species of *Pleurotus ostreatus* fruited and was used in the subsequent experiments.

Colonisation and pinning period. The spawn run period for bean straw spawn when inoculated with wheat grain spawn was 23 days; while for maize cob spawn it was 25 days. When wheat straw was inoculated with wheat grain spawn, the colonisation period was 25 days. However, when wheat straw was inoculated with maize cob and saw dust spawn the colonisation period was 27 days. There were significant differences ($P<0.05$) in colonisation of the six types of substrates used in spawn production in (Table 1). However, there was no significant differences in pinning periods for all the spawn types, when inoculated on bean and wheat straw substrates (Table 1). Bean straw gave the best spawn compared to other crop residues, since the colonisation and pinning periods were much shorter in both bean and wheat straw substrates when inoculated with bean straw spawn (Table 1).

Wheat is the most common material for spawn production (Rai, 2003; Dadwal and Jamaluddin, 2004). However bean straw spawn proved to be a promising alternative substrate for spawn production. The lower performance in yield by bean straw, sawdust and maize cob spawn when inoculated on wheat straw substrate could be due to low lignolytic and cellulytic activity of the substrates (Pathak and Goel, 1988).

The greatest difference in colonisation was obtained between maize cob and wheat grain spawn, when inoculated on bean straw; which resulted into colonisation period of 25 and 23 days, respectively (Table 1). Similar results were obtained when maize cob spawn and wheat grain spawn were inoculated on wheat straw, where colonisation in wheat straw took a shorter period (25 days), when inoculated with wheat grain spawn as compared to 27 days when inoculated with maize cob spawn.

The significant difference ($P<0.05$) in colonisation period between wheat and bean straw, when inoculated with different spawn, may be attributed to variation in the chemical composition and C: N ratio of the different types of substrates used as reported by Bhatti *et al*. (1987). Yildiz *et al.* (2002) reported that the natural substrates (woods on which *Pleurotus* species grow) are very poor in nitrogen content, in spite of the fact that the mushroom mycelia colonisation takes place. Thus, the insufficient nitrogen may be one of the factors affecting the overall colonisation values in wheat straw, when inoculated with sawdust spawn. The delayed colonisation of bean straw substrate by maize cob spawn (Table 1) might be due to agro waste clumps in maize cob, which reduced total point of inoculum per gramme of spawn or due to their complex chemical nature as reported by

TABLE 1. Effect of spawn substrate on colonisation and pinning period on bean and wheat straw

Spawn substrate	Colonisation period (days)	Pinning period (days)	Pinning period (days)	Colonisation period (days)
	Bean straw	Bean straw	Wheat straw	Wheat straw
Bean straw	23 [a]	5[a]	5[a]	25[b]
Barley straw	24[ab]	5[a]	6[a]	25[b]
Maize cob	25 [b]	6[a]	6[a]	27[a]
Sawdust	23 [a]	5[a]	5[a]	27[a]
Wheat grain	23[a]	5[a]	5[a]	25[b]
Wheat straw	24[ab]	6[a]	5[a]	25[b]

Means followed by same letter in the same column are not significantly different at $P<0.05$

Siddhant *et al*. (2013). Another factor for the low performance by maize cob and sawdust spawn may be due to slower break down of cellulosic and lignin substrates as reported by Pokhrel *et al*. (2013). Thus, from the present study, the interaction of poor spawn type (maize cob and sawdust) with the spawned substrates (wheat straw) may have affected the colonisation. According to Philippoussis *et al*. (2001), the nature and nutrient composition of the substrate affects mycelium growth, mushroom quality, and crop yield of this value-added biotransformation process.

Therefore, the nature and nutrient composition of the substrates used in spawn production and the spawned substrates which were bean straw and barley straw may have led to the difference in colonisation. The colonisation periods recorded on bean and wheat straw substrates were similar to those of Shah *et al*. (2004), who reported that the spawn ran for 16-25 days after inoculation.

Among mushroom fungi, *L. edodes* and *Pleurotus* species are known to be more efficient in degrading a wide range of lignocellulosic residues, such as wheat straw, cotton wastes, coffee pulp, corn cobs, sunflower seed hulls wood chips and sawdust, peanut shells, vine prunings and others into mushroom protein (Stamets, 2000; Philippoussis *et al*., 2001). Their mycelium can produce significant quantities of enzymes, which can degrade lignocellulosic residues and use them as nutrients for their growth and fructification (Bushwell *et al*., 1996; Elisashvili *et al*., 2008). The production of enzymes by the mushroom mycelia could have favoured colonisation and, hence the insignificant difference in colonisation.

Cereal grains are generally used as spawn substrates and one attribute of good quality cereal grain is the ability to be evenly spread in the final substrate (Siddhant *et al*., 2013). This is possible only when the grains are not clumped together. During spawning, $CaCO_3$ is added so as to buffer the pH; while $CaSO_4$ is added to keep the grains apart (Siddhant *et al*., 2013). Wheat grains act as a reservoir of carbohydrates, which offer sufficient nutrition for mycelia growth and provide a vehicle for the even distribution of mushroom inoculants. This is the reason why the colonisation period for bean and wheat straw inoculated with wheat grain spawn was shorter. The major disadvantage of cereal grains is the presence of less food material in their endosperm, and their intrinsic problems of being very prone to contamination and yet being attractive to rodents (Bahl, 1998).

The time for pin head initiation recorded in Table 1 for all the spawn substrates indicates no significant difference ($P>0.05$) in pinning periods for both wheat straw and bean straw substrate, when inoculated with different spawn substrates. This is in agreement with Ahmed *et al*. (2009), who reported that *P. ostreatus* completed spawn running in 17-20 days on different substrates, and time for pinhead formation was noted at 23-27 days. Narain *et al*. (2009) reported that mushroom mycelia growth and primordial development were dependent on the ligno-cellulosic materials, especially the C:N ratio. According to the findings of Sanchez and Royse (2001), the C:N ratio in the substrate decreases from spawn run to fruitification, and there was a decrease in C:N observed in bean straw and wheat straw substrates after cultivating and harvesting the mushrooms.

According to Stamets (1993), C:N ratio and equilibrium values favour optimal fungal growth; while lack of C:N equilibrium decreases or halts fungal development. Nitrogen is essential for mushrooms colonisation and pinning; hence its concentration in the substrate has a direct effect on their development. This accounts for the absence of a significant difference in pinning period in both bean and wheat straw. It is possible that there was C:N ratio equilibrium that favoured pinning of mushrooms in both substrates.

The insignificant differences in pinning period in the present study conforms to that of Hernandez et al. (2011), who reported that in bean and wheat straw substrates, lignin is continuously broken down during the primordium formation stage, with 31.8% (bean straw) and 34.4% (wheat straw) degradation. Breakdown of lignin in wheat and bean straw most likely favoured pinning in the present study. Changes in N concentration modify substrate pH and affect the ability of the mushroom to establish (Leatham and Stahmann, 1989; Przybylowicz and Donoghue, 1990). Rajarathnam *et al*. (1987) showed that *Pleurotus flabellatus,* cultivated on rice straw, also promoted a continuous increase in sugars during initial mycelial growth; followed by a decrease during the fruiting stage. This is possibly associated with energy requirements for initiating pinning. Mushrooms need easily assimilable sources of carbon and available sugars (Przybylowicz and Donoghue, 1990). These accelerate mushroom growth, degradation of the medium and also reduce fruitification time, since the mycelium converts these carbohydrates into reserves for fruiting (Przybylowicz and Donoghue, 1990). The ability of mushroom mycelia to assimilate sufficient carbon and sugars from the spawn substrates used, as well as the spawned substrates (bean and wheat straw), could be the reason for the short pinning period in the present study.

In addition, the combined activities of cellulases, hemicellulases and ligninolytic enzymes may explain the accelerated vegetative growth and primordium formation in vine pruning's (Silva *et al*., 2005). This could explain the insignificant difference in pinning by both wheat and bean straw, since they contain these enzymes. Finally spawn from crop residue substrates could have had an impact on colonisation, since the substrates had the above mentioned enzymes capable of accelerating vegetative and primordial formation.

Mushroom yield. Wheat grain spawn when inoculated on bean straw gave 1st flush yield of126.1 ± 18.1^{a}, which was different from sawdust spawn that gave a 1st flush yield of 84.1 ± 27.8^{bcd}. Wheat straw inoculated with wheat grain spawn gave a 1st flush of 106.7 ± 18.7^{ab} which was different from sawdust spawn that gave a 1st flush 65.4 ± 17.4^{d} maize cob that gave a 1st flush of 67.5 ± 20.8$^{cd.}$

On bean straw substrate, there was a significant difference (P<0.05) in flush 1 yields from sawdust spawn (Table 2). On the other hand, there were no significant differences (P>0.05) for the other five spawn types; namely wheat straw, bean straw, barley straw, maize cob and wheat straw. When wheat and bean straw substrates were inoculated with the six spawn types, there was no significant difference (P<0.05) in flush 2 yields. This lack

TABLE 2. Mushroom yield (g) on bean and wheat straw when inoculated with different spawn types

Spawn substrate	Bean straw		Wheat straw	
	1st flush	2nd flush	1st flush	2nd flush
Bean straw	125.2 ± 17.3^{a}	52.1 ± 9.7^{a}	98.9 ± 25.1^{abcd}	47.2 ± 12.8^{a}
Barley straw	92.1 ± 24.4^{abcd}	44.3 ± 6.2^{a}	112.7±16^{a}	51.7 ± 9.1^{a}
Wheat straw	99.5 ± 22.7^{abcd}	48.1 ± 7.5^{a}	104.7 ± 20.1^{abc}	56.2 ± 7.4^{a}
Wheat grain	126.1 ± 18.1^{a}	58.1 ± 13.6^{a}	106.7 ± 18.7^{ab}	60.2 ± 3.8^{a}
Saw dust	84.1 ± 27.8^{bcd}	37.9 ± 8.5^{a}	65.4 ± 17.4^{d}	36 ± 13.9^{a}
Maize cob	95.6 ± 24.2^{abcd}	29.3 ± 7.5^{a}	67.5 ± 20.8^{cd}	37.8± 18.9^{a}

Means followed by same letter are not significantly different at P<0.05

of significant difference in the flush 2 yield performance as compared to wheat grains (control) confirms that crop residues can be used as substrates for spawn production. This is in agreement with report by Chang (2009) that most agricultural wastes can be used to prepare mushroom spawn.

The variation in yields obtained when different spawn substrates were inoculated on bean and wheat straw substrates (Table 2) may be attributed to difference in lignolytic and cellulolytic activity of the substrates (Pathak and Goel, 1988). This could be the reason why wheat straw as a substrate gave significantly lower yields compared to bean straw. Furthermore, the higher protein content in bean straw than in wheat straw could have been responsible for the greater mushroom yields in the former than in the latter.

Hernandez *et al*. (2005) showed that fiber in substrate is mainly composed of cellulose, hemicellulose and lignin, and that the percentages of fibre (based on dry matter) for the substrates inoculated with *Lentinula edodes* were wheat straw (69.1%), bean straw (87.1%) and vine prunning (71.9%). The initially high fibre content in bean straw (87.1%) coincides with the high mushroom production on this substrate, as previously reported by Hernandez *et al*. (2005). A high flush 1 yield of 126.1 g from bean straw substrate may be attributed to high fibre in bean straw substrate. In the present study, there was a significant difference in flush 1 yield on bean straw substrate when sawdust and wheat grain spawn were used. Sawdust gave a low flush 1 yield of 84.1 g compared to wheat grain that gave a yield of 126.1 g. There was also a significant difference in flush 1 when wheat straw was inoculated with wheat grain, sawdust and maize cob spawn. Sawdust gave a yield of 65.4 g; while maize cob gave 67.5 g and wheat grain 106.7 g. Stamets (2000) found that many cereal grains can be used for spawn preparation; and that selection depended on the availability of raw materials, yield and experience of the spawn makers. This conforms to our study since wheat grain spawn had better yields. Stamets (2000) also found out that sawdust obtained from furniture workshops was either too fine to be used or mixed with wood shavings and, thus compromised as a substrate. As such, it is difficult to use, difficult to replicate and poor in quality. In the present study, poor quality of sawdust could have caused the low flush 1 yields when inoculated in bean and wheat straw substrates; its inconsistency could have compromised the spawned bean and wheat straw substrates.

CONCLUSION

Bean straw, sawdust, maize cob, barley straw and wheat straw can be used as alternative substrate for spawn production. However, spawn produced from bean straw residues produced higher yields in first flush when inoculated on bean straw substrates while spawn from barley straw residues produced a higher yield in first flush when inoculated on wheat straw substrates. There is a need to evaluate other crop residues including rice straws, sugarcane bagasse and coffee husks for enhancing better spawn production that result to high yield of mushrooms. More research could explore the potential of more agricultural residues combinations for production of mushroom spawn. Use of crop residues in mushrooms cultivation is an eco-friendly, cost-effective practice for mushroom production for food security and as agribusiness for smallholder farmers in developing countries.

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