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EFFECT OF MIXING CEREAL AND LEGUME STRAWS ON YIELD OF GREY OYSTER MUSHROOM UNDER CONTROLLED CONDITIONS

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ABSTRACT

In Zimbabwe, yield of oyster mushroom (*Pleurotus ostreatus*) grown on sole substrates of maize or sorghum straw is low, <75% biological efficiency translating to less than a kilogramme of fresh mushroom per kg of dry substrate. This study aimed at determining the effect of mixing sorghum or maize straw with various proportions of bean straw, on the yield of grey oyster mushroom. Maize and sorghum straws were singly mixed with 0, 20, 40, 60, 80 and 100% bean straw, followed by a spawning rate of 5%. Eighteen days after incubation in a dark room, bags were hanged in a mushroom growing house with controlled temperatures (25-26^{o}C). Mixing bean with maize straw had a significantly higher oyster mushroom yield (1, 229 g) than with sorghum straw (1,138 g); leading to biological efficiency of 83 and 74%, for maize straw and sorghum straw, respectively. Yield and biological efficiency decreased from 1,357 to 977 g plot^{-1} and 97 to 61%, respectively, when the proportion of bean straw was increased from 0-100%; while number of days to second and third harvest increased. There were significant interactions (P<0.05) for the number of pins, days to second and third harvest, between maize or sorghum straw with bean straw. Maize straw gave better performance with high percentages of bean straw than sorghum straw. Results showed that maize straw mixed with 20% bean straw produced better yield of grey oyster mushroom.

Key Words: Biological efficiency, maize straw, *Pleurotus ostreatus*

RÉSUMÉ

Au Zimbabwe, le rendement de pleurotes (*Pleurotus ostreatus*) produites sur les substrats des pailles du maïs ou du sorgho est faible, < 75% de l'efficience biologique traduisant à moins d'un kg des champignons frais par kg du poids sec du substrat. Cette étude visait à déterminerl'effet du mélange des pailles du maïs et du sorgho avec diverses proportions de la paille du haricot, sur le rendement du pleurote gris. Les pailles du maïset du sorgho ont été singulièrementmélangées avec 0, 20, 60, 80 et 100% de la paille du haricot, suivie par un taux de 5% de mycélium. Dix-huit jours après incubation dans une chambre noire, les sacs ont été suspendus dans une chambre de croissance de champignon avec des températures contrôlées (25-26^{o}C).Le mélange de la paille du haricot avec la paille du maïs a eu un rendement significativement plus élevé du pleurote (1 229 g) qu'avec la paille du sorgho (1 138 g) ; entrainant une efficience biologique de 83 et 74%, pour la paille du maïs et du sorgho, respectivement. Le rendement et l'efficience biologique ont décru du 1 357 à 977 g plot^{-1} et 97 à 61%,respectivement, quand la proportion de la paille du haricot a augmenté de 0-100% ; alors que le nombre de jours à la deuxième et troisième récolte a augmenté. Il y a eud'interactions significatives (P<0.05) pour le nombre de broches, le nombre de jours à la deuxième et troisième récolte, entre la paille du maïs etdu sorgho avec celle du haricot. La paille du maïs a donné de meilleures performances avec des pourcentages élevés de la paille du haricot que celle du sorgho. Les résultats ont montré que la paille du maïs mélangée avec 20% de la paille du haricot ont produit de meilleur rendement du pleurote gris.

Mots Clés: Efficience biologique, paille du maïs, *Pleurotus ostreatus*

INTRODUCTION

Oyster mushrooms (*Pleurotus* spp.) have high value and short life cycle of up to 30 days (Chitamba, 2007) that results in quick returns; coupled with a huge local demand. Its production can be done irrespective of age, gender or skill (Gume *et al*., 2013). Thus, mushroom cultivation is a potential income generating activity for the poor particularly in sub-Saharan Africa (Ahmed *et al*., 2013; Gume *et al*., 2013). Other advantages of oyster mushroom are that it is rich in all essential amino acids, and minerals and vitamins such as ascorbic acid, thiamine, riboflavin, folic acid and niacin (Pathmashini *et al*., 2008; Yehia, 2012; Ahmed *et al.*, 2013; Gume *et al*., 2013). Mushrooms have some medicinal properties against non-communicable diseases such as cancer and cardio-vascular diseases (Tikdari and Bolandnazar, 2012; Gume *et al*., 2013).

Oyster mushroom does not require large capital investments (Tikdari and Bolandnazar, 2012). Almost all crop residues are suitable as oyster mushroom substrates (Aguilar *et al.*, 2010; Yehia, 2012). Wheat, rice, maize and sorghum straws are abundantly available in most countries in sub-Saharan Africa because every farmer has to grow at least one of them for food, depending on the agro-ecological region. Oyster mushrooms are mainly saprophytes that get their nutritional requirements from a host substrates or from the agricultural wastes that are rich in lignin, cellulose and hemicelluloses (Tikdari and Bolandnazar, 2012). According to Yehia (2012), most *Pleurotus* species have the ability to utilise cellulose, hemicelluloses and lignin because of their ability to produce lignocellulosic enzymes. These enzymes digest complex carbohydrates into simple sugars, which are readily absorbed and utilised by the mushrooms as carbon sources (Aguilar *et al.*, 2010). This helps to transform inedible residues into edible biomass that is of high market value (Yang *et al*., 2013). The waste produced after mushroom production can be used as compost and is good in controlling nematodes (Aguilar *et al*., 2010).

In countries like Zimbabwe, maize straw and corn cobs are readily available since maize is a staple food crop (Chitamba, 2007). Also, sorghum is common in the drier parts of the country, where maize production is limited. Moreover, bean and cowpeas have become the most common legume crops that are found readily available with the majority of the farmers. Straw such as from the cereals (maize and sorghum) are rich in carbon and the legumes such as beans are rich in proteins; hence, low biological efficiencies of less than 100% were previously reported when sole substrates were used to grow mushrooms (Zireva *et al*., 2007). Oyster mushrooms in Zimbabwe are grown mostly on sole substrates; thus they rarely give high yields, and bear biological efficiency of less than 125%. However, worldwide, there are reports of biological efficiencies of up to 250% (Yehia, 2012) due to appropriate use of substrate combinations.

Given a wide occurrence of various substrates on farms, there is need to investigate the yield benefits of using different substrate combinations. Substantial research has been done in Zimbabwe to find out the performance of different agricultural wastes as mushroom substrates (Chitamba, 2007). Farmers grow several crops that include the protein rich leguminous crops. This raises possibilities of increasing the yield of oyster mushroom produced by combinations of any one of the common grass such as maize or sorghum with a protein rich substrate such as beans. The objective of this study was to determine the best cereal-legume substrate combination that can produce high yield of grey oyster mushroom in Zimbabwe.

MATERIALS AND METHODS

Experimental site. The experiment was conducted in the mushroom growing house in the Department of Crop Science, University of Zimbabwe during 2016/2017 summer season. The mushroom house had a concrete floor, lined with sand (3 cm thick) in order to retain moisture (70% relative humidity). The house was roofed with iron sheets and had a wooden ceiling. It had double doors made up of wood lined with black polythene to prevent insects and its windows were lined with black shade-cloth screens to avoid the flies from flying in. The mushroom growing house was disinfected with 5% sodium hypochlorite solution a week before using it. The temperatures in the growing house were maintained at around 25-26 ^{o}C, throughout the growing cycle and this was typical of the summer conditions in Zimbabwe.

Substrate collection and preparation. Maize (*Zea mays* L.) straw and white sorghum (*Sorghum bicolor* L.) straw were obtained from the fields at the Department of Crop Science, University of Zimbabwe after the harvesting of the previous experiments. Sugar beans (*Phaseolus vulgaris* L.) straw was obtained from a farm in Glen Forest, about 50 km north of Harare. The maize, bean and sorghum straws were separately dried in an oven at 72^{o}c for two days and ground into 3-4 cm, a recommended size, using a diesel powered hammer mill.

Substrate combinations and spawning. Two substrate types (maize variety SC513 and sorghum straw variety Macia) were combined separately with various proportion of bean straw to form mixes of dry substrate. The bean straw proportions in the combinations were 0, 20, 40, 60, 80 and 100% in each of the 3kg bags. Maize straw is a lignocellulosic biomass which contains components such as cellulose (34.0%), hemicellulose (37.5%), and lignin (22%) (Feng *et al.*, 2012). The carbon-nitrogen ratio (C/N ratio) for maize straw is about 66.31% (Hills and Roberts, 1981). This composition decrease in sorghum and bean straw residues (Feng *et al.*, 2012).The combinations were based on the dry mass using a digital scale (Uni Bloc Shimadzu UW6200H). Dry substrates mixtures were separately packed in hessian sacks and immersed in tap water in 100 litre metal drums, for 8 hours. The wet substrates were boiled while in hessian bags (almost at 100 ^{o}C) using a 200 litre capacity steel drum for two hours. Mushrooms tend to lower the pH of the substrate; thus calcitic lime (1 g per kg of wet substrate) was added to maintain the required pH of the substrate around 7 (Yehia, 2012). After boiling, excess water was drained from the substrate by simply leaving free water to drain away. The bags of steamed substrates were emptied on a plastic sheet previously disinfected with 5% sodium hypochlorite and allowed to cool to a room temperature. Calcitic lime (supplied by the Zimbabwe Fertiliser Company) was added to each substrate combination at a rate of 1% on wet mass basis.

Calcitic lime is a well-known antiseptic because of its cytotoxic effects on many bacterial strains (Pathmashini *et al.*, 2008). A scale was used to establish the wet mass for each substrate combination to allow estimation of the lime rates. Spawning was done separately for each substrate combination at a rate of 5% on wet mass basis. The spawned substrate were thoroughly mixed, packed into 3 kg microbial-free plastic bags, tightly pressed manually and tied at the topmost part of plastic bag using rubber bands. Many tiny needle sized holes were punched into the bags to drain off excess water and permit gaseous exchange, using a sterile needle. The spawned bags were placed on shelves and incubated in a completely darkroom at 25-26 °C.

Experimental design and crop management. After eighteen days, the bags (each bag representing an experimental unit) were removed from the dark room and hanged on wires lined in a light room. The experiment was a 2 x 6 factorial, laid out in a randomised complete design, with three replications. The blocking factor was the direction to the door way to capture variation caused by the effects of external conditions when the doors were opened during operations. The bags were 20 cm apart on wires. The gap between the wires was 80 cm to allow all the mushroom management operations. At the time of hanging, the substrate appeared completely white as a result of successful spawn run. Sterile blades were used to slit surface of the bags, at a distance of 10 cm apart to allow the pin-heads to come out. The bags were exposed to an eight hour cycle of natural light. About 80-85% relative humidity was maintained in the dark room by spraying the floor with clean borehole water. No flooding of the floors was allowed. The room was kept closed and a footbath with chlorinated water was put at the door way in order to avoid introducing contaminants. The temperature in the mushroom house was maintained at 25-26 °C, the normal summer temperatures at the study location.

Data collection. The data were collected on the number of pins; days to first, second and third harvest; yield at first, second and third harvest, total yield and biological efficiency. The number of pins observed per bag was recorded two days after first notice of the bag producing pins. The number of days to the first, second and third harvest was noted from the first day of substrate incubation in the dark room. Total mushroom yield was obtained by summing up the total yield from the first, second and third harvests.

Biological efficiency was estimated as the percentage of total yield per bag, divided by the substrate dry weight (Gume *et al.*, 2013). Harvesting of mature mushrooms marked by the concave shape was done by gently twisting the fruiting bodies by clean hands previously washed with 5% sodium hypochlorite solution. Disturbance of the growing substrate was minimised because the second and third flushes were to be harvested. Mushrooms were harvested while their rims were still concave, which is the correct harvest maturity.

Data analysis. All the data collected were subjected to analysis of variance (ANOVA) using GenStat 17th Edition statistical package (GenStat, 2014). The Least Significant Difference was used to separate the means of the main effects where significance was detected at $P<0.05$. The scatter and the joint biplots (Yan and Kang, 2002) were generated using GenStat 17th Edition statistical package (GenStat, 2014) as described by Yan and Tinker (2006) to display the relationships based on the interactions of the substrate type and bean straw percentage combinations on the number of pins, days to second harvest and days to third harvest.

RESULTS

Type of substrate was not significant for the number of days to first harvest, but was significant ($P < 0.05$) for the rest of the parameters recorded (Table 1). Maize had significantly higher mushroom yield than sorghum, for the three harvests, total yield and biological efficiency (Table 2). In all cases, sorghum gave about 7% less yield and biological efficiency than maize.

The proportion of bean straw in the substrate mix was highly significant ($P<0.001$) for all parameters (Table 1). In general, increasing the proportion of bean straw in the substrate mix, increased the days to first harvest from 21 to 26 days; however, it reduced mushroom yield. Increasing the

TABLE 1. Mean squares and their significance for the various parameters recorded on grey oyster mushroom

Source of variation	DF	Number of pins	Days to 1st harvest	Days to 2nd harvest	Days to 3rd harvest	Yield at 1st harvest (g plot^{-1})	Yield at 2nd harvest (g plot^{-1})	Y ield at 3rd harvest (g plot^{-1})	Total yield (g plot^{-1})	Biological efficiency (%)
Replication	2	0.08	0.53	0.53	0.19	451.7	1306	86.2	1050	28.37
Bean percentage	5	0.77^{ns}	20.84***	34.84***	47.63***	13121.00***	20678.00***	7580.70***	114729.00***	620.01***
Substrate type	1	152.11***	1.78^{ns}	5.44*	6.25*	9287.50**	9407.00*	5979.90***	73274***	1091.68***
Substrate type* bean straw percentage	5	9.91***	0.44^{ns}	3.11**	3.85*	727.40^{ns}	2351.00^{ns}	232.10^{ns}	2411.00^{ns}	25.88ns
Error	22	0.3	0.68	0.63	1.41	744.2	1367	186.9	3010	23.46

*** Significant at 0.01%, **significant at 1%, * significant at 5%

TABLE 2. Effects of substrate type on the growth and yield of grey oyster

Straw type	Days to 1st harvest	Yield at 1st harvest (g plot^{-1})	Yield at 2nd harvest (g plot^{-1})	Yield at 3rd harvest (g plot^{-1})	Total yield (g plot^{-1})	Biological efficiency (%)
Maize	22.56	488.70	455.30	284.60	1228.60	82.65
Sorghum	23.00	456.50	423.00	258.80	1138.40	74.35
Mean square for substrate type	1.78	9287.50	9407.00	5979.90	73274.00	620.01
Mean square error	0.68	744.20	1367.00	186.90	3010.00	23.46
F-Probability value	0.12	0.002	0.016	<0.001	<0.001	<0.001
5% Least significance difference	0.57	18.86	25.56	9.45	37.93	3.35
Coefficient of variation (%)	3.60	5.80	8.40	5.00	4.60	6.20
Error variance component	0.68	744.20	1367.00	186.90	3010.00	23.46
Substrate type variance component	0.37	2847.77	2680.00	1931.00	23421.33	198.85
Total variance component	1.05	3591.97	4047.00	2117.90	26431.33	222.31
Repeatability (%)	35.02	79.28	66.22	91.17	88.61	89.45

proportion of bean straw from 0 to 100% reduced the grey oyster mushroom yield from 524.4 to 407.2 g at first harvest, 521.9 to 346.2 g at second harvest, 310.8 g to 223.70 g at third harvest, 1357.1 g to 977.1 g for total yield, and 97 to 61% biological efficiency. This trend generally shows at least 20% yield and biological efficiency reduction if the percentage of bean straw is increased from 0-100%.

Non-significant interactions were observed for days to first harvest, yield at first up to third harvest, total yield and biological efficiency (Table 1). However, significant interactions ($P < 0.05$) of substrate type and percentage of bean straw were observed on number of pins, and days to second and third harvest (Table 1, Figs. 1- 4).

The angle between the imaginary vectors of maize and sorghum from the biplot origin was almost equal to 90° (Fig. 1). Maize produced the highest number of pins when the proportion of bean straw was high (even better when bean is alone, 100% beans). However, sorghum required less bean straw to produce the highest number of pins (Fig. 2). In general, maize residues mixed with bean straw produced more yield than sorghum residues (Fig. 3). A high proportion of bean

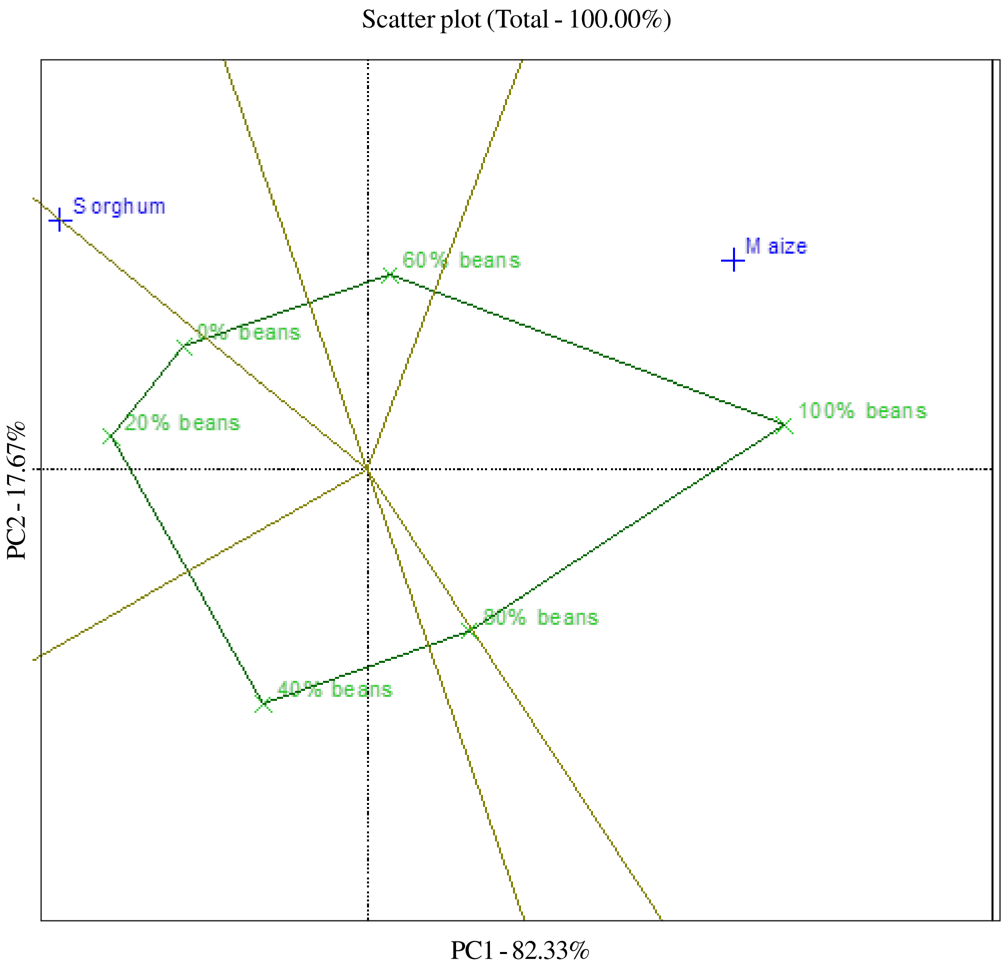

Figure 1. The interaction effect of substrate type and mix on the number of mushroom pins produced.

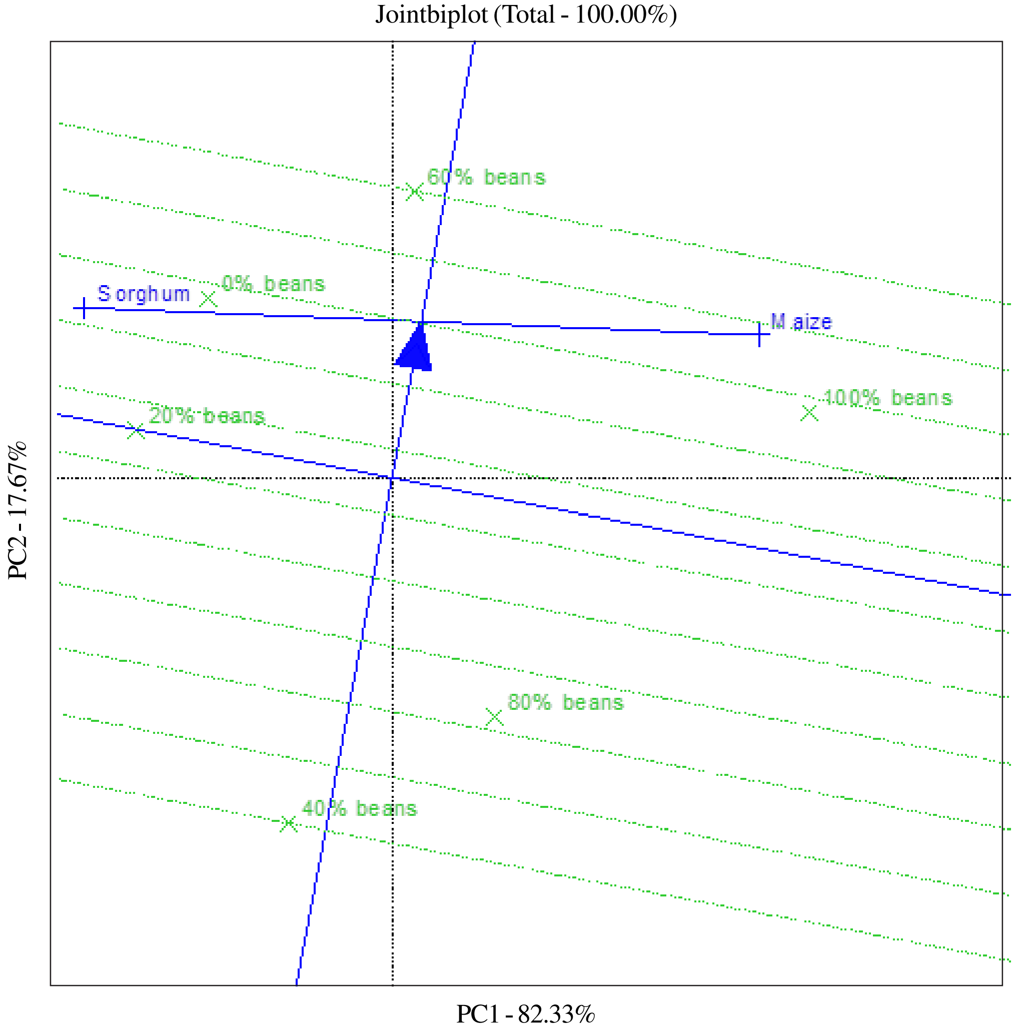

Figure 2. A joint biplot showing the percentage of bean straw that favours a given substrate type.

straw (80% and 100%) resulted in more time to the second and third harvests (Figs. 3 and 4) for both maize and sorghum.

DISCUSSION

Maize straw in combination with bean straw as mushroom substrates constantly led to higher yield than sorghum because of several reasons. Maize has high water holding capacity due to the presence of the pulp in its stem, while sorghum is mainly hollow, and thus has less water holding capacity. Substrate drying as the water in the substrate is used up by the mushrooms is one of the factors that results in low mushroom yield. The humidity in a growing room should be kept constantly high, and maintaining such humidity with the use of traditional methods such as hand spraying is difficult since it has to be done frequently. Therefore, substrates such maize with high water holding capacity will tend to favour higher mushroom yield than sorghum. Furthermore, the substrate composition for maize could be better than that for sorghum. Owing to its pulpy stem, the sorghum has a

Scatter plot (Total - 100.00%)

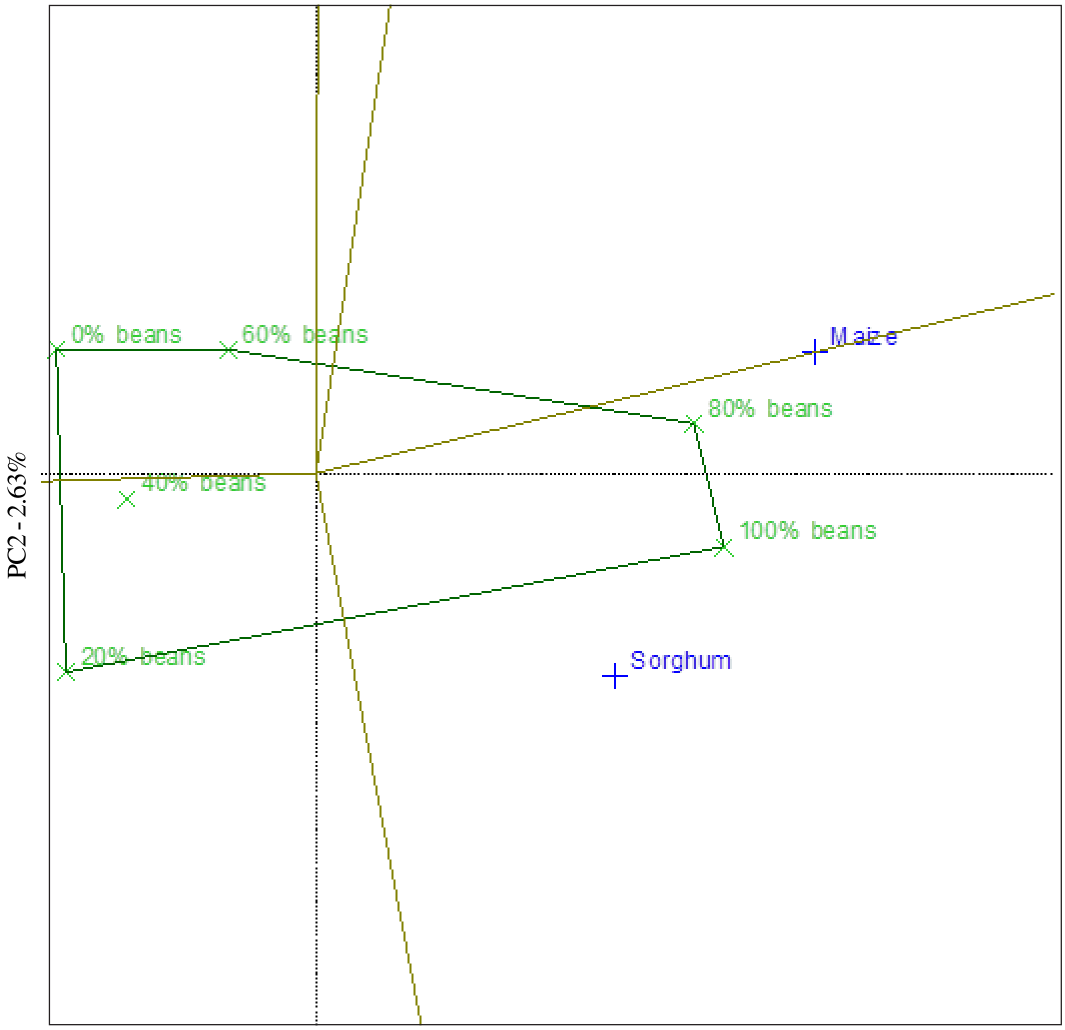

Figure 3. The interaction effect of substrate type and mix of the number of days to second harvest.

very loose texture that is not preferred by the mushrooms (Chitamba, 2007; Zireva *et al*., 2007). For this reason, the use of sorghum alone is widely discouraged (Chitamba, 2007; Zireva *et al*., 2007). The most important factor is that, maize tends to have more digestible cellulose than sorghum (Aguilar *et al*., 2010). The yield of mushroom is mainly dry matter accumulation that is mainly contributed by highly digestible cellulose, richer in maize than sorghum (Aguilar *et al*., 2010). Oyster mushroom has the ability to digest lignocellulosic materials that is more common in maize followed by sorghum and release glucose and other sugars while it uses the proteins common in the bean straw (Aguilar *et al*., 2010). It would be better for farmers to use the maize straw than the sorghum straw in mushroom production. However, in drier parts of Zimbabwe, where sorghum dominates, Scatter plot (Total - 100.00%)

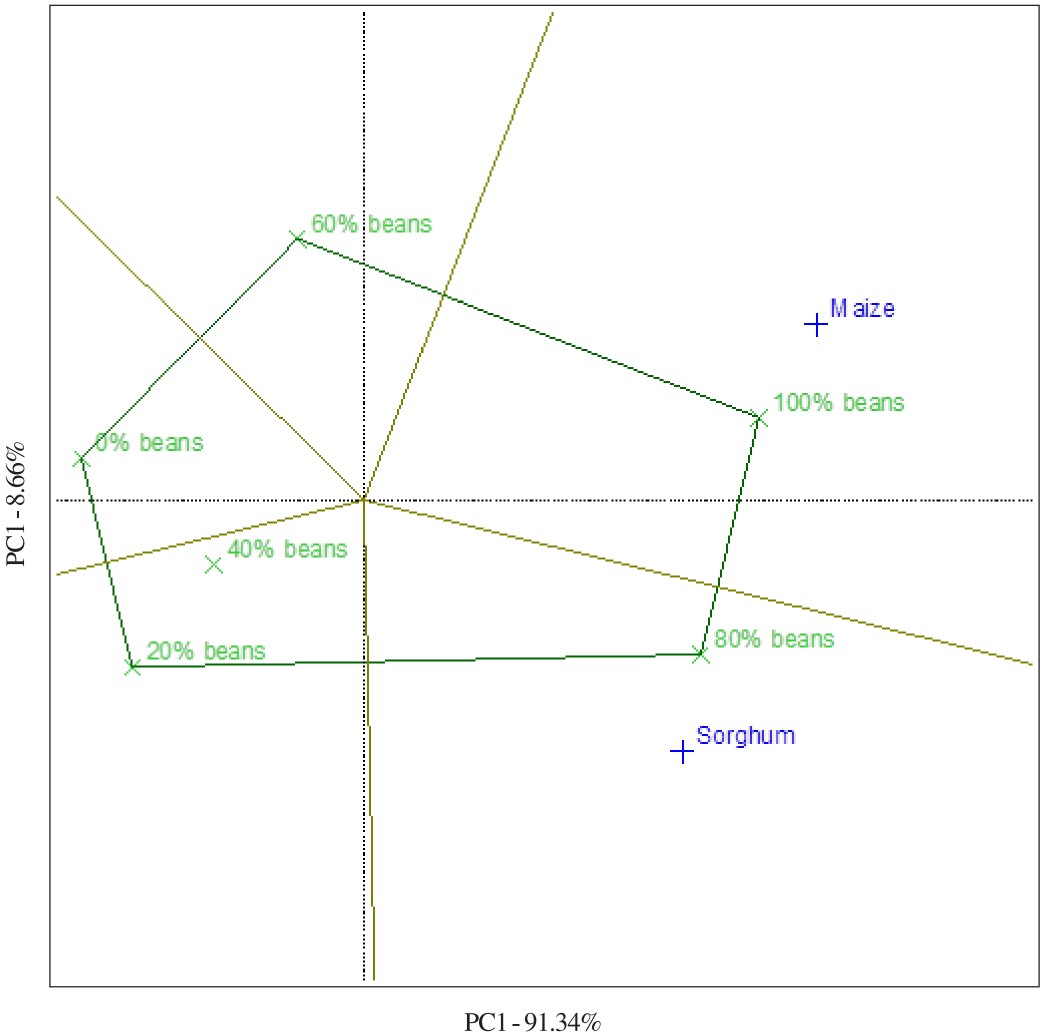

Figure 4. The interaction effect of substrate type and mix on the number of days to the third harvest.

there will be limited options by the farmer and the trade-off will be the 7% loss in mushroom yield. In this situation, some reliable and effective supplements must be identified such as bean straw and some inorganic supplements (Aguilar *et al.*, 2010).

In general, bean straw alone caused inferior yields to cereals (Table 1). This is because mushrooms require high cellulose as commonly found in cereals. Aguilar *et al.* (2010) and Yang *et al.* (2013) reported that oyster mushrooms require materials which are high in lignocellulosic materials. The optimum substrate combination was a 4:1 ratio of maize straw to bean straw. Thus, increasing bean percentage increases proteins at the expense of substrate structure and yield due to reduced carbon source for the mushroom. Furthermore, bean straw has poor structure, because it is too compact, especially when used at high percentages. Compact substrates do not only limit the exchange of important gases such as oxygen, carbon dioxide and methane but also reduce the growth speed of the mycelium (Yang *et al.*, 2013). Reduced growth rate of mycelium translate into increased numbers of days to harvesting, as observed in this study (Table 3).

A right angle formed between maize and sorghum straw mixtures (Fig. 1) shows that the performance of these substrate types is uncorrelated (Yan and Kang, 2002; Yan and Tinker, 2006). However, both belong to the cereal group and one would expect them to behave similarly. Crop residues vary in nutritional composition, with some being more nutritious than others. Mushroom growth is favoured by substrates that are high in lignocellulose (Tikdari and Bolandnazar, 2012). However, the amount of lignocellulose vary with the crop, even crops that belong to the same species can show huge differences in their phyto-biochemistry. In fact, the nutritional composition found in the residues are related to the soil fertility from which the crop was grown. This also results in the variation in the nutritional composition from the same crop species. To this regards, the observed variation in the performance of maize and sorghum is expected though the materials belong to the same family of grasses.

The straw that is ideal for the growth of mushroom should contain high proportions of carbon and nitrogen compounds (Aguilar *et al.*, 2010) which was typical of a combination between maize and bean straws. The maize straw is rich in cellulose while bean residue is not only nutritionally rich in cellulose, but also contain other macro- and micro-elements needed in the proper growth of mushrooms.

Several authors have recommended supplementing the cereals with bean straw in order to achieve high yields. For example, Tikdari and Bolandnazar (2012) stated that small quantities of protein rich additives are recommended to boost yield. The supplementation of cereals with beans becomes very crucial under the smallholder sector. Most smallholder farmers lack capacity to manage their soil fertility and, thus the cereal residues which they produce will be of low nutritional quality. Thus, at this point, the mixing of maize and cereals become very important and mixing 4:1 ratio of maize straw to bean straw was found to be optimum.

An increase in the bean residues proportions resulted in an increased days to harvest of the mushroom (Figs. 3 and 4). Supplementation of cereals with bean residues improves the nutrition. When nutrients are abundant in the right proportions, the mushroom mycelia will grow vegetatively, inter-twine and utilise the available nutrients. However, when one or more of the essential nutrients are scarce, the mushroom shifts from vegetative to the reproductive stage, and attempts to complete the lifecycle, produce spores that are more resilient and thus ensures that its species is perpetuated. A combination of beans and a cereal (either maize or sorghum) provides a good mix of the required nutrients such as high levels of cellulose (carbon) and nitrogen. Beans have both ligno-cellulose, and proteins (Tikdari and Bolandnazar, 2012), while cereals are mainly rich in ligno-cellulose (Chitamba, 2007). This explains why when the percentage of beans is increased; the numbers of days to the harvest are increased for all the harvests (first, second and third). In general farmers should minimise the amount of bean residue which they supplement to cereal and then probably reduce the number of days which the mushroom crop would take to harvest. However, it should be noted that there could be a penalty in total yield that result from the reducing the bean amount in the substrate mixture, mainly due to poor nutrition, especially needed for pin formation and general mushroom growth. Furthermore, the mushroom nutritional value may decrease if the amount of bean residue is reduced (Yang *et al.*, 2013).

A mushroom may not derive all the nutrients from a cereal crop alone. To this regards, use of low (at most 20%) of bean straw is encouraged for both maize and sorghum if the farmer is to derive substantial gains in yield. Another possibility which explains low yield

TABLE 3. Effect of bean straw percentage in the substrate on the growth and yield of grey oyster

Substrate combination	Days to 1st harvest	Yield at 1st harvest (g plot^{-1})	Yield at 2nd harvest(g plot^{-1})	Yield at 3rd harvest (g plot^{-1})	Total yield (g plot^{-1})	Biological efficiency (%)
Bean straw (0%)	21.17	524.4	521.9	310.8	1357.1	96.79
Bean straw (20%)	21.5	522.1	468.4	296.9	1287.4	89.28
Bean straw (40%)	21.33	480.7	453.8	296.7	1231.2	82.61
Bean straw (60%)	22.5	466.3	429.9	264.7	1160.9	72.17
Bean straw (80%)	24.67	435	414.7	237.5	1087.2	69.66
Bean straw (100%)	25.5	407.2	346.2	223.7	977.1	60.5
Mean square for percentage bean straw	20.84	13121	20678	7580.7	114729	1091.68
Mean square for error	0.68	744.2	1367	186.9	3010	23.46
F-probability value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
5% Least significant difference	0.99	32.66	44.27	16.37	65.69	5.8
Coefficient of variation (%)	3.6	5.8	8.4	5	4.6	6.2
Error variance component	0.68	744.2	1367	186.9	3010	23.46
Bean straw percentage variance component	6.72	4125.6	6437	2464.6	37239.7	356.07
Total variance component	7.4	4869.8	7804	2651.5	40249.7	379.53
Repeatability (%)	90.82	84.72	82.48	92.95	92.52	93.82

with bean straw could be that, as the bean percentages is increased, there is increased compactness, resulting in reduced gaseous exchange and mycelium growth. Mycelium would take a long time to colonise thus resulting into longer days to the second and third harvest as the bean straw percentage is increased. However, although the biological efficiency observed in this study was less than 100%, the figures of up to 96% obtained from this study are higher than those previously reported in Zimbabwe (Zireva *et al*., 2007). This shows that more work must not only focus on the media type and mixes but must consider the nutritional composition of the media used and also the climatic conditions.

Maize straw gives higher yield than sorghum straw (Table 2). Increasing the bean straw percentage in the substrate mix would reduce the yield and lengthen the days to harvest. When mixing the substrate, the bean percentage must be kept at most 20%. Farmers must use low percentages of beans (at most 20%) when mixing with maize or sorghum in order to achieve high yields.

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