

Evaluation of Tea Wastes as an Alternative Substrate for Oyster Mushroom Cultivation

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Abstract

Oyster mushroom (*Pleurotus sajor caju*) is locally cultivated on sugarcane bagasse substrate. Due to decreasing supply of bagasse, there was an urgent need to identify alternative substrates. The aim of this study was to evaluate tea wastes as an alternative substrate for oyster mushroom cultivation. The first step was to assess growth of oyster mushroom mycelium on PDA containing tea wastes extract. Antifungal substances found in tea wastes slowed mycelial growth but was not lethal to it. As positive results were obtained, substrate mixtures containing different proportions of tea wastes and sugarcane bagasse were assessed in fruiting bags to identify the most promising substrate mixture on which the effects of supplements such as lime and crushed maize seeds were assessed independently. Substrate containing 75% sugarcane bagasse and 25% tea wastes showed promising results in terms of mycelial growth and fructification. This substrate mixture was selected for further trials with lime and crushed maize. Highest yield of mushroom was obtained with substrate mixture supplemented with 5% lime whereas the substrate mixture gave the highest yield at 0% crushed maize. Results obtained showed that tea wastes can be successfully used to cultivate oyster mushroom but it need to be mixed with sugarcane bagasse for the source of cellulose. Substrate mixture containing 75% sugarcane bagasse and 25% tea wastes, supplemented with 5% lime, yielding about 200g of mushroom can be proposed to growers.

Keywords: Oyster, mushroom, substrate, alternative substrate, tea wastes.

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INTRODUCTION

Mushrooms, which belong to the Kingdom Fungi, are the principal recyclers of our planet as they degrade organic wastes and return the nutrients to the ecosystem (Stamets, 1993). Mushrooms were one of man's earliest foods. Long ago, mushrooms were reserved only for the wealthy people but today, they are consumed both by the rich and the poor (Quimio, 2004). Long ago, mushrooms were collected from nature and were consumed by man only after visual identifications of edibility. Collection of wild mushrooms from nature presented some risks of mushroom poisoning since there was no appropriate method to accurately differentiate edible mushrooms from poisonous ones.

Cultivation packages for several edible mushrooms are now available worldwide. These cultivation packages have allowed safe consumption of mushrooms by eliminating the risk of mushroom poisoning. Mushroom cultivation also allows harvesting of mushroom as and when needed and in the required places.

Mushrooms are an important component of the diet of the Mauritian people. Button mushrooms are the most popular locally. Failures of local trials to cultivate button mushrooms were principally due to unsuitable cultivation methods and inappropriate substrate formulations (Huzar Fatty, 2003). After several assessments, oyster mushroom (*Pleurotus sajor caju*) (Plate 1) was found to be most adapted to the local conditions and to the available substrate, sugarcane bagasse (Cangy, 1990). A cultivation package of oyster mushroom has been proposed to local growers and currently approximately 60 tonnes of fresh oyster mushroom are produced annually for the local market (Huzar Fatty Beejan, pers. comm.).

Oyster mushrooms can be grown on almost all agro wastes including sunflower seed hull, rice bran, wheat bran, sugarcane bagasse, sawdust, cotton wastes, banana leaves, corn cob and coconut coir, among others (Kang, 2004).

Substrates used for cultivating oyster mushrooms should provide all the necessary nutrients for growth. Cellulose, hemicelluloses and lignin are the main sources of nutrition for oyster mushroom which requires high carbon and lower nitrogen input, thus needing a high C/N ratio (Kang, 2004). Optimal C/N ratio of substrates used for growing oyster mushroom is 350-500: 1 (Choi, 2004). To achieve the appropriate C/N ratio for growing oyster mushroom, the substrate must therefore be supplemented since most of the substrates used are generally poor in nitrogen (Kang, 2004). In Mauritius, the bagasse based substrate is supplemented with crushed maize to optimise the C/N ratio. The optimal pH for growing oyster mushrooms is 5-6.5. Highly acidic substrates may be supplemented with gypsum or lime (Oei, 1991). A low pH is known to inhibit mycelial growth while a pH higher than the optimum does not affect mycelial growth since the pH drops when the mycelium grows but however it decreases mushroom yield (Cangy, 1990).

Supplements are added to substrates to promote mycelial growth and increase the yield. C/N ratio of the substrate gives an indication of level of supplements to be used. High nitrogen content of substrates usually decreases the level of

supplementation to be used (Oei, 1991). Protein rich materials are generally used as supplements and these include rice bran, wheat bran, crushed maize seeds, soybean meal and ground corn among others (Stamets, 1993). Each supplement has specific nutritional composition.

When supplementing substrates with the nutrient rich supplements, there is increased risk of contaminations by microbes and thus proper hygiene should be maintained at all times. Contamination of substrates eventually decreases the yield of mushrooms. Substrate, supplements and water should be uniformly mixed to prevent contamination and ensure uniform mycelial growth. Excessive use of supplements cause rapid accumulation of heat in the fruiting bags and adversely affects fructification (Cangy, 1990).

Locally, sugarcane bagasse is highly appreciated as substrate in mushroom cultivation since it is available in large amounts and also because it does not need any pre- treatment unlike other substrates such as banana leaves and pseudo-stem which need to be chipped prior to use.



Plate 1: Oyster mushroom



Plate 2: Mixing of substrate



Plate 3: Fruiting bags ready for pasteurisation

In Mauritius, oyster mushroom is still being cultivated on the bagasse based substrate. The standard substrate mix contains 80% sugarcane bagasse, 10% lime and 10% crushed maize seeds on a dry weight basis. Tap water is then added and the materials are thoroughly mixed (Plate 2). The relative humidity should be approximately 60 %. The mixture is filled in polypropylene bags of known capacity. The open ends of the bags are tied with a raffia piece (Plate 3) and the fruiting bags are pasteurised at 70°C for 3 hours.



Plate 4: Incubation



Plate 5: Mushroom primordia

After cooling, the substrate is inoculated with oyster mushroom spawn. After incubating for 2-4 weeks, colonisation is complete (Plate 4). Fructification is initiated by humidifying the substrate 2-3 times daily. Mushroom primordia (Plate 5) take 3-5 days to mature into fruit bodies. 3- 4 flushes of fresh oyster mushroom can be obtained from each bag. One kg of substrate yields about 250-300 g of oyster mushroom. The spent substrate can be composted for use in crop production.

The main constraint being faced by local mushroom growers is the decreasing availability and increasing purchase cost of sugarcane bagasse which is mainly due to its use for electricity generation and decrease in acreage of sugar cane cultivations (Huzar Fatty Beejan, 2007). Thus, the need has been felt to identify alternative substrates for mushroom growers.

In Mauritius, the Agricultural Research and Extension Unit (AREU) is responsible for research in mushroom cultivation and AREU also promotes mushroom cultivation through the sales of fruiting bags, spawn bags and mother spawns.

Local researches in mushroom cultivation are targeted towards identification of suitable alternative substrate for oyster mushroom cultivation, mutation breeding of oyster mushroom and also cultivation of other mushrooms such as shiitake, wood ear mushroom, reishi and milky mushrooms. A number of substrates have been evaluated which include elephant grass, banana leaves and pseudo stem amongst others. However, the potential of tea wastes have not been exploited for mushroom cultivation in Mauritius. Tea wastes obtained directly from tea factories represent a potential substrate for oyster mushroom cultivation since about 100 tonnes are available annually. Some of the tea wastes obtained from tea factories are utilised in floriculture but the majority of the wastes is either dumped or burned.

Tea leaves are composed of fibres and are known to be rich in nitrogen and lignin. They have a low C/N ratio, making tea wastes a possible nitrogen supplement in spawns and compost preparation (Fung *et al.*, 1981).

Element	Total content / g of dry matter
Nitrogen (%)	0.43 ± 0.02
Phosphorus(ppm)	1655.00 ± 4.80
Potassium(ppm)	2807.33 ± 6.43
Sodium(ppm)	409.00 ± 7.94
Calcium(ppm)	5445.00 ± 4.36
Magnesium(ppm)	4272.67 ± 3.06
Zinc(ppm)	42.67 ± 2.52
Copper(ppm)	20.67 ± 2.08
Iron(ppm)	162.00 ± 2.65
Manganese(ppm)	88.67 ± 1.53
Organic matter (%)	55.67 ± 3.51

Table 1: Chemical analysis of tea wastes

Tea wastes are inappropriate as a single substrate in mushroom cultivation due to their poor content in cellulose (19-39%) and hemicelluloses (9-16%) (Fung *et al.* (1981)). Experiments carried out abroad on use of tea wastes for oyster mushroom cultivation has revealed that these mushrooms can be cultivated on tea wastes after combination with a cellulosic substrate as discussed by Upadhyay (2004).

The aim of this study was to assess the suitability of tea wastes obtained from local tea factories for oyster mushroom cultivation and eventually propose a cultivation package for mushroom growers.

MATERIALS AND METHODS

Tea wastes were purchased from Corson Tea Factory for the experiment. Tea wastes, a by product of tea production was collected and filled in large waterproof bags. Production trials were set up at the Mushroom Unit of the Agricultural Research and Extension Unit (AREU) located at La Brasserie, Curepipe. Chemical analyses of the substrates were conducted at the Soil Laboratory of the Faculty of Agriculture of the University of Mauritius.

In order to assess suitability of tea wastes as a substrate for oyster mushroom cultivation, several trials were necessary.

1. Mycelial growth of oyster mushroom on agar containing tea waste extract

Tea wastes extract was incorporated into Potato Dextrose Agar (PDA) which was poured into sterile petri dishes (14 cm diameter). After solidification and cooling, the agar medium was inoculated with agar plugs of oyster mushroom. After sealing with cling film, the petri dishes were incubated at 25°C. Growth patterns and colonization of agar medium was observed.

2. Fruiting bag assessment of different mixtures of tea waste and bagasse

Substrate mixtures containing different proportions of tea wastes and sugarcane bagasse were assessed in fruiting bags (Table 2). Growth of mycelium followed by fructification was assessed. The most promising substrate mixture was selected as a result of this experiment for further trials. This experiment had a completely randomized design, with 6 treatments and 5 replicates per treatment.

<i>Tea wastes (%)</i>	<i>Sugarcane bagasse (%)</i>
100	0
0	100
50	50
75	25
25	75
Control: 80% bagasse + 10% lime + 10% crushed maize	

Table 2: Substrate mixtures assessed

Number of days taken for colonization of the fruiting bags, occurrence of contamination and yield of bags were observed.

3. Study of the effect of lime on potential mixtures of tea wastes and bagasse

As result of trial 2, substrate mixture containing 25% tea wastes and 75% sugarcane bagasse was selected for further trials. The selection was based on colonisation and fructification results. The effect of increasing level of lime on this substrate mixture was assessed in fruiting bags (Table 3). The experiment was made up of 9 treatments in a randomised block design with 4 blocks and 5 replicates of each treatment in each block.

Substrate mixture = 75 % sugarcane bagasse+ 25 % tea wastes	
Mixture (%)	Lime (%)
100.0	0.0
97.5	2.5
95.0	5.0
92.5	7.5
90.0	10.0
87.5	12.5
85.0	15.0
82.5	17.5
80.0	20.0

Table 3: Substrate mixture assessed

Parameters observed included number of days taken for colonisation of the fruiting bags, occurrence of contamination and yield of bags (number of mushrooms, total weight of mushrooms per fruiting bag)

4. Study of the effect of crushed maize on potential mixtures of tea wastes and bagasse

As result of trial 2, substrate mixture containing 25% tea wastes and 75% sugarcane bagasse was selected. The effect of increasing level of crushed maize as supplement on this substrate mixture was assessed in fruiting bags.

The experiment was made up of 9 treatments in a randomized block design with 4 blocks and 5 replicates of each treatment in each block (Table 4).

Substrate mixture = 75% sugarcane bagasse + 25% tea wastes	
Mixture (%)	Crushed maize (%)
100.0	0.0
97.5	2.5
95.0	5.0
92.5	7.5
90.0	10.0
87.5	12.5
85.0	15.0
82.5	17.5
80.0	20.0

Table 4: Substrate mixture assessed

Parameters observed included number of days taken for colonization of the fruiting bags, occurrence of contamination and yield of bags (number of mushrooms, total weight of mushrooms per fruiting bag).

RESULTS

1. *Mycelial growth of oyster mushroom on agar containing tea waste extract*

Petri dishes from the treatment containing tea wastes extract took 14 days for colonisation by oyster mushroom (*Pleurotus sajor caju*) mycelium compared to those with the standard treatment (without tea wastes extract) were colonised in 7 days. The petri dishes containing the standard treatment showed the traditional white linear mycelial growth that became cottony with time (Plate 6) while petri dishes containing agar with tea wastes extract showed white zonate, cottony mycelium (Plate 7). No initial radial growth was observed in treatment containing tea wastes extract. No contamination was observed in either treatment.



Plate 6: Mycelial growth of standard treatment



Plate 7: Mycelial growth of treatment containing tea wastes extract

2. Fruiting bag assessment of different mixtures of tea wastes and bagasse

Substrate mixture	Mean number of days for colonisation	Mean weight(g)	Mean number of mushrooms
100% bagasse	24 ± 1.3 ^{ab}	113.4 ± 13.4 ^b	15.4 ± 2.7
50% tea wastes + 50% sugarcane bagasse	42 ± 1.6 ^c	114.6 ± 6.6 ^b	18.2 ± 7.0
25% tea wastes + 75% sugarcane bagasse	30 ± 0.9 ^b	104.4 ± 10.1 ^b	12.2 ± 7.0
80% sugarcane bagasse + 10% lime + 10% crushed maize seeds (control)	21 ± 1.5 ^a	191.8 ± 17.1 ^a	20.2 ± 3.3
LSD	5.4	16.7	-

Table 5: Table of results

± Standard deviation

Means followed by a common letter are not significantly different at 5% level

Fruiting bags containing 100% tea wastes and those containing mixture of 75% tea wastes + 25% sugarcane bagasse showed poor mycelial growth that eventually stopped, leading to failure of colonisation. These fruiting bags were discarded. At 5% significance level, it was observed that substrate mixtures used had a significant effect on number of days taken for successful colonisation of fruiting bags by oyster mushroom mycelium (Table 5). At 5% significance level it was also observed that substrate mixture used had a significant effect on mean weight of mushrooms harvested whereas at 5% significance level, substrate mixtures did not have any significant effect on mean number of oyster mushroom harvested.

3. Study of the effect of lime on potential mixtures of tea waste and bagasse

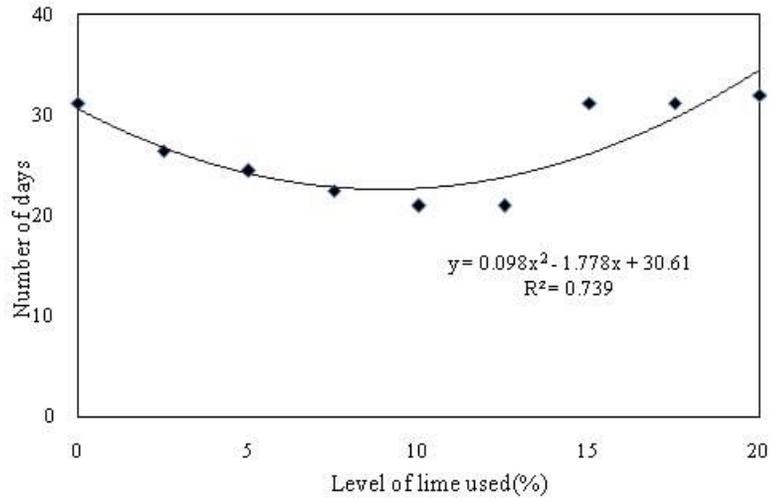


Figure 1: Colonisation of fruiting bags

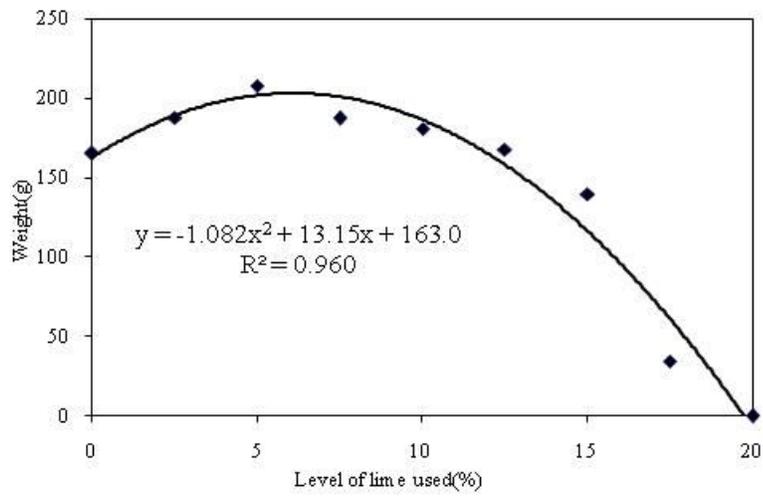


Figure 2: Weight of mushrooms harvested

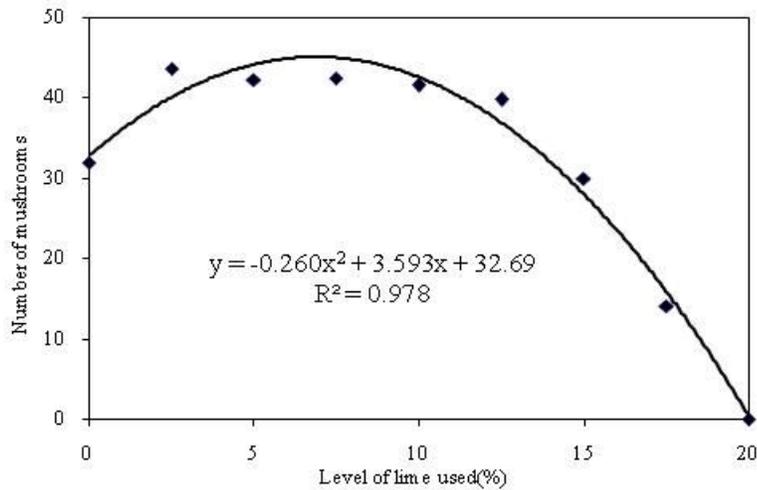


Figure 3: Number of mushrooms harvested

Level of lime (%)	pH
0	5.53 ± 0.01
2.5	7.26 ± 0.06
5	7.43 ± 0.02
7.5	7.93 ± 0.03
10	8.14 ± 0.02
12.5	8.71 ± 0.12
15	9.56 ± 0.03
17.5	10.13 ± 0.03
20	10.56 ± 0.02

Table 6: pH of substrate mixtures

The effect of level of lime on mean number of days taken for colonisation of fruiting bags is illustrated in Figure 1. The optimal level of lime is 9% in which fruiting bags take 23 days for colonisation. The effect of level of lime on mean weight of mushrooms harvested is illustrated in Figure 2. At 5% significance level, the level of lime used had a significant effect on weight of mushrooms harvested.



Plate 8: Mushrooms harvested at 5% lime

The effect of level of lime on mean number of mushrooms harvested is illustrated in Figure 3. At 5% significance level, level of lime used had a significant effect on mean number of fruits harvested.

4. *Study of the effect of crushed maize on potential mixtures of tea waste and bagasse*

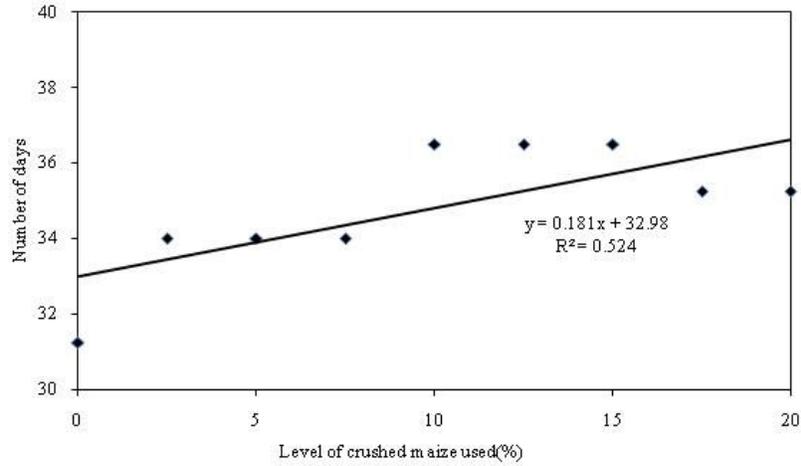


Figure 4: Colonisation of fruiting bags

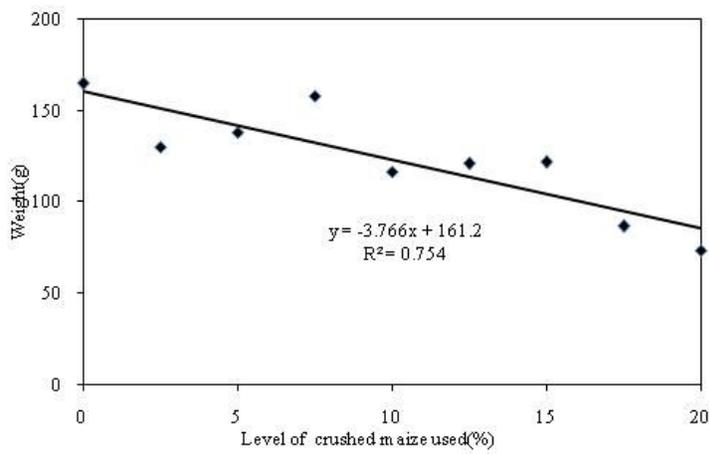


Figure 5: Mean weight of mushrooms harvested

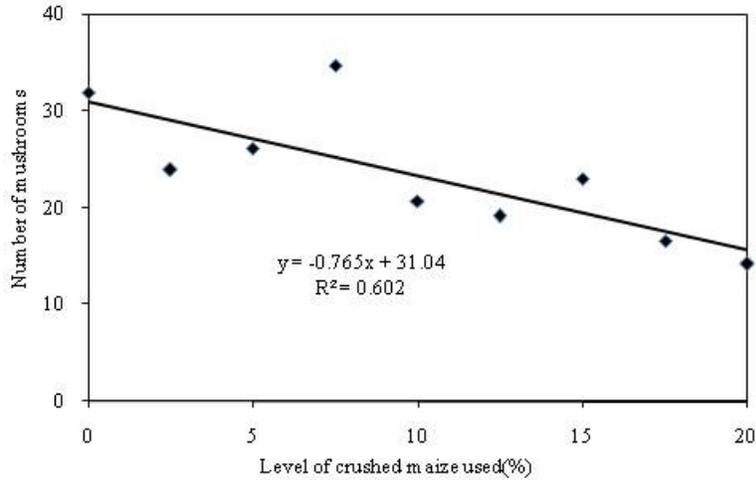


Figure 6: Mean number of mushrooms harvested

Level of crushed maize (%)	pH
0	5.47 ± 0.03
2.5	5.63 ± 0.04
5	5.66 ± 0.03
7.5	5.68 ± 0.04
10	5.74 ± 0.02
12.5	5.76 ± 0.03
15	6.04 ± 0.05
17.5	6.06 ± 0.03
20	6.34 ± 0.05

Table 7: pH of substrate mixtures

Figure 4 shows the effect of level of lime on mean number of days taken for colonisation of fruiting bag. The effect of level of crushed maize on mean weight of mushrooms harvested is illustrated in Figure 5. At 5% significance level, treatment had a significant effect on mean weight of mushrooms harvested.

The effect of level of crushed maize on mean number of mushrooms harvested is illustrated in Figure 6. At 5 % significance level, level of crushed maize used had a significant effect on number of mushrooms harvested.



Plate 9: Mushrooms harvested at 0% crushed maize

DISCUSSION

1. Mycelial growth of oyster mushroom on agar containing tea waste extract

Development of mycelium of oyster mushroom (*Pleurotus sajor caju*) on agar containing tea wastes extract indicates that the tea wastes extract are not lethal to the mushroom mycelium even though growth is slowed and distorted. The different growth patterns (Plates 6 & 7) and the extra number of days taken for colonisation as compared to standard treatment suggests that the antifungal substances present in tea wastes slow mycelial growth as discussed by Kharare *et al.* in 1994. The standard treatment (without tea wastes extract) showed the traditional growth of oyster mushroom of initial linear growth, changing to cottony growth with time. Zonate mycelial growth on agar containing tea wastes extract is an indication in natural changes in age of mushroom mycelium as described by Stamets (1993).

2. Fruiting bag assessment of different mixtures of tea wastes and bagasse

When mixtures of tea wastes and sugarcane bagasse were assessed, it was noted that the standard treatment (80% sugarcane bagasse + 10% lime + 10% crushed maize) yielded the highest weight of mushrooms in 3 flushes as compared to treatments containing tea wastes. Mixtures containing lower level of tea wastes showed higher yield than those containing higher levels of tea wastes. 100 % tea wastes failed to sustain mycelial growth and produce mushrooms since the substrate did not have sufficient celluloses and lignocelluloses necessary for mushroom development. This effect can be decreased by mixing tea wastes with a substrate rich in celluloses and lignocelluloses. This implies that tea wastes contain substances that slow mycelial growth while at the same time they contain insufficient amounts of celluloses and hemicelluloses necessary for mushroom production. Thus mushroom production was adversely affected.

3. Study of the effect of lime on potential mixtures of tea wastes and bagasse

All the treatments assessed contained the same proportions of substrate materials (75% sugarcane bagasse + 25% tea wastes). The highest yield obtained from substrate mixture supplemented with 5% lime can be associated to the pH which is near optimum (Table 6). Beyond the optimum, with increasing level of lime, the total weight of mushrooms harvested decreased. This decrease in weight of mushrooms harvested can be associated with the increasing pH that adversely affects fructification. The decrease in the number of mushrooms with increasing level of lime can be related to the fact that at higher pH, mushroom primordia are aborted and hence only the larger and most vigorous primordia survive.

4. Study of the effect of crushed maize on potential mixtures of tea waste and bagasse

Production of mushrooms in all the treatments could be correlated to successful colonisation and use of crushed maize as supplements that provided nutrients for mushroom growth and development. Fructification in substrate mixture without any crushed maize can be correlated to the important content of nitrogen in tea wastes. Highest yield was obtained in this mixture and the yield of mushrooms harvested decreases with increasing level of crushed maize as supplements. Use of crushed maize seeds does not affect pH of substrates considerably (Table 7). Excessive use of supplements does not necessarily lead to higher yields of mushroom. Decreasing yield with increasing level of crushed maize could be due to competition with other organisms. Mixture without crushed maize gave the highest number of mushrooms harvested and the number of mushrooms harvested decreases with increasing level of crushed maize seeds. Level of crushed maize used has a significant effect (at 5% level) on number of mushrooms harvested.

Mushroom quality

Fruiting bags supplemented with either maize or lime yielded mushrooms which were friable when humidification was carried out 3 times daily. The friable quality of mushrooms harvested from substrates containing tea wastes could be attributed to poor adaptation of mushroom mycelium on tea wastes since the spawns contained sugarcane bagasse. For further acclimatisation on tea wastes, it is proposed to produce mother spawn on tea wastes which can be used to inoculate spawn produced with tea wastes. This will ensure adaptation of *Pleurotus sajor caju* mycelium on tea wastes, hence improving yield and quality of mushrooms harvested from tea wastes substrates.

Comparison of effect of lime and crushed maize seeds

Substrate mixtures with increasing level of lime from 0 % to 20 % took fewer days for colonisation than the fruiting bags of treatments containing increasing level of crushed maize seeds and pH. The main reason behind the delayed colonisation in

substrates containing crushed maize seeds can be correlated with the slightly acidic nature of the substrates (pH range-5.47 to 6.34) containing crushed maize seeds. Substrates from treatments with increasing level of lime showed more rapid colonisation even though the pH was higher. This may be attributed to the fact that during colonisation, the pH is brought down to optimal as discussed by Cangy (1990).

Substrates from treatments containing lime gave higher mean weight of mushrooms than the treatments with crushed maize but mixtures containing 17.5 % lime and those containing 20% lime had lower mean yield than treatments with crushed maize. This could suggest that nitrogen supplementation may not necessarily increase the weight of mushrooms harvested whereas certain substrate materials rich in nitrogen such as tea wastes could boost yield of mushrooms.

On the whole, the treatments with substrates containing lime yielded more mushrooms as compared to treatments with crushed maize seeds as supplement. The number of mushrooms harvested can again be related to pH. Substrates with additions of lime were more alkaline as compared to substrates supplemented with crushed maize seeds. Acidity below optimum results in abortion of mushroom primordia.

CONCLUSION

From the results obtained from the trials that were set, it could be noted that opportunities exist for use of tea wastes in oyster mushroom cultivation in Mauritius only after mixing it with a cellulosic substrate. Results obtained in the experiment show that tea wastes contain antifungal substances that slows mycelial growth. A cultivation package of oyster mushroom on substrate mixture containing 75 % sugarcane bagasse + 25 % tea wastes supplemented with 5 % lime yielding about 200g of fresh oyster mushroom can be proposed to local mushroom growers since this package gives approximately the same yield as the standard treatment used in Mauritius (80 % sugarcane bagasse + 20 % crushed maize seeds + 10 % lime). The additional benefit of using tea wastes was that it excluded the need to add crushed maize in the substrate mixture. This would be a positive outcome since it will lower the cost of fruiting bag preparation since the cost of purchase of crushed maize has increased.

RECOMMENDATION

It is proposed that mixtures of tea wastes and sugarcane bagasse (1:3) can be supplemented with 5% lime. However, there is still the need to further decrease the volume of sugarcane bagasse used. Bagasse, being scarcer can be replaced with other potential substrates that can be obtained locally. Such potential substrate includes agro wastes are banana leaves, banana pseudo stems, corn wastes, coconut coir and grasses. Experiments could be set up to assess mixtures of tea wastes with the above mentioned substrates to decrease reliance on sugar cane bagasse.

The species of *Pleurotus* that was assessed was *sajor caju*. Since different species have different performances on particular substrates, other available species of *Pleurotus* can be assessed for their suitability of being grown on tea wastes substrates.

Other mushrooms still on trial at AREU such as Wood Ear Mushroom, Shiitake Mushroom and *Ganoderma* mushroom can be experimented using tea wastes as substrate, alone and in combination with other locally available substrates along with suitable supplements.

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