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Comparative study of growth and yield of edible mushrooms, *Schizophyllum commune* Fr., *Auricularia polytricha* (Mont.) Sacc. and *Lentinus squarrosulus* Mont. on lignocellulosic substrates

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Abstract

Schizophyllum commune Fr., *Auricularia polytricha* (Mont.) Sacc. and *Lentinus squarrosulus* Mont. are edible mushrooms which are also proven to be medicinally important. This study was carried out to investigate the potential of locally available substrates to grow these mushrooms. Alternative substrates including dried banana leaves, coconut leaves, paddy straw and coir dust were compared with sawdust (rubber) which is the commonly used substrate for commercial production of mushrooms. Banana leaves, coconut leaves and paddy straw were cut into 1 cm × 0.2 cm pieces. To each substrate, 10% (w/w) rice bran, 2% (w/w) $CaCO_3$ and 0.2% (w/w) $MgSO_4$ were added. The mixture was filled into 200 gauged polypropylene bags (22 cm × 12.5 cm) and autoclaved. Each Bag was inoculated with a 1 cm^2 block from the actively growing region of the mushroom culture maintained on PDA. After incubating under dark conditions at room temperature (28 ± 2 ^{0}C) and 78 – 80% relative humidity, mycelial growth rate and once fructification commenced, yield was determined. Highest rate for mycelial growth was observed in mixtures containing banana leaves for all three mushrooms. It was 10.345 ± 0.02 cm/week for *S. commune*, 7.818 ± 0.31 cm/week for *A. polytricha* and 10.895 ± 0.30 cm/week for *L. squarrosulus*. Highest mushroom yield for *S. commune* was obtained in coconut leaf (9.589 ± 0.66 g) and coir dust (9.182 ± 0.17 g) containing mixtures. A yield of 25.054 ± 5.18 g was recorded for *A. polytricha* in the medium prepared from banana leaves and sawdust (rubber) substrate was preferred by *L. squarrosulus* with a significantly higher yield of 54.079 ± 3.61 g.

Key words – alternative substrates – banana leaves – coconut leaves – mushroom yield – mycelial growth

Introduction

Edible mushrooms are the earliest form of microbial food known to mankind. They have gained wide popularity during recent times as "functional food" to complement and supplement a healthy diet as well as for their significant role in human disease control (Chang 1999, Khan et al. 2009).

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Schizophyllum commune Fr. is one of the commonly found fungi and has been isolated from all continents except Antarctica. The fungus usually grows abundantly during the rainy season and frequently appears on dead wood. It is an edible mushroom which belongs to the family Schizophyllaceae. *S. commune* is known to be a very good source of proteins, vitamins, lipids and mineral elements (Adejoye et al. 2007). The medicinal value of *S. commune* is also under investigation. A beta-glucan extracted from *S. commune* demonstrated potential anti-cancer effects when used in combination with other chemotherapeutic agents (Galena & Vaghefi 2008). *Auricularia polytricha* (Mont.) Sacc. is also known to be a famous medicine in China which is ideal for dietetic prevention of hyperlipldaemia (Yang et al. 2002, Xu & Yun 2003). It is rich in P, Mg, K and Se and with high dietary fiber content more than 50% of the net weight (Ghorai et al. 2009). Research on *Lentinus squarrosulus* Mont. has shown that it is also medicinally important due to its excellent antimicrobial activity (Giri et al. 2012). According to Jonathan et al. (2011), the fresh fruiting body of *L. squarrosulus* consists of 16.87% crude protein, 22% crude fat, 6.73% crude fiber and 6.88% sugars and Potassium which is the most abundant mineral element. The protein content of *L. squarrosulus* is reported to be six times that of oranges (Adesina et al. 2011).

Commercial production of fresh edible mushrooms is a rapidly growing industrial activity that can be carried out in a large or small scale. It is an efficient and relatively short biological process of food protein recovery from negative value lignocellulosic materials, utilizing the degrading capabilities of mushrooms (Martínez-Carrera et al. 2000).

This study investigated the potential use of locally available substrates to grow *S. commune*, *A. polytricha* and *L. squarrosulus* especially sawdust, a frequently used substrate for growth of edible mushrooms in Sri Lanka. These species are not commercially grown in Sri Lanka currently and has a potential to be a viable industry with the development of value added products.

Materials and methods

Mushroom strains

Fruiting bodies of *S. commune* and *A. polytricha* were collected from IFS Sam Popham Arboretum Dambulla, Sri Lanka and *L. squarrosulus* from Matara, Sri Lanka. Specimens were initially identified by comparing morphological characters with published keys and guides (Coomaraswamy 1979, Coomaraswamy & Kumarasingham 1988, Keizer 1998, Jordan 2004, Young 2005). Identity was confirmed by DNA extraction and sequencing (Ediriweera et al. 2014). The resultant sequences were analyzed and submitted to GenBank database (GenBank Accession Nos.: *S. commune* - KR706163, *A. polytricha* - KP943500 and *L. squarrosulus* - KP982902).

Tissue culturing was carried out in the laboratory where the stipe is surface sterilized and inner tissues (3 mm × 3 mm) were cut and placed on the Potato Dextrose Agar (PDA) containing petriplates. Cultures were incubated at room temperature and subsequently pure cultures were prepared by sub culturing into new PDA plates (Leon et al. 2009).

Effect of alternative substrates for mushroom production

Several alternative substrates such as dried banana leaves (BL), coconut leaves (CL), paddy straw (PS) and coir dust (CD) were compared with Rubber sawdust (SD/control). One kilogram of above substrates was used separately. Banana leaves, coconut leaves and paddy straw were cut into 1 cm × 0.2 cm pieces. To each substrate, 10% (w/w) rice bran, 2% (w/w) $CaCO_3$ and 0.2% (w/w) $MgSO_4$ were added. The mixture was filled into 200 gauged polypropylene bags with dimensions of 22 cm × 12.5 cm. The bags were autoclaved for 15 minutes at 121 ^{0}C / 15 psi and were left to cool overnight. Thereafter each bag was inoculated with a 1 cm^2 block from the actively growing region of the mushroom culture maintained on PDA. Three replicates were maintained for each treatment.

The inoculated bags were incubated under dark conditions at room temperature (28 ± 2 ^{0}C) and weekly mycelial growth was measured to determine the mycelial growth rate.

Yeild data

Growth rates were determined by measuring the mycelia growth front distal from the inoculum and by averaging the growth measurements at five equi-distant points around the circumference of each bag (Rajapakse et al. 2007). Subsequently the bags were cut opened and placed on a rack in a cool shed in which the humidity was well maintained (80 – 82%) at room temperature (28 ± 2 ^{0}C). Water was sprayed on the cut opened surfaces of the bag to induce fructification. Once the fructification commenced, fully grown fruiting bodies (four days after primordial emergence) were harvested. Fresh weight of each harvest/flush was recorded (Oghenekara et al. 2009). The weekly mushroom yield was calculated accordingly.

Statistical analysis

The experiment was laid on a completely randomized design. The data was analyzed using MINITAB 14 statistical package.

Results and Discussion

Highest rate for mycelia growth for *S. commune* (10.345 ± 0.02 cm/week), *A. polytricha* (7.818 ± 0.31 cm/week) and *L. squarrosulus* (10.895 ± 0.30 cm/week) was observed in mixtures containing banana leaves (Figure 1). Even though *L. squarrosulus* grow in all the used substrates *S. commune* showed no signs of mycelial growth in the medium containing paddy straws and *A. polytricha* did not grow in the substrate prepared by sawdust (Fig. 1).

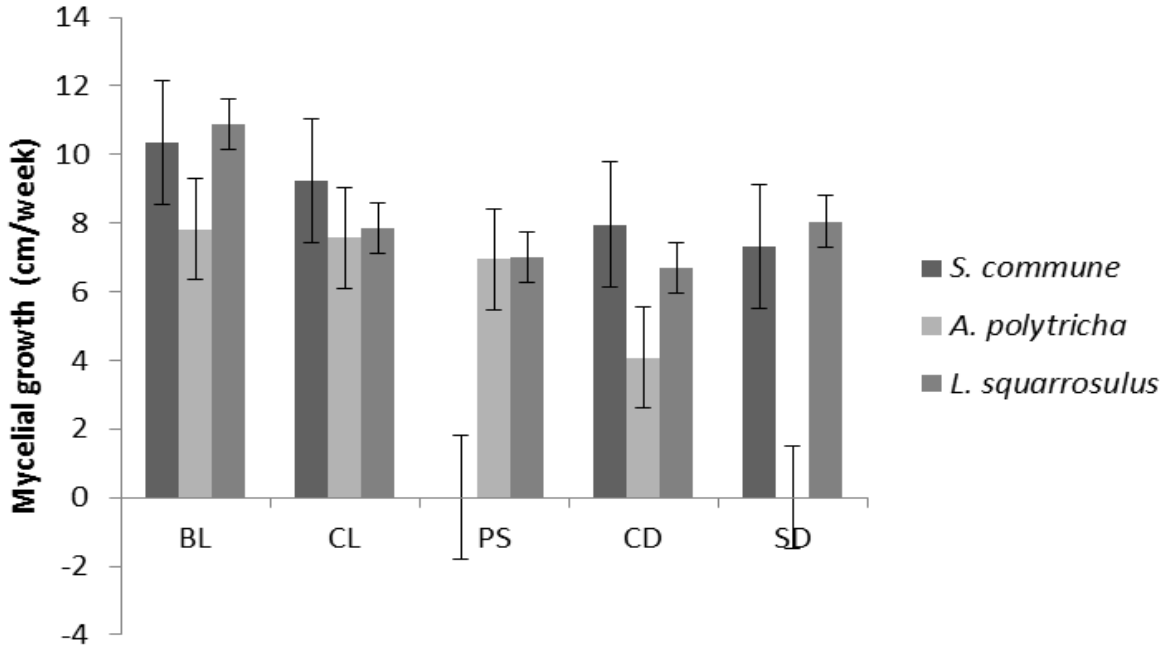

Fig. 1 – Mean mushroom mycelia growth in different mediums. BL - Banana leaves, CL - Coconut leaves, PS - Paddy straw, CD - Coir dust, SD - Sawdust (control)

Fruiting bodies of *S. commune* occurred after 20 – 25 days of inoculation and it took 8 – 15 days for one cycle production. Highest mushroom yield for *S. commune* was obtained for coconut leaves (9.589 ± 0.66 g) and coir dust (9.182 ± 0.17 g) containing mixtures which are significantly different from other used substrates (P = 0.05). Banana leaves containing substrate gave the highest yield for *A. polytricha* (25.054 ± 5.18 g) while paddy straw and coir dust containing media gave intermediate yields (Table 1, Figure 2). Fruiting bodies of *A. polytricha* occurred within 40 – 45 days of inoculation while one cycle of production is 20 – 25 days.

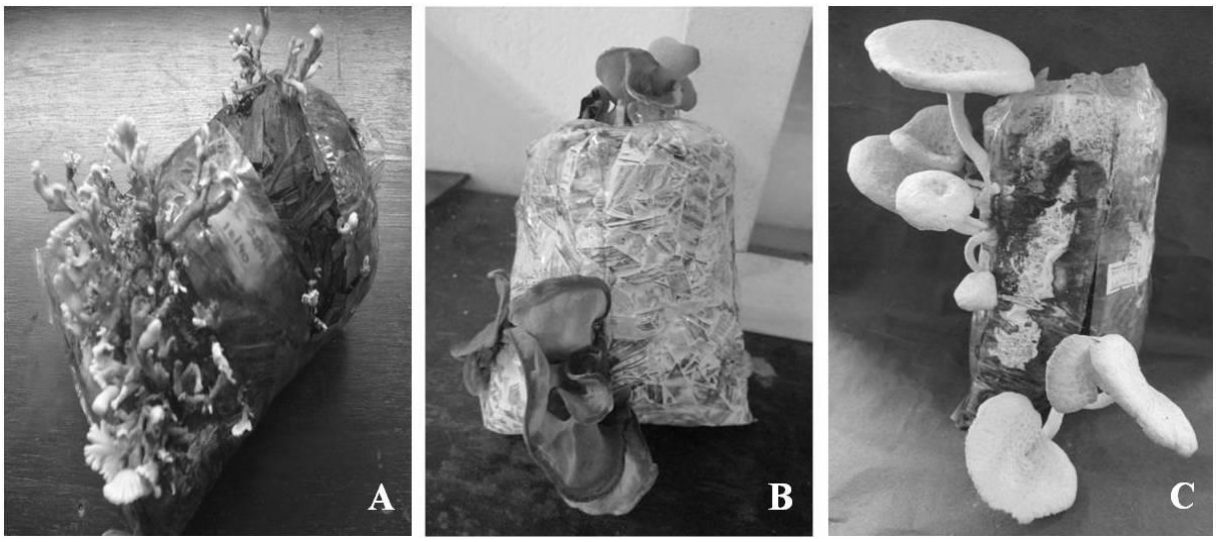

Fig. 2 – A. *S. commune* growing on the substrate prepared by coconut leaves, B. *A. polytricha* growing on the substrate prepared by banana leaves, C. *L. squarrosulus* growing on the substrate prepared by rubber sawdust.

When considering *L. squarrosulus*, most successful substrate was rubber sawdust containing medium with a significantly higher yield of 54.079 ± 3.61 g (Table 1, Figure 2). In *L. squarrosulus* 50 – 60 days were taken for initiation of fruiting after inoculation while one cycle of production is 15 – 20 days.

Table 1 Mean mushroom yield in different mediums

Main Ingredient in the medium	**Mean Yeild (g)**		
	S. commune	***A. polytricha***	***L. squarrosulus***
Banana leaves (BL)	$3.294^{a} \pm 0.03$	$25.054^{a} \pm 5.18$	$9.800^{a} \pm 2.55$
Coconut leaves (CL)	$9.589^{b} \pm 0.66$	$0.000^{b} \pm 0.00$	$0.000^{a} \pm 0.00$
Paddy Straw (PS)	$0.000^{c} \pm 0.00$	$9.946^{b} \pm 4.89$	$27.351^{b} \pm 5.17$
Coir dust (CD)	$9.182^{b} \pm 0.17$	$11.097^{ab} \pm 1.15$	$5.525^{a} \pm 1.28$
Saw dust (SD)	$3.726^{a} \pm 0.63$	$0.000^{b} \pm 0.00$	$54.079^{c} \pm 3.61$

Each data point represents the mean of 3 replicates ± standard error. Mean values denoted by the same letters along a column are not significantly different at 0.05 alpha levels.

In *A. polytricha* even though coconut leaves containing media exhibited highest mycelial growth rate, no fructification occurred in that medium. Similarly in a study carried out in Ghana for *Pleurotus ostreatus*, rice straw medium showed the highest yield while rice husk containing medium gave the fastest mycelia growth rate (Obodai et al. 2003). This indicates that mycelial growth and yield do not relate to each other since they have different requirements.

Same kind of study in Nigeria showed that *L. squarrosulus* can be cultivated in the laboratory on fruit tree waste especially the leaves of *Spondias mombin* and *Citrus sinensis* (Adesina et al. 2011). It was also successfully cultivated on cassava peels and *Andropogon* straw (Adesina et al. 2011).

To obtain a higher yield, the size of the bag could be increased, such as polypropylene bags with the dimensions 32.5 cm × 17.5 cm which is the recommended size for the commercial production of oyster mushrooms by the Department of Agriculture, Sri Lanka. In addition for *S. commune* bags with a smaller diameter can also be tested since the fruit body is relatively small. Furthermore substrate can be placed on shallow trays and the yield can be compared as it increases the surface area for fructification.

Substrate preparation is known as the "heart of process" thus a substrate that can provide adequate nutrients for mycelia growth and fructification plays a key role in determining the success of the cultivation (Rajapakse et al. 2007). Adding nutritional supplements can also induce a better yield. According to Oghenekaro et al. 2009 when *L. squarrosulus* was cultivated on the sawdust of five economic tropical tree species *Brachystegia nigerica* sawdust supplemented with 1% $CaCO_3$, 1% sugar and 20% wheat bran gave the highest yield. Some of the additives which were proven to be inducing fruiting are rice bran, cassava peels, carbohydrates such as glycogen, natural extracts like yeast and malt extract, ground pigeon pea, soybean, wheat, rye, millet, refined and crude vegetable oils and fish oil (Nawanze et al. 2005).

More experiments should be carried out based on these results for the production of reliable spawns so that this product can be commercialized. With the availability of a large amount of agricultural and forest waste as well as conducive climatic conditions this can be improved as a profitable industry in a country like Sri Lanka. It plays an important and a silent role as an environmental protection strategy. Most of the agricultural waste are either left in the field to rot or burned. By cultivating mushrooms on them those inedible wastes are transformed into edible biomass of high market value (Obodai et al. 2003). Furthermore the spent substrate from mushroom cultivation can be used as an animal feed (Obodai et al. 2003).

Based on mycelia growth rate and fruiting body yield obtained in this investigation, coconut leaves and coir dust based media can be recommended for the cultivation of *S. commune*, substrate made out of banana leaves for *A. polytricha* and rubber sawdust medium for *L. squarrosulus.*

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