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COMPARITIVE STUDY OF VARIOUS SUBSTRATE SUPPLEMENTS IN THE GROWTH AND YIELD OF *AGROCYBE AEGERITA*, BLACK POPLAR MUSHROOM

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ABSTRACT

The present study was conducted to standardize the spawn production and cultivation technology of *Agrocybe aegerita,* Black poplar mushroom. The mushroom culture procured was grown in Malt Agar (MA) medium and the culture was preserved in MA slants. The slant mushroom culture was used for the production of mushroom spawn. White sorghum seeds were used as a substrate to produce mushroom spawn. The quality spawn produced after complete mycelium spreading was used for the cultivation of mushroom in the mushroom cultivation chamber. The paddy straw substrate was supplemented with various amendments and the potency of each supplement in growth and yield of mushroom were studied. The various steps involved in the cultivation process was repeated and standardized for the optimum production of fruiting bodies with paddy straw as the substrate. The mushroom beds were monitored for their growth and fruiting bodies were harvested. The result indicated the rice bran supplementation of 10% to the paddy straw gave the maximum yield and bio efficiency in par with other supplements.

KEYWORDS: *Agrocybe aegerita***,** cultivation, spawn run, paddy straw, rice bran

INTRODUCTION

For centuries, mushrooms have been appreciated as sources of food nutrients and pharmacologically important compounds useful in medicine (Sagakami *et al*., 1991).

A mushroom is a macrofungus with a distinctive fleshy fruiting body that can be either hypogeous (underground) or epigeous (above ground), large enough to be picked up by hand (Kirk *et al*., 2001). Most of the edible mushrooms are basidiomycetes with the exceptions of the truffles and morels that are ascomycetes (Chang 1999;Yun and Hall, 2004). Within their varied natural habitats, fungi are usually the primary decomposers present, being of crucial importance in the breakdown of the vast amounts of organic carbon produced annually by photosynthesis, and thus are the main recyclers. However, they are also among the strongest and most aggressive opportunists, not restricting their habitats to naturally occurring dead wood and leaves (Dix and Webster, 1995).

Mushroom cultivation has also been successfully done on various industrial wastes (Singhal *et al*., 2005; Kulshreshtha *et al*., 2010; Dulay *et al*., 2012 and Kulshreshtha *et al*., 2013b). Applications of mushroom as mycoremediation tool in the bioconversion of these industrial wastes into protein rich mushroom carpophores (fruiting bodies of mushroom), on one hand provides mushroom and on the other hand helps in solving pollution problems, which their disposal may otherwise cause (Kulshreshtha *et al.*, 2014).

Mushroom is a tremendous boon to the idea of using this for mycoremediation process as a real-world solution. The cultivation of edible mushroom on agricultural and industrial wastes may thus be a value added process capable of converting these discharges, which are otherwise considered to be wastes, into foods and feeds (Kulshreshtha *et al.*, 2014).

For millennia, mushrooms have been valued as edible and healthy food, medical source, psychoactive drugs, and religious symbols for humankind (Alexopoulos *et al*., 1996, Rühl and Kües, 2007). Mushroom utilization as food or in medical application has been practised traditionally in Asian countries for centuries (Chang, 1993). A wide variety of fungi can recycle lignocellulosic waste materials into edible and/or medicinal mushrooms (Rühl and Kües, 2007). In the last years, with the popularization of mushroom farming, mushroom production is steadily increasing. It is estimated that more than 10,000 tones of edible and medicinal mushrooms were produced only in China in 2003, which is the leading country in mushroom production in the world (Chang, 2005). The environmental friendly cultivation of specialty mushrooms on lignocellulosic wastes represents one of the most economical and cost-effective organic recycling processes (Poppe, 2000).

Black poplar mushroom – *Agrocybe aegerita* (Brig.) Sing. (=*A. cylindracea* (Brig.) Sing) is an edible mushroom common in the forests of southern Europe, United States and similar climatic zones of the Far East. In nature, *A. aegerita* grows saprophytically on living and decaying stumps of mostly deciduous trees such as: poplar, willow, black poplar, ash, elderberry and black locust (Wright and Alberto, 2002). Black poplar mushroom is characterized by high content of protein, easily digested in human gastrointestinal tract (Bauer Petrovska and Kulevanova, 2000; Yildiz *et al*., 2005). Extracts from *Agrocybe aegerita* was found to have antioxidant properties (Cheung *et al*., 2003; Lo and Cheung, 2005; Tsai *et al*., 2007; Mujic *et al*., 2010).

Obtaining high yields and good quality of the carpophores is one of the most important issues modern mushroom growers focus on choosing the substrate for cultivation. This is because even well designed cultivation factors such as temperature, relative air humidity, CO_2 concentration and substrate moisture are insufficient when the substrate is either badly chosen or poor in nutrients. The Black poplar mushroom is a species with a wide spectrum of substrates that could be used for its cultivation such as: sawdust from broad–leaf trees, wheat straw, barley, rice husks, sunflower, cotton wastes or peanut shells (Philippoussis and Diamantopoulou, 2000; Philippoussis *et al*., 2001).

Black poplar mushroom is reported to utilize preferably cellulose before hemicelluloses and lignin, which might suggest that this species is a brown rot fungi (Wang *et al*., 2000; Kempken, 2002). The aim of this study was to determine the best substrate supplement for the cultivation of *A. aegerita* among rice bran, wheat bran and horse gram.

MATERIALS AND METHODS

Collection and Maintenance of *A. Aegerita* Culture

The pure culture of *A. aegerita* was obtained from the Directorate of Mushroom Research (DMR), Chambaghat, Solan, Himachal Pradesh and were used for mass culture production and utilized for spawn preparation and mushroom production. The culture was frequently sub cultured and maintained for further studies. The culture of *A. aegerita* was grown and preserved in Malt Agar (MA) medium. The prepared media was inoculated with 1 cm dia mycelial disc and incubated at room temperature for 12 - 15 days (Aprna Sabharwal and S Kapoor, 2013).

Spawn Preparation

The mushroom spawn was prepared on white sorghum grain. The mature grain procured from local market was well cleaned and boiled in water for 30 min. The boiled grain was mixed with 2% calcium carbonate. 300g of calcium carbonate mixed grain was filled in polypropylene bags of size 11 inch x 5 inch and sterilized for 15 psi for one hour. The sterilized bags were cooled to room temperature and inoculated with the mushroom culture maintained in slants (Ram *et al.*, 2013). The culture inoculated bags were kept undisturbed at room temperature for 30 - 35 days. After 30 - 35 days, completely mycelium spreaded spawn bags were ready for preparation of mushroom beds.

Bed Preparation

Beds were prepared using paddy straw as substrate following the method described by Sharma *et al.*, 2006 and Krishnakumari *et al.*, 2014 with certain modifications. Paddy straw was soaked in water overnight (12hours). After 12 hours, the water was drained and paddy straw was dried to 60 % moisture level in shade. 250g of shade dried paddy straw was filled in polypropylene bags (25 cm x 40 cm) with difference substrate supplements like rice bran powder, wheat bran powder and horse gram powder at 5% and 10 % of paddy straw content. All the supplements were prior sterilized in autoclave at 15 psi for 1hr. The bags filled with paddy straw and respective supplements at two concentrations serves as treatment and bags filled with paddy straw alone serves as control. All the substrate filled bags were plugged and sterilized at 15 psi for 1hr. The sterilized bags were left undisturbed for 24 hours.

Each of the sterilized bags were inoculated with 100g of well matured spawn and incubated at room temperature. The relative humidity of 80 – 85% was maintained constantly for each experiment. After complete spreading of mycelium, the polypropylene bags were opened and temperature of 24 $^{\circ}$C and relative humidity of more than 85% were maintained in the cultivation chamber. The bags were monitored regularly for any contamination and growth suppresion.

Days for spawn run, days for pin head formation and days for first, second and third harvest and total yield were recorded for all the bags of varied treatments and control. The bioefficiency of the mushroom on different substrate supplements were calculated. The morphological characters of mushrooms were also recorded.

The bioefficiency (%) of the mushroom can be calculated using the formula,

Bioefficiency (%) = Yield of fresh mushroom (g) / Total weight of dry substrate used (g) x100

RESULTS AND DISCUSSION

The present study was aimed in finding out the suitability of substrate supplement in the Growth and yield of *Agrocybe aegerita* black poplar mushroom. The morphological characters viz., mycelial character, presence of annulus, cap character and diameter, stem length and colour, arrangement of gills, spore characters were recorded. The mycelial character of *Agrocybe aegerita* was found to be cottony, linear, white colour initially and turned to brown to dark brown later on. The cap was hemispheric, smooth, and brown with diameter of 1.5 - 3 cm. The stem was creamy white in colour measuring 6 -10 cm in length. Gills were seen radiating from centre to periphery and of brown in colour.

The fig. 1 depicts the slant culture, mycelium spreaded pertriplate and well matured spawn of *Agrocybe aegerita*, Black poplar mushroom. The mycelium spreading bed, appearance of pin headed structures and fruiting bodies of *Agrocybe aegerita* was shown in fig. 2.

The observations of the growth pattern of *Agrocybe aegerita* at various stages are illustrated in table. I

Table I. Growth Pattern of *Agrocybe Aegerita*, Black Poplar Mushroom

S. No	Substrate	DFSR	DFPA	DFFH
1	Paddy straw	35.2 ± 7.07	44.93 ± 4.66	53.1 ± 3.14
2	Paddy straw + rice bran (5%)	40.05 ± 1.82	41.4 ± 5.43	45.43 ± 2.66
3	Paddy straw + rice bran (10%)	30.1 ± 5.47	35.5 ± 4.58	36.03 ± 3.45
4	Paddy straw + wheat bran (5%)	31.75 ± 5.17	42.63 ± 2.87	50.06 ± 3.85
5	Paddy straw + wheat bran (10%)	36.5 ± 5.19	41.57 ± 3.16	46.2 ± 3.16
6	Paddy straw + horse gram (5%)	33.41 ± 5.27	40.67 ± 4.25	47.3 ± 2.88
7	Paddy straw + horse gram (10%)	34.5 ± 3.61	39.4 ± 3.77	46.3 ± 1.04

All the values are expressed as mean ± SD of triplicates

DFSR - Days for spawn run DFFH - Day for 1st harvest DFPA - Days for pin headed appearance

The mushroom bed inoculated with 10% rice bran supplementation to the paddy straw showed minimum period for spawn run during 30.1 ± 5.47 days and also for pin headed formation during 35.5 ± 4.58 days consequently earlier harvest of mushrooms at 36.03 ± 3.45 days when compared with the other treatments and control. Rice bran 5% supplemented treatment showed complete spawn run at 40.05 ± 1.82 days, pin headed formation during

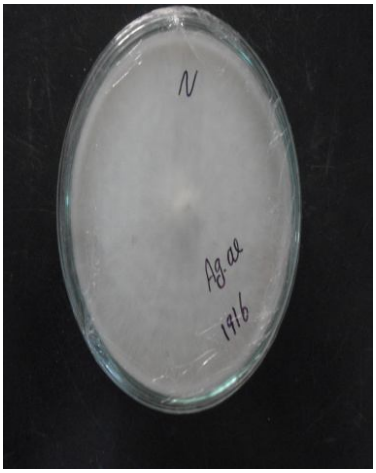
41.4 ± 5.43 and first harvest during 45.43 ± 2.66 days and this treatment showed shortest period for pin headed formation and fruiting body development.

Effect of various substrate supplements on the growth and yield of *Agrocybe aegerita*, Black poplar mushroom with their level of bioefficiency are tabulated in table. II

Table II. Yield pattern of *Agrocybe aegerita*, Black poplar mushroom

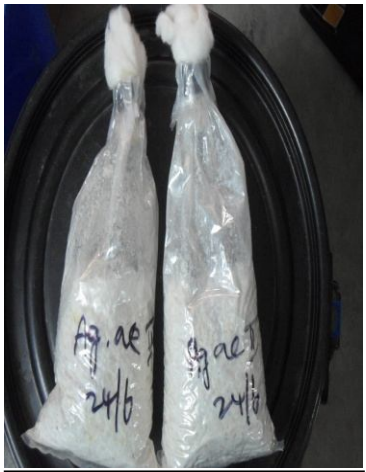
S.No	Substrate	Yield (g)			Total yield (g)	Bioefficiency(%)
		I	II	III		
1	Paddy straw	29.5 ± 3.12	29.17 ± 2.84	20.13 ± 4.74	78.79	31.52
2	Paddy straw + rice bran (5%)	57.3 ± 2.40	45.17 ± 4.16	40.63 ± 3.38	143.01	57.24
3	Paddy straw+ rice bran (10%)	65.33 ± 4.53	38.83 ± 5.01	44.4 ± 8.15	148.56	59.42
4	Paddy straw + wheat bran (5%)	53.73 ± 3.22	48.9 ± 3.21	18.86 ± 4.08	121.49	48.6
5	Paddy straw+ wheat bran (10%)	39.83 ± 3.05	31.53 ± 4.33	32.97 ± 2.45	104.33	41.73
6	Paddy straw + horse gram (5%)	48.1 ± 2.84	33.86 ± 3.46	24.6 ± 3.58	106.56	42.62
7	Paddy straw + horse gram (10%)	49.53 ± 3.61	34.5 ± 3.7	31.47 ± 3.5	115.49	46.2

All the values are expressed as mean ± SD of triplicates



Fully grown culture

Malt agar slants



Well matured spawn

Fig 1. Steps of *Agrocybe Aegerita* Spawn Production

Rice bran at 10% level supplementation with paddy straw gave more yield in the first (65.33 ± 4.53 g), second (38.83 ± 5.01 g) and third harvest (44.4 ± 8.15 g) consequently higher total yield (148.56 g) and enhanced bio efficiency (59.42 %) when compared with other treatments and control. This was followed by 5% supplementation of rice bran to the

substrate which gave 57.3 ± 2.40 g, 45.17 ± 4.16 g and 40.63 ± 3.38g in the first, second and third harvest. The total yield was 143.01 g with bioefficiency of 57.24%. Wheat bran (5%), horse gram (10%), horse gram (5%), wheat bran (10%) and paddy straw supplementations followed the 5% rice bran supplementation treatment for the harvest and bioefficiency of the mushroom.

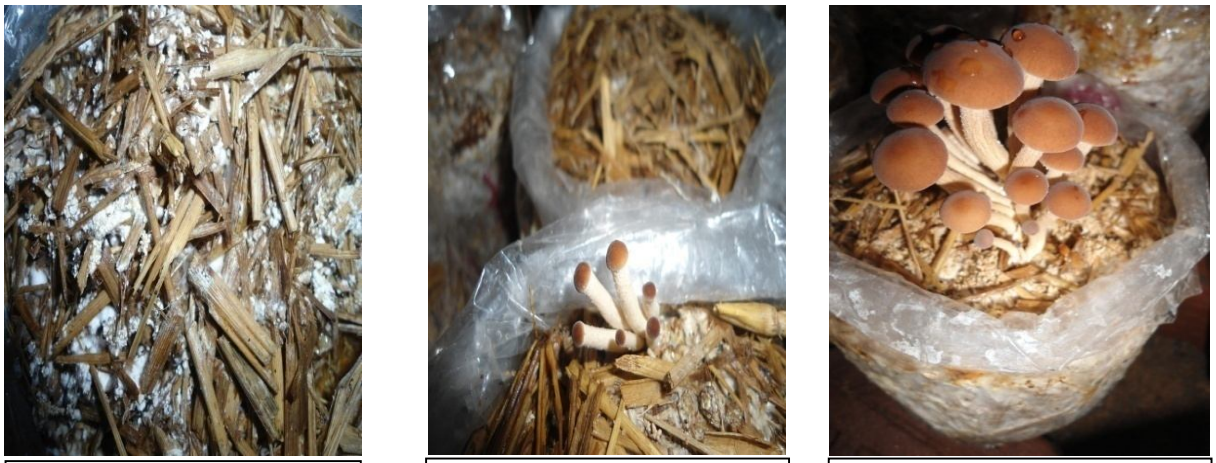


Fig 2. Various Stages of *Agrocybe Aegerita* Mushroom Growth

CONCLUSION

Mushroom cultivation is a useful method of environmental waste management and waste disposal. Many agricultural and industrial by-products can find uses in mushroom production (Chinda, and Chinda,2007)

Mushroom cultivation is highly suitable for Indian condition due to the availability of agricultural wastes in plenty, labour availability and varied agro-climatic conditions exists in different parts of the country. Considering many vital aspects, cultivation of medicinal mushrooms is a good avenue for the diversification of Indian horticulture and generation of employment opportunities (Veena SS and Meera Pandey, 2012).

The results showed that mycelial growth and yield of black poplar mushroom depended on the substrate and substrate supplement used in the experiment. The maximum yield of the mushroom was in the paddy straw substrate supplemented with the rice bran (10%). This black poplar mushroom, *Agrocybe aegerita* can be one of the mushrooms that can be grown for commercial purpose and exploitation of nutraceuticals.

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