International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(11) November -2015

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THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 11, pp. 7472-7476, November, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

EFFECT OF SPAWN MATURITY PERIOD ON THE GROWTH AND YIELD OF *AGROCYBE AEGERITA*, BLACK POPLAR MUSHROOM

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ARTICLE INFO

Article History:

Received 06th August, 2015
Received in revised form
14th September, 2015
Accepted 23rd October, 2015
Published online 28st November, 2015

Key words

Agrocybe aegerita, paddy straw substrate, spawn, steam sterilization, cultivation, bio-efficiency.

ABSTRACT

This study was designed with an objective of analysing the effect of spawn maturity period on the growth and yield of *Agrocybe aegerita*, black poplar mushroom. The spawn of *Agrocybe aegerita* was prepared and used at three different days of maturity viz. 25 days, 35 days and 45 days. The days of spawn run, pin headed appearance, first harvest, second harvest and third harvest were observed and recorded. Similarly, the effect of different days of spawn maturity on the yield and bio-efficiency of the mushroom were also analysed. The study showed that, beds inoculated with 35 days spawn gave more yield of mushrooms with higher bio-efficiency when compared to other two groups of spawn inoculated beds. This mushroom possess potent anti-cancer biochemical and phytochemical compounds which on cultivation yields great benefit to the society.

INTRODUCTION

Mushrooms are defined as macrofungi with distinctive and visible fruiting bodies that may grow above or below ground (Miles and Chang, 1997). It is estimated that there are approximately 1.5 million species of mushrooms in the world of which approximately 70,000 species are described. About 10,000 of the known species belong to the macrofungi of which about 5,000 species are edible and over 1,800 species are considered to have medicinal properties (Bratkovich and Stephen, 2004). Mushroom is being widely used as food and food supplements from ancient times. They are increasingly being recognized as one of the important food items for their significant roles in human health, nutrition and diseases (Chang, 1996). Experimental evidence indicates that mushrooms contain many biologically active components that offer health benefits and protection against degenerative diseases (Barros *et al*., 2008). Their biochemical composition, with significant contents of proteins, carbohydrates, lipids, enzymes, minerals, vitamins and water, has attracted attention also as functional health promoters (Chang, 2008). Mushrooms have also become an attractive source for the development of drugs and nutraceuticals (Lakhanpal and Rana, 2008).

Mushrooms are recognized as the alternative source of good quality protein and are capable of producing the highest quantity of protein per unit area and time from the worthless agro-wastes (Chadha and Sharma, 1995). Mushrooms can substantiate the sufferings from malnutrition to some extent, because they produce large quantities in a short time and provide more protein per unit area than other crops (Hossain *et al.,* 2007). Large quantities of agro-industrial wastes that are produced worldwide often cause environmental and health problems (Garg and Gupta, 2009). In addition, the ever-growing need of cheap nutritious food, and the lack of protein in developing countries led to the development of the mushroom cultivation industry (Sivaprakasam and Kandasawamy, 1981; Levanon *et al*., 1993; Yildiz *et al*., 1997; Croan, 2000; Zervakis *et al*., 2001). *Agrocybe aegerita*, an edible aromatic and flavoursome mushroom, is popular in Asia as a nutritional delicacy (Diyabalanage *et al*., 2008; Li *et al*., 2014). *A. aegerita* is rich in nutrient value with high protein and reduced fat content, and containing 8 kinds of essential amino acids and abundant vitamins and minerals like selenium, potassium. The fresh and dried fruiting bodies are eaten traditionally in meals or administered to patients with hypertension, cardiovascular disease and obesity in Chinese

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traditional medicine. In addition, co-products from this mushroom are used in the food industry (Brennan *et al*., 2012).

The basidiomycete *Agrocybe aegerita* is unique among the cultivated mushrooms, as it is easy to control its complete life cycle in *vitro* (Barroso *et al*., 1995). The black poplar mushroom, *Agrocybe aegerita*, is an edible basidiomycete belonging to the order Agaricales. It is one of the constituents in the popular gourmet mix produced by mushroom cultivators and known to possess anti-fungal and anti-tumor properties. Previous studies of the fruiting body of this species have reported the presence of palmitic acid, linoleic acid, ergosterol, mannitol and trehalose (Zang *et al*., 2003). The genus Agrocybe is also reported to contain several bioactive metabolites, such as indole derivatives with free radical-scavenging ability (Kim *et al*., 1997), polysaccharides with hypoglycemic activity (Tadashi *et al*., 1994) and agrocybin, a peptide with anti-fungal activity (Ngai *et al*., 2005).

MATERIALS AND METHODS

Mushroom culture

The mushroom culture of *Agrocybe aegerita* was procured from Directorate of Mushroom Research (DMR), Indian Council of Agricultural Research (ICAR), Chambaghat - 173213, Solan, Himachal Pradesh, India. The species was sub cultured and maintained in Potato dextrose agar medium (PDA) at room temperature as slants and in pertriplates (Sivaprakasam and Kandaswamy, 1983).

Mushroom spawn production

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. The culture inoculated bags were kept undisturbed at room temperature for 20 - 25 days. After complete mycelium spreading spawn bags were ready for preparation of mushroom beds.

Cultivation technology of Agrocybe aegerita, Black poplar mushroom (Krishnakumari et al., 2014)

Paddy straw aged between 3 to 9 months were chosen as the substrate for mushroom cultivation. The soil debris and unwanted materials should be removed from the paddy straw. After that paddy straw is to be cut into small pieces of 3 – 5 cm length using a paddy straw cutter or any small arrangement for cutting the paddy straw. The cut paddy straw is filled in perforated polyethylene sacs to half of its level and tied. The tied bags should be soaked in water overnight. Overnight soaked paddy straw was taken and washed thoroughly for the complete removal of the dust particles and the unwanted matters. The washed paddy straw is dried in a clean place free from dust. The dried paddy straw at 50-60% moisture content were packed in the polypropylene bags (25 cm x 40 cm). 250 g of dried paddy straw was packed in each bag and the bags were plugged with cotton and sterilized in the autoclave 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 for mycelium spreading. After complete spreading of mycelium, the polypropylene bags were opened and placed in the mushroom cultivation chamber for the growth of mushrooms. The chamber was maintained with a temperature of 24°C and relative humidity of more than 85%. The bags were monitored regularly for any contamination and growth suppression. The mushrooms after attaining full growth was harvested and used for the further studies. The average crop cycle was 60-70 days. The pin headed structures appear after complete mycelium spreading and fully grown mushrooms can be harvested at an average of three times. The total yield of the mushroom was recorded by calculating the weight of each mushroom harvested in all the three harvests. The bioefficiency of the mushrooms was calculated using the formula, Bioefficiency(%) = Yield of fresh mushroom (g) / Total weight of dry substrate used (g) x 100 .

Statistical analysis

Statistical comparison was done at significance level, $P<0.05$ using SPSS package version 20. One way ANOVA followed by post hoc analysis of DMRT was performed.

RESULTS AND DISCUSSION

The growth, yield and bioefficiency of *Agrocybe agerita* was studied with the inoculation of 25 days, 35 days and 45 days spawn in paddy straw substrates sterilized by steam sterilization method.

Table 1 Effect of days of spawn maturity on the growth of *Agrocybe aegerita*

Days of spawn maturity	DFSR	DFPA	DFFH	DFSH	DFTH
25	40.17 ± 0.76^{c}	50.5 ± 1.32^{c}	60.67 ± 1.53^{c}	70.17 ± 1.26^{c}	77.17 ± 2.02^{c}
35	35.2 ± 1.07^{a}	44.93 ± 1.66^{a}	53.1 ± 1.14^{a}	58.17 ± 0.76^{a}	63.17 ± 2.02^{a}
45	37.83 ± 1.04^{b}	48.17 ± 1.26^{b}	58.17 ± 0.76^{b}	63.5 ± 1.32^{b}	69.17 ± 0.76^{b}

All the values are expressed as mean ± SD; n=6 Mean values in the same column followed by different alphabets in the superscripts are significantly different ($P<0.05$, ANOVA, DMRT).

DFSR - Days for spawn run DFFH - Day for 1st harvest

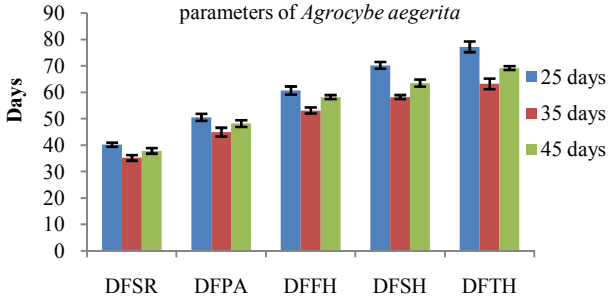
DFPA - Days for pin headed appearance DFSH - Day for 2nd harvest

DFTH - Day for 3rd harvest

The days for spawn run in 25 day spawn inoculated beds were during 40.17±0.76 days, in 35 day spawn inoculated beds were during 35.2±1.07 days and in 45 day spawn inoculated beds were during 37.83±1.04 days and all the values are significantly different. The days for pin headed appearance in 25 day spawn,

35 day spawn and 45 day spawn inoculated beds were during 50.5±1.32 days, 44.93±1.66 days and 48.17±1.26 days respectively and all the values are significantly different.

Fig. 1 Effect of spawn maturity period on the growth parameters of *Agrocybe aegerita*



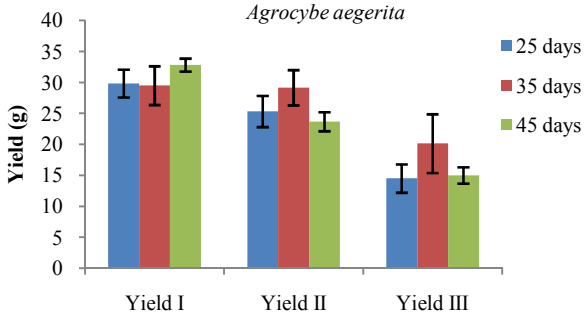
The days for first harvest in 25 days, 35 days and 45 days spawn inoculated beds were during 60.67±1.53 days, 53.1±1.14 days and 58.17±0.76 days respectively. The days of second harvest in 25 days, 35 days and 45 days spawn inoculated beds were during 70.17±1.26 days, 58.17±0.76 days and 63.5±1.32 days respectively. Similarly, the days of third harvest in 25 days, 35 days and 45 days spawn inoculated beds were during 77.17±2.02 days, 63.17±2.02 days and 69.17±0.76 days respectively. There observed a significant difference in the days for spawn run, days for pin headed appearance, days for first harvest, second harvest and third harvest between the beds inoculated with 25 days, 35 days and 45 days spawn. The age of spawn greatly determined the mycelium spreading in the beds of *Agrocybe agerita* and the 35 days spawn showed better efficiency in all growth parameters.

Table 2 Yield and bio efficiency of *Agrocybe aegerita* in paddy straw substrate

Days of spawn maturity	Yield			Total yield (g)	Bioefficiency (%)
	I	II	III		
25	29.83±2.25^{a}	25.33±2.52^{a}	14.5±2.29^{a}	69.66	27.86
35	29.5±3.12^{a}	29.17±2.84^{b}	20.13±4.74^{b}	78.79	31.52
45	32.83±1.04^{b}	23.67±1.53^{a}	15±1.32^{a}	71.5	28.60

All the values are expressed as mean ± SD; n=6
Mean values in the same column followed by different alphabets in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

Fig. 2 Effect of spawn maturity period on yield of *Agrocybe aegerita*



The first, second and third yield in 25 day spawn inoculated beds were 29.83±2.25g, 25.33±2.52g and 14.5±2.29g respectively with a total yield of 69.66 g and bioefficiency of 27.86%. The first, second and third yield in 35 days spawn inoculated beds were 29.5±3.12g, 29.17±2.84g and 20.13±4.74 g respectively with a total yield of 78.79g and bioefficiency of 31.52%. Similarly, the 45 day spawn inoculated beds yielded 32.83±1.04 g, 23.67±1.53g and 15±1.32g in the first, second and third yield respectively with a total yield of 71.5g and bioefficiency of 28.60%.

The 35 days spawn inoculated beds gave three yields of similar quantity with a total bioefficiency of 31.52% followed by 45 days spawn inoculated beds with a total bioefficiency of 28.60% and in 25 days spawn inoculated beds the bioefficiency was 27.86%. The bioefficiency of mushroom obtained in 25 days and 45 days spawn inoculated beds does not show significance rather both these groups are significant with 35 days spawn inoculated beds. Hence, the 35 day spawn has a great impact on the growth, yield and bioefficiency of *Agrocybe aegerita.* The high yield in 35 day old spawn inoculated beds can be correlated to the presence of high vigour mycelium in 35 day spawn which gave more yield of mushrooms. *Agrocybe aegerita* mushrooms have a unique flavor, are highly nutritious food and are of medicinal value (Zhao *et al*. 2003). Fruit bodies have been produced on several lignocellulosic based substrates consisting of barley, maize and wheat straw, cotton seed shells and sawdust, orange peel, grape stalk, reed, sunflower, cotton waste, peanut shells (Nicolini *et al*., 1987; Wang *et al*., 2000; Zervakis *et al*., 2001; Philippoussis *et al*., 2001). However, mushroom yields in *A. aegerita* are generally lower than yields reported for many other cultivated mushrooms; hence its large scale or commercial cultivation is not widespread (Wang *et al.,* 2000). *Agrocybe aegerita* is relatively understudied for its cultivation and biotechnological applications when compared to other mushrooms species of similar organoleptic properties. Ullrich and Hofrichter (2005) reported that during vegetative growth, *A. aegerita* produces a peroxidase which catalyzes the oxidation of 2,6-dimethoxyphenol or 2,20 -azinobis-(3-ethylbenzothiazoline-6-sulfonate).

CONCLUSION

Searching the equilibrium between the social, economic and environmental aspects, the reuse of wastes has taken on an extremely important dual purpose: elimination or reduction of wastes from the environment and giving them added value through the production of low cost food (Villas-Boas *et al*., 2002). Mushrooms, as an emerging high value crop, are gaining popularity in the world today with great opportunities for income generation. Mushroom cultivation is a profitable agribusiness. India has a great potential to cultivate mushrooms (Koshy, 2012). Cultivation of *A. aegerita* in large scale can provide a better bioremediation solution for lignocellulosic substrates and production of medicinally and nutritionally important food supplement for humans.

Acknowledgement

Our sincere gratitude to University Grants Commission (UGC), New Delhi, India (F. No. 42 - 648/2013(SR)) and Management of Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India for the financial support offered to carry out the research work.

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How to cite this article:
Kathiravan Subramanian, Krishnakumari Shanmugasundaram and Nagalakshmi Muthu., Effect of Spawn Maturity Period On The Growth And Yield of Agrocybe Aegerita, Black Poplar Mushroom. *International Journal of Recent Scientific Research Vol. 6, Issue, 11, pp. 7472-7476, November, 2015*

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ISSN 0976-3031