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RESEARCH ARTICLE

A STUDY ON THE GROWTH CHARACTERISTICS OF *PLEUROTUS SAJOR-CAJU* WITH VARYING SUBSTRATE STERILIZATION METHODS AND DAYS OF SPAWN MATURITY

Kathiravan, S.[1,2,*] and Krishnakumari, S.[1]

[1]Department of Biochemistry, Kongunadu Arts and Science College (Autonomous), Coimbatore – 641 029. Tamil Nadu, India.

[2]Department of Biochemistry, Dr. NGP Arts and Science College (Autonomous), Coimbatore – 641048, Tamil Nadu, India.

ABSTRACT

The research work carried out investigated the influence of various substrate sterilization methods and days of spawn maturity on the growth characteristics of *Pleurotus sajor-caju*, grey oyster mushroom. The paddy straw substrate was sterilized by various sterilization methods such as chemical sterilization, boiling water sterilization and steam sterilization. The spawn of the mushroom produced were used at three different days of maturity viz. 25 days, 35 days and 45 days. The substrate and spawn inoculated beds were maintained at defined environmental conditions in the mushroom cultivation chamber and the growth performance were monitored and the characteristics were recorded. The study revealed that the steam sterilized substrate and the 35 days old spawn gave the maximum yield compared to the beds inoculated with substrates sterilized with other two methods of sterilization and spawn of 25 days and 45 days old. The results gave a reliable information regarding the preference of the substrate sterilization method and the days of spawn maturity for the cultivation of the *Pleurotus sajor-caju*, grey oyster mushroom.

Keywords: *Pleurotus sajor-caju*, spawn run, paddy straw, sterilization, cultivation.

1. INTRODUCTION

Mushrooms are defined as macrofungi with distinctive and visible fruiting bodies that may grow above or below ground (1). Higher Basidiomycetes represent a taxonomically, ecologically, and physiologically extremely diverse group of eukaryotic organisms. Recently, extensive research on these fungi has markedly increased, mainly due to their potential use in a variety of bio¬technological applications, particularly for the pro¬duction of food, enzymes, dietary supplements, and pharmaceutical compounds (2-4). 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 areconsidered to have medicinal properties (5).

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 (6). 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 (7). 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 (8).

Edible mushroomsare widely consumed in many countries as a food. Owing to their attractive taste, aroma and nutritional values, edible mushrooms are valuable components of the diet, whose culinary and commercial value is mainly due to theirorganoleptic properties such as their texture and flavour, being possible to distinguish edible mushroom species on the basis of their characteristic odour or aroma (9,10). Their nutritional value is due to high protein, fiber, vitamin and mineral contents and a low-fat level (11-14). The amino acid compositions of mushroom proteins are comparable to animal protein (15,16) which is of particular importance to counterbalance a high consumption of protein animal food sources, especially in developed countries. In addition, edible mushroomscharacteristically contain many different bioactive compounds such as eritadenine and phenolic compounds (13,14,17).

Cultivation of edible mushrooms might be the only current process that combines the production of protein-rich food with the reduction of environmental pollution (18). It represents one of the most efficient biotechnological processes for lignocellulosic organic waste recycling (19).

*Correspondence: S. Kathiravan, Department of Biochemistry, Dr. NGP Arts and Science College (Autonomous), Coimbatore – 641048. Tamil Nadu, India. E.mail: kathiravanbiochem@gmail.com

The present study was designed to study the effect of various substrate sterilization methods viz. chemical sterilization, boiling water sterilization and steam sterilization and days of spawn maturity on the growth parameters of *Pleurotus sajor-caju*.

2. MATERIALS AND METHODS

2.1. Mushroom culture

The culture of *Pleurotus sajor-caju* was procured from Vijaya Mushrooms, Coimbatore. The species was sub cultured and maintained in Potato dextrose agar medium at room temperature as slants and in pertriplates (20).

2.2. Mushroom spawn production (21)

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 11inch 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 mycelium running. After mycelium running, spawn at different age levels viz. 25 days, 35 days and 45 days were used for preparation of mushroom beds.

2.3. Cultivation technology (22)

Paddy straw was chosen as the substrate for cultivation of P.sajor-caju. The cut paddy straw was soaked in water overnight and washed in water thoroughly. Sterilization is being done in three ways. In chemical sterilization of paddy straw, chopped paddy straw was soaked in water containing formalin, bavastin and malathion overnight. In boiling water sterilization, Paddy straw was allowed to boil in water for 45 min. In steam sterilization of substrate, the washed paddy straw was steam sterilized for 45 min and shade dried. The matured spawn of *Pleurotus sajor-caju* was taken and dispersed carefully in a sterile bowl. The polypropylene bag was taken and initially, a handful of paddy straw was taken and dispersed at the bottom of the bag which forms the first layer. A handful of spawn was taken and dispersed over the first layer of paddy straw. Thus, first layer is made. Next, a layer of paddy straw was made with the spawn spreaded over it. Likewise, alternate layers were made with spawn and paddy straw. The packed bag was tied with the rope and hanged in the mushroom unit for mycelium spreading. The mushroom unit was maintained at a temperature of 18 – 23 °C.

Bioefficiency (%) = Yield of fresh mushroom (g) / Total weight of dry substrate used (g) x 100

2.4. Statistical analysis

Statistical analysis was carried out at 5% significance level using SPSS package version 20.0. One way ANOVA followed by DMRT analysis of LSD was done.

3. RESULTS AND DISCUSSION

The cultivation of *Pleurotus sajor-caju* was done by following three different substrate sterilization methods viz. Chemical sterilization, boiling water sterilization and steam sterilization. The spawn used for cultivation were of 25 days, 35 days and 45 days old spawn. The days of spawn run, days of pin headed appearance, days for first harvest, days for second harvest, days for third harvestwere recorded and tabulated as follows.

Table. 1 Effect of various substrate sterilization methods and days of spawn maturity on the days for spawn run of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	15.17 ± 0.29^{be}	14.83 ± 0.76^{be}	13.83 ± 1.04^{bd}
35	14.17 ± 0.58^{af}	13.33 ± 0.29^{ae}	12.67 ± 0.29^{ad}
45	14.83 ± 0.29^{be}	14.17 ± 0.76^{be}	13 ± 0.87^{abd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different ($P<0.05$, ANOVA, DMRT).

The days for spawn run of *Pleurotus sajor-caju* in the packed mushroom beds varied with different substrate sterilization methods and also with the different age of spawn. In the 25 day aged spawn inoculated beds, spawn run took 15.17±0.29 days in chemically sterilized substrate, 14.83±0.76 days in boiling water sterilized substrate and 13.83±1.04 days in steam sterilized substrate. In the 35 day aged spawn inoculated mushroom beds; the spawn run was on 14.17±0.58days in chemically sterilized substrate, 13.33±0.29days in boiling water sterilized substrate and 12.67±0.29

days in steam sterilized substrate. Similarly, the 45 day aged spawn inoculated beds took 14.83±0.29days, 14.17±0.76 days, and 13±0.87 days for spawn in chemically sterilized substrate, boiling water sterilized substrate and steam sterilized substrate respectively. The days of spawn run in the 35 day aged spawn inoculated beds with paddy straw sterilized chemically was significant with the days of spawn run recorded in 25 day and 45 day aged spawn inoculated beds with substrate sterilized chemically. Similarly, days for spawn run in the 35 day aged spawn inoculated beds with boiling water sterilized substrate was also statistically significant with that of 45 day and 25 day aged spawn inoculated beds. The days of spawn run in the 35 day aged spawn inoculated beds with steam sterilized substrate was almost same with that of 45 day aged spawn inoculated and does show any significance, whereas it was significant with 25 day aged spawn inoculated beds.

Table. 2 Effect of various substrate sterilization methods and days of spawn maturity on the days for pin headed appearance of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	16.67±0.29^{be}	16.67±0.29^{ce}	15.17±0.76^{bd}
35	15.33±0.29^{af}	14.83±0.29^{ae}	14.33±0.29^{ad}
45	16.67±0.29^{be}	15.67±0.76^{bd}	15.17±0.29^{bd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

The days of pin headed appearance in the beds with substrate sterilized by three different methods and effect of age of spawn maturity on the same was tabulated in the table.19. The 35 day aged spawn inoculated beds showed pin headed structures soon as compared to 25 day and 45 day aged spawn inoculated beds. 35 day aged spawn inoculated beds showed pin headed structures during 15.33±0.29 days, 14.83±0.29 days and 14.33±0.29 days in chemical, boiling water and steam sterilized substrates respectively, whereas 45 day aged spawn inoculated beds pin headed appearance took place during 16.67±0.29 days, 15.67±0.76 days and 15.17±0.29 days in chemical, boiling water and steam sterilized substrates respectively. Similarly, in the 25 day aged spawn inoculated beds, the pin headed structures appeared in the range of 15.17±0.76days to 16.67±0.29 days. Among the methods of substrate sterilization, steam sterilization was found to be effective as pin headed structures appeared first only in the steam sterilized substrate bed irrespective of spawn age and as age of spawn maturity is concerned, 35 day aged spawn was vigour enough in producing pin headed structures before 45 day and 25 day aged spawn irrespective of substrate sterilization method. The days of pin headed appearance in the 35 day aged spawn inoculated beds with substrates sterilized by all the three methods were statistically different with that of 45 day and 25 day aged spawn inoculated beds of all three substrate sterilization methods.

Table.3 Effect of various substrate sterilization methods and days of spawn maturity on the days for first harvest of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	17.83±0.76^{be}	17.67±0.29^{ce}	16.67±1.04^{bd}
35	16.5±0.5^{ae}	15.83±0.76^{ad}	15.5±0.5^{ad}
45	17.83±0.29^{be}	16.5±0.5^{bd}	16.83±0.29^{bd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

Table. 4 Effect of various substrate sterilization methods and days of spawn maturity on the days for second harvest of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	26.17±1.61^{bf}	24.33±1.04^{be}	22.33±1.04^{bd}
35	23.17±1.04^{af}	21.83±1.04^{ae}	20.17±0.76^{ad}
45	25±0.5^{be}	21.67±0.76^{ad}	21.83±1.04^{bd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

The days of first, second and third harvest of *Pleurotus sajor-caju* in all the three different substrate sterilization methods and effect of different spawn age on the production was tabulated in Tables 3,4 and 5.

3.1. Chemical sterilization method

In the 25 day aged spawn inoculated beds, the first harvest were during17.83±0.76days, second harvest during 26.17±1.61days and third harvest during 29.17±0.76days, where as in 35 day spawn inoculated beds, the three harvests were on a range from 16.5±0.5 days to 26.83±1.26days, for that of 45 day aged spawn the range of all the three harvests were from 17.83±0.29days to 28.17±0.76days.

Table. 5 Effect of various substrate sterilization methods and days of spawn maturity on the days for third harvest of *Pleurotus sajor-caju*

Days of spawn maturity	Chemical sterilization	Boiling water sterilization	Steam sterilization
25	29.17±0.76^{be}	28.17±0.76^{bd}	27.83±0.76^{bd}
35	26.83±1.26^{ad}	27.5±0.5^{ad}	26.67±1.04^{ad}
45	28.17±0.76^{bd}	27.83±0.29^{abd}	27.5±0.87^{abd}

All the values are expressed as mean ± SD; n=6

Mean values in the same column followed by different alphabets (a-c) and mean values followed by different alphabets (d-f) in the same row in the superscripts are significantly different (P<0.05, ANOVA, DMRT).

3.2. Boiling water sterilization method

The first, second and third harvests in the 25 day aged spawn inoculated beds were during 17.67±0.29days, 24.33±1.04days and 28.17±0.76 days respectively. In 35 day aged spawn inoculated beds; first, second and third harvests were during 15.83±0.76days, 21.83±1.04days and 27.5±0.5days respectively. For 45 days aged spawn inoculated beds, the three harvests ranged from 16.5±0.5days to 27.83±0.29days.

3.3. Steam sterilization method

In the steam sterilization method, the first, second and third harvests of the mushroom in the 35 day aged spawn inoculated beds were during 15.5±0.5 days, 20.17±0.76 days and 26.67±1.04 days respectively. In 25 day aged spawn inoculated beds; first, second and third harvests were during 16.67±1.04 days, 22.33±1.04 days and 27.83±0.76 days respectively. In 45 day aged spawn inoculated beds, the three harvests ranged from 16.83±0.29 days to27.5±0.87 days.

In the first harvest, days of harvest in the 35 day aged spawn inoculated beds with substrates sterilized by all the three methods were almost significant with that of the days of harvest recorded in the 45 day and 25 day aged spawn inoculate beds with substrates sterilized by all the three methods.

In the second harvest also, the days of harvest in the 35 day aged spawn inoculated beds of all the three substrate sterilization methods were almost significant with that of the 45 day and 25 day aged spawn inoculated beds.

Like days of first and second harvest, days of harvest in 35 day aged spawn inoculated beds were almost significant with that of rest of the treatments.

Chang and Hayes, 1978 (23) stated that successful cultivation of mushroom often requires pasteurization of the substrate, prior to inoculation with spawn. Among all the three substrate sterilization methods, steam sterilized substrate gave better results followed by boiling water and chemical sterilized substrates. The better results of *Pleurotus sajor-caju* in steam sterilized substrate can be attributed to the fact that steam sterilized substrate retains all the nutrients and makes it available to the mushroom growth.

4. CONCLUSION

Biological approaches based on industrial and environmental biotechnology is focusing on the development of “clean technologies" which emphasizes on the maximum production, reduced waste generation, treatment and conversion of waste in some useful form (24). Mushroom cultivation is one of such component which addresses the issues of waste utilization, nutritional product formation and economical stability for the population involved in it.

The cultivation of oyster mushrooms is popular in our country and it has more advantages than any other types of mushroom in the way that it is disease resistant, simpler cultivation technology, wide variety of substrate utilization and gained popularity among the common mass. So the substrate sterilization methods also play a role in the growth of the mushrooms. As an inference of this study, it is clear that steam sterilized substrate is better for the optimum growth of mushrooms in a more convenient way.

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