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# Effect of Natural Probiotic Supplementary Feed and Consequent Cultivation of *Pleurotus sajor-caju* (Berks and Br.)

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**Abstract:** *Plerrotus sajor-caju* is an edible mushroom that highly appreciated and has a commercial potential in many countries. *P. sajor-caju* was cultivated and with the help of four altered substrates. This study shows that the effect of four different kinds of supplementary diets, mycelium linear growth, quantity of sporocarp, moisture content level yield, chemical constituents and chemical composition of fruiting bodies were estimated. The mycelium linear growth was 1.9, 1.7 and 1.6 mm day$^{-1}$ with no significant differences at level of 10% bran addition to sawdust, soybean straw, sugar cane bagasse and rice straw, respectively. The data declare that moisture content of fruit bodies ranged between 87.63 and 90.26% and no significant differences were detected between those grown on sawdust, soybean straw and sugarcane bagasse. The obtained data show that soybean, contained significantly highest amount being (281. 45 g) followed by sawdust being 216.90 g while rice straw sugar cane bagasse recorded significantly lower values being 38.60 and 30.00, respectively.

**Key words:** *Pleurotus sajor-caju*, rice bran, paddy straw, saw dust, sugarcane bagasse, yield

## INTRODUCTION

The earliest times, mushrooms have been treated as a special kind of food, it has been also considered as the oldest microbial food (Magnigo *et al*., 2004). Mushroom is an attractive crop to cultivate in developing countries for many reasons. Very recently, one of the most charming rounds would be that they are grown on agricultural wastes (Alam *et al*., 2010). It enables us to acquire substrate materials at low prices or even for free and to conserve the environment by recycling wastes (Singh *et al*., 1989). Most of all oyster mushroom (*Pleurotus* sp.). Formerly, Caglarlrmak (2007) reported that mushrooms are good source of vitamins and minerals. Although, mushrooms cannot be an alternative protein source instead of meat fish and egg. Agricultural residues have been used to produce the edible mushroom *Pleurotus* sp., also known as oyster mushroom, hiratake, shimeji or houbitake (Chang, 2007).

Several researchers had been depicted the *Pleurotus sajor-caju* mushroom successfully cultivated on many agricultural and agro-industrial wastes including various sawdust, wheat straw, cotton waste, peanut shells, sugar cane bagasse, wheat, rice bran, millet straw and soybean straw (Peng *et al*., 2000; Moda *et al*., 2005; Okano *et al*., 2007; Alam *et al*., 2008). The cultivation of oyster mushrooms is gaining importance in tropical and subtropical region due to its simple way of cultivation and high biological efficiency in paddy straw which gave a good yield than other substrates and useful for the degradation and recycle of biowastes (Peng *et al*., 2000; Alam *et al*., 2008).

The mostly cultivating *Pleurotus* sp., in India is *P. djamor*, *P. citrinopileatus*, *P. flabellatus*, *P. oystereatus*, *P. sapidus*, *P. sajor-caju* and *P. florida* (Kirbag and Akyuz, 2008a, b). *Pleurotus sajor-caju* is having high nutritional value potential of host mediated response and may be act as antimicrobial agent (Hassan, 2007). Besides these mushroom have been used extensively in traditional, medicine for curing various types of bacterial infection, gastrointestinal disorder, bleeding, high blood pressure, etc. (Oei, 2003). Mycelium growth and the yield of *P. sajorcaju* greatly affected by the supplementation type and rate for the media substrate (Oei, 2003). The daily intake of this mushrooms helpful in combating common bacterial infections and also strengthens the immune system. Previously (Chang, 2007) demonstrated that many of mushrooms produce a range of metabolites of intense interest to the nutraceutical and pharmaceutical (e.g., anti-tumor, immunomodulation agents and hypocholesterolaemic agents) and food (e.g., flavor compound) industries. Most of the mushroom species does not contain biologically active polysaccharides fruiting bodies, submerged broth are

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sources of the bioactive compounds (Stamets and Chilton, 1983; Caglarlrmak, 2007). Among higher fungi group Pleurotus is well acknowledged as an economically important genus. This may be attributed to its world-wide dispersal, its broad adaptability in growing under various conditions and of course to its dietetic properties. Thus, Pleurotus mushrooms were now cultivated with the help of many agricultural wastes and industrial by products providing nutritious foods (Synytsya *et al*., 2008).

Amendments or mixtures of various straws were tried to assess possibilities for main substrates to increase the yield of *P. sajor-caju*. The objective of the present study is to compare the effect of rice bran in combination with paddy stray and saw dust in the cultivation of *Pleurotus sajor-caju*. Still now, number of researches have been done this mushroom though ample of study were conducted this kind of mushroom species. Hence, the present study having the main objective is evaluating the growth of the *P. sajor-caju* with four different supplementary medium and its chemical constituents, quantity of sporocarp production, etc.

## MATERIALS AND METHODS

**Source of oyster mushroom:** Spawn of *P. sajor-caju* was purchased from Krishi Vigyan Kendra, Mitraniketan and Vellanad. The culture was used for producing the grain spawn by the convenient method. The prepared spawn were stored at 40°C until using for cultivation.

**Processing of substrate:** Large bundle of paddy straw were spread and dried well under sun for 3 days. It was stored in moisture free atmosphere as stock. From that 1 kg of straw was chopped into 3 inch pieces and dipped in clear water for 12 h. Then the straw was autoclaved at 121°C and 15 pounds per square inch for 11/2 h. Then this straw was spread out on a clean platform for cooling and controls the moisture level.

**Bagging of substrate and spawning:** About 1 kg of dry substrate was used in each bag. Size and gauge of the polythene bag used was 12×20 and 100, respectively. Spawning was done in five layers at the rate of 3% of net substrate (30 g spawn). Spawning was inoculated into substrate bag manually after disinfecting the bottle necks with an alcohol flame and with disinfected gloves. After filling, the bed is pressed to make it compact and the top is tightly covered. A small pinch of cotton is plugged in all tiny pores of polythene tube inorder to avoid the insect attack before spawn run fluctuation. These bags were incubated at room temperature (27±2°C) in a dark room. After the completion of spawn run, the bed was transported to cropping room. When pin head appears upper (anterior portion) and lower parts of polythene cover is split with a razor blade to expose the mycelium and to encourage the transformation of primordial (pin head) into mature fruiting bodies. Cropping room should be maintained with proper ventilation and humidity. Humidity was maintained by spraying water 3-4 times a day.

**Fruiting and harvest:** After opening the bags, primordial (pin head) started to form and were ready for harvesting in another 2 days. The mushroom was collected in 3 flushes and after each flush a small layer of substrate was scrapped off from all the side of the substrate and kept it without watering for 2 days. After 2 days, watering continued till the harvest.

**Growing media and supplementation level:** Sawdust, soybean straw, sugar cane bagasse and rice straw were used as substrate growing media, wheat bran supplementation level and mycelium linear growth were determined. This experiment was carried out to determine the most suitable wheat bran supplementation ratio on the mycelium growth of *P. sajor-caju* on different substrate formulae each medium substrate was mixed with 5% Calcium Carbonate ($CaCO_3$), supplemented with (5, 10 15, 20, 25, 30 and 35%) wheat bran and moisture content was adjusted to be approximately 69-71% then the mixture was packaged in polypropylene bags (10 bags, 2 kg for each). The bags were autoclaved at 121°C for 1 h.

After the bags were cooled down to room temperature they were inoculated with *P. sajor-caju* using previously prepared grain spawn at rate of 4% incubated at 25-27°C after 5 days from inoculation (base line) the mycelium linear growth was measured in mm day$^{-1}$. The gathered data were statistically analyzed. The suitable supplementation level for each substrate was used for the further experiment for the cultivation of *P. sajor-caju*. Other unsuitable supplementation levels were excluded.

**Cultivation of *P. sajor-caju:*** Each media substrate was mixed with 5% calcium carbonate and the suitable level of wheat bran, moisture content was adjusted to approximately 69-71% then each medium was filled in polypropylene bags each one contained 2 kg medium. The bags were autoclaved at 121°C for 1 h and inoculated after cooler down with *P. sajor-caju* grain spawn at 4% level and incubated at 25-27°C till complete medium colonization by the mycelium. At the end of incubation period, the bags were opened and subjected to the fruiting conditions, i.e., exposure to scattered light, watering by daily water spraying, good ventilation, adjusting relative humidity to 85-90% and temperature was maintained around 20°C. The crop was harvested after 15-17 days from the end of incubation period in three consecutive flushes at intervals of 10-15 days. The further cultivation experiment was carried out in three consecutive trials to get actual and reliable results.

**Statistical analysis:** The obtained data was statistically analyzed using ANOVA procedure of the SPSS statistical package.

## RESULTS

**Effect of supplementation level on mycelium growth:** The mycelium growth of mushroom specie is mainly dependents upon the substrate, nutrients and the growing conditions. The data in Table 1 indicate that at 5% wheat bran supplementation level, no evident growth for *P. sajor-caju* was detected for all tested substrates. The mycelium linear growth was 1.9, 1.7 and 1.6 mm day$^{-1}$ with no significant differences shown at the level of 10% bran addition to sawdust, soybean straw, sugar cane bagasse and rice straw, respectively.

The mycelium linear growth of *P. sajor-caju* increased to 4.0, 3.3 and 4.7 mm day$^{-1}$ for the aforementioned substrate consecutively by increasing the addition level of wheat bran to be 15%. Addition of 20% wheat bran to the tested substrates approximately doubled the amount of mycelium linear growth for all substrates and differed significantly compared to 15% wheat bran level.

Increasing wheat bran level to 25% significantly increased the mycelium linear growth to be 7.9, 7.4 and 6.2 mm day$^{-1}$ for sawdust, soybean straw, sugar bagasse and rice straw, respectively. Meanwhile increased the wheat bran level to 30 or 35% did not significantly increased the mycelium growth for all substrates except for sugar cane bagasse which reached the maximum significant growth at 30% level. It could be observed that the amount of mycelium linear growth and its increment with the increase of medium level was much pronounced in sawdust and soybean straw with no significant differences followed by rice straw while the

Table 1: Effect of supplemental level on mycelium linear growth of *P. sajor-caju* wheat bran addition level (%) Mycelium linear growth

| | Growth level | | |
|---|---|---|---|
| Supportive medium | 1st | 2nd | 3rd |
| Sawdust | $1.9^{Ca}$ | $1.7^{Da}$ | $1.6^{Da}$ |
| Straw | $4.0^{Ca}$ | $3.3^{Ca}$ | $4.7^{Cab}$ |
| Sugar cane bagasse | $7.8^{Ba}$ | $6.2^{Bb}$ | $3.4^{Cc}$ |
| Rice straw | $7.9^{ABa}$ | $7.4^{Aa}$ | $6.2^{Bc}$ |
| Soybean straw | $9.0^{Aa}$ | $8.4^{Aab}$ | $8.8^{Ac}$ |

mycelium linear growth in the sugar cane bagasse was the lowest. The results of Table 2 dealt for maximum and minimum amount of sporocarp has been effectively produced on soybean medium (281.45 g) and Sawdust (216.90 g). For instant, optimum as well as minimum weight of sporocarp been produced on rice straw (38.60 and 30.00) and sugarcane bagasse medium.

From the tested chemical constituents, more amounts was noted in saw dust and total nitrogen as well as ash content in soybean (0.85 and 4.87) finally maximum carbohydrates (3.94) present in rice straw diet (Table 3). Table 4 shows that among the four different category of supplemented diet for growth of this oyster mushroom mainly sugarcane bagasse possessed the high moisture content then followed by rice straw and saw dust. Eventhough, first and third trial clearly shows the rice straw had moisture content was high (71.01and 70.39). When compared with the four diets soybean and rice straw contains significantly higher moisture content than the remaining two supplemental diets (Table 5). From Table 6 stands for main chemical constituents percent of *P. sajor-caju* mushroom fruit a body. The maximum as well as more or less similar crude prote in were seen on both soybean and sugarcane bagasse based supplementary diet followed by total fiber content ash material also been increased on soybean diet than the remaining three kinds of diets such as sawdust, sugarcane

Table 2: Effect of media substrate for weight of sporocarp from *P. sajor-caju*

| Harvest duration | Sawdust (g) | Soybean straw (g) | Sugar cane bagasse (g) | Rice straw (g) |
|---|---|---|---|---|
| First flush | 45.20 | 159.95 | 27.30 | 33.90 |
| Second flush | 216.90 | 281.45 | 30.00 | 38.60 |
| Third flush | 033.50 | 087.10 | 25.60 | 38.00 |
| Total | 295.60 | 528.50 | 82.90 | 110.50 |
| Average | 98.54 | 176.16 | 27.64 | 36.84 |

Table 3: Effect of different supplements and their levels of assessment of the economical yield of *P. sajor-caju*

| Substrate | Initial assessment | Medium assessment | Final assessment |
|---|---|---|---|
| Sawdust | $30\pm1.4^{h}$ | $32\pm1.5^{gf}$ | $30\pm1.8^{h}$ |
| Soybean straw | $33\pm1.7^{g}$ | $34\pm1.3^{efg}$ | $36\pm1.7^{e}$ |
| Sugar cane bagasse | $41\pm2.4^{a}$ | $37\pm1.2^{b}$ | $39\pm1.2^{ab}$ |
| Rice straw | $35\pm1.5^{d}$ | $37\pm1.5^{c}$ | $38\pm1.5^{bc}$ |

Table 4: Several chemical constituents percent of the tested substrates

| Substrate | Moisture content | Total nitrogen | Ash content | Carbohydrates |
|---|---|---|---|---|
| Sawdust | $8.21^{c}$ | $0.31^{c}$ | 0.35 | 0.54 |
| Soybean straw | 3.64 | 0.85 | 4.87 | 0.67 |
| Sugar cane bagasse | 0.96 | 0.57 | 0.71 | 0.67 |
| Rice straw | 0.54 | 0.67 | 0.64 | 3.94 |

Table 5: Moisture content on growing media percent for *P. sajor-caju*

| Media | First trial | Second trial | Third trial |
|---|---|---|---|
| Sawdust | $69.84^{abcd}$ | $70.47^{abc}$ | $69.18^{cd}$ |
| Soybean straw | $70.19^{abcd}$ | $68.79^{d}$ | $70.04^{abcd}$ |
| Sugar cane bagasse | $69.59^{bcd}$ | $71.22^{a}$ | $70.33^{abc}$ |
| Rice straw | $71.01^{ab}$ | $68.82^{d}$ | $70.39^{abcd}$ |

Means have different superscript letter are differ significantly

Table 6: Main chemical constituents percent of *P. sajor-caju* mushroom fruit bodies

| Media moisture | Crude protein | Total fiber content | Ash | Total carbohydrates |
|---|---|---|---|---|
| Sawdust | 88.91±0.56 | 22.17±0.84 | 3.06±0.26 | 6.94±0.38 |
| Soybean straw | 90.26±0.67$^{a}$ | 24.08±0.33$^{a}$ | 3.78±0.11$^{a}$ | 7.66±0.22$^{a}$ |
| Sugar cane bagasse | 90.17±0.60$^{a}$ | 21.33±0.06$^{b}$ | 2.96±0.28$^{b}$ | 6.54±0.24$^{b}$ |
| Rice straw | 87.63±0.62$^{b}$ | 22.75±0.47$^{ab}$ | 3.16±0.17$^{b}$ | 8.02±0.20$^{a}$ |

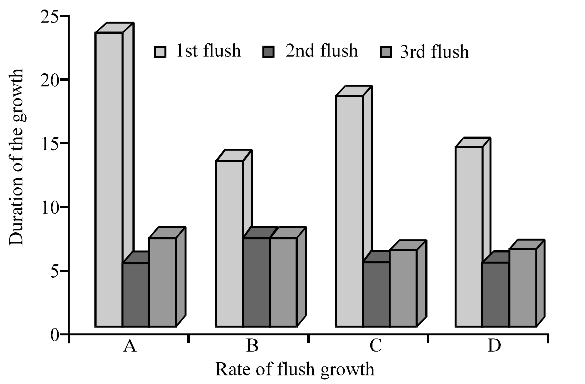


Fig. 1: Influence of various supplemental diet and its growth performance of *P. sajor-caju* mushroom species

bagasse and rice straw. Though, a total carbohydrate was optimum (7.66±0.22) and minimum amount observed on rice straw diet and sugarcane bagasse 8.92. However, sawdust and sugar cane diet contained minimum (6.9±0.38 and 6.54±0.24) carbohydrate was observed. Among the four supplementary diets, soybean diet should be provide a signinificantly better growth to *P. sajor-caju* oyster mushroom.

Furthermore, Fig. 1 indicates duration of the required days >20-25 days for initial stage of the 1st flush growth. But another two flushes having short duration 1st-8th days for growth compared with initial flush growth. The significantly dominant flush growth and the efficiency of fruiting bodiy have seen on sugarcane bagasse followed by soybean media also.

Though, very little flush as well as budding growth formation takes place with the medium of rice straw and saw dust (Fig. 2). Means within the same column have different superscript capital letter are differ significantly. Means within the same raw have different superscript small letter are differ significantly. Means have different superscript letter or are differ significantly.

**Statistical analysis:** Despite the result of the four types of substrate used, the maximum mushroom fresh weights were produced on media B rice straw. Subsequently, averages of 176 g fresh-weight mushrooms were harvested per kg of dry-weight substrate saw dust (A).


Fig. 2: Fruiting bodies of four various media developed *P. sajor-caju*; a) Saw dust, b) Rice straw, c) Sugar cane bagasse and d) Soybean

Similarly, the highest number of fruit bodies per bag was recorded in the bed (A). The number of fruit bodies was 161 bag$^{-1}$.

## DISCUSSION

These results are in accordance to Manzi *et al.* (2004) who found that rice straw and sawdust contained 19.7 and 0.6% ash as well as 0.51 and 0.16% nitrogen content, consecutively also, Hassan (2007) stated that rice straw, soybean straw and sawdust contained 6.99-9.58% moisture 0.2-0.74% total nitrogen and 0.62-17.3% ash, respectively. The similar kind of research has been done with another kind of mushroom such as *P. eryngii* by Akyuz and Yildiz (2008).

The present results coincide with those obtained by Peng *et al.* (2000) who used supplementation of rice bran from 0.0-47.95%, Zervakis found that linear growth and colonization rate of *P. erryngii* differed from supplemented substrate to another. Medany found that increasing wheat bran to 25% for different media substrates increased mycelium linear growth of *Flammulina vetutips* and *Lentinus edodes* mushroom to be >4 mm day$^{-1}$. While the results were Cangy and Peerally who stated that the mycelium growth of *P. florida, P. columbinus* and *P. cornucopiae* and *P. sajor-caju* was day and this may be due to different species. So, 25% wheat bran level was used to supplement sawdust, soybean and rice straws while sugar cane bagasse was supplemented by 30% wheat bran for preparing the growing media used for cultivation of *P. eryngii* during the later three trials.

The data in Table 3 declare that the moisture content of the tested growing media ranged between 68.79 and 71.22% in the three cultivation trails with a little significant difference among the substrates and trials. These results are confirmed by those reported by Akyuzy and Yildiz (2007, 2008) and Kirbag and Akyuz (2008a, b) who cultivated *P. eryngii* successfully on different media with 70% moisture content. Hassan (2007) stated that the moisture content of the growing mushroom media is considered as one of the most important factors hence, proper level encourage the growth, meanwhile, higher or lower ones had a negative effect on growth. Among all tested substrates, sawdust differed significantly and recorded the shortest incubation period being 30, 32 and 30 days in 1st, 2nd and 3rd trials, respectively without significant differences between the three trials. On the other hand, sugar cane bagasse had the longest incubation period 41, 37 and 39 days which significantly differed from other substrates. As for soybean straw medium, it could be observed that it has shorter incubation period (33, 34 and 36 days) through the 3 trials compared to rice straw medium (35, 37 and 38 days). Similar findings also been reported by Shashirekha *et al.* (2002), Moda *et al.* (2005) and Alam *et al.* (2008). It could be observed that substrates had high mycelium growth rate such as sawdust and soybean straw recorded short incubation period while low mycelium growth rate of sugar cane bagasse and rice straw resulted in long incubation period. These results are nearly in the range of the data reported by many researchers.

Akyuz and Yildiz (2007, 2008) who recorded that the longest mycelium growing period for *P. eryngii* was 27 days on wheat straw and millet straw (1:1) +15% rice bran. According to Dubost *et al.* (2007), cultivated *P. eryngii* on sugar beet or wheat straw in porous bags (gas exchange more easily) and traditional bags. They found that incubation period was 45 days in porous bags while it increased up to 90 days for traditional one. On the other side, Akyuz and Yildiz (2007) recorded shorter incubation period for *P. eryngii* being 13 days on wheat straw +20% rice bran, 18 days on wheat straw and soybean straw (1:1 ) +5% rice bran.

## CONCLUSION

Cultivation of *Pleurotus sajor-caju* under local conditions and using the available cheap lignocellulosic wastes in India is an important achievement, since this type of mushroom is highly prized for their nutritive and medicinal value.

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