Comparative evaluation studies of different species of *Pleurotus* for Radial growth, Biomass and Yield parameters.

ABSTRACT

Aim: This study was undertaken with the major objective of determining the best yielding mushroom among the three species of *Pleurotus spp*. i.e., *P. florida*, *P. djamor* and *P. sajorcaju*.
Study design: The experiment was laid out in Completely Randomized Design (CRD) with five treatments in three replications.
Place and duration of study: The experiment was conducted at Plant Pathology laboratory of Dau Kalyan Singh College of Agriculture & Research Station, Bhatpara, Chhattisgarh.
Methodology: Pure culture was prepared from the spawn and then observations were recorded for radial growth and Biomass by subculturing the isolates in freshly prepared Potato Dextrose Agar (PDA) medium. Then, after making spawn of all isolates, they were cultivated for determining the yield of particular isolates.
Result: The results indicated that *P. sajorcaju* is significantly superior with Radial growth -78 mm, Biomass - 4.884 gm & Yield- 804 gm followed by *P. florida*and then *P. djamor*respectively.
Conclusion: The results obtained from the present study can help the mushroom growers in selecting superior isolate for cultivation, leading to enhanced quality of spawn thereby increasing the yield of this nutritious & medicinal crop

Key Words - *Radial growth, Biomass, yield potential, biological efficiency.*

1. INTRODUCTION

Mushrooms are often called as “boneless vegetarian meat” due to its delicious taste, nutraceutical nature and unusual taste [1]. *Pleurotus sp*. are edible mushrooms containing high nutritional values: rich in proteins,dietary fibres, minerals and low-fat contents [2]. Cultivation of mushroom can be viewed as an effective way to extract bioresources residual in agriculture and as a comprehensive environment protection strategy [3].Nowadays,even waste paper is also being used for cultivation of oyster mushroom by some added additionalresources[4], itsas an effective way of recycling, reusing and reducing waste. Oyster mushroom (*Pleurotus*) is amongst the most farmed species belonging to the family Agaricaceae and class Basidiomycetes. It was first cultivated in Germany by Flack in 1917 on tree stumps and wood logs. It presently ranks as second highest cultivated edible mushroom after button mushroom. The fluted, graceful oyster shell’s shape and delicate, briny flavour are best praised in a poem written during the Sung dynasty that calls the oyster “the mushroom of flower heaven”. Oyster mushroom has different shades of white, black, pink, blue, yellow, cream, depending on the species and has the clashing fragrance of benzaldehyde. *Pleurotus* is one of the few known carnivorous mushrooms whose mycelia can kill and digest nematodes by secreting volatile ketones [5]. Oyster mushrooms are the most uncomplicated and cost-effective mushroom to grow because they are highly acclaimed for turning crop wastes into food protein and are considered as potential source of income, alternative food production, provision of employment and for recycling of agricultural wastes and to lower environmental pollution [6].The yield and nutritional content of mushrooms depends on the various factors, majorly involved are environmental conditions, quality of substrate and spawn, stage of mushroom at the time of harvesting and skilled manpower. Different high through-put techniques are now being involved in mushrooms cultivationto improve the overall quality and yield of mushrooms and reduce manual work, time, and other resource by adopting the automized temperature and humidity control systems,internet of things (IoT),machine learning, lights, irrigation system[7].Oyster mushroom is highly nutritive with less cultivation resources; hence this experiment was conducted to find out the best one among the above-mentioned species of oyster mushroom for the various growth parameters namely radial growth, biomass and spawn production.

2.MATERIAL AND METHOD

The major stages involved in cultivating mushroom are primarily divided into:1. substrate preparation, 2. Spawn preparation, 3. incubation, 4. fruiting and finally 5.Harvesting [8].

2.1 Isolation of pure culture

The spawn was inoculated in the freshly prepared PDA media aseptically in the laminar air flow. Then, the culture plates were incubated for 1-2 days till the growth of mycelium is visible from the spawn. The subculturing was done, for formation of pure culture and incubated in BOD incubator at the temperature range of 28^0 C for 10-15 days.

2.2 Determination of Radial growth and Biomass

The culture was extracted from each isolatei.e.,*P.sajorcaju, P. florida*, *P.djamor*and 6 mm mycelium disc was cut with the help of a sterilized cork borer and then transferred into petri plates containing freshly prepared PDA media with the help of sterilized inoculation loop.Within a week or two, the petri plates were completely filled by the mycelium.Inoculation of mycelial disc is done into the conical flask containing potato dextrose broth. Then plugged the mouth of flask using cotton plug and incubated in BOD incubator at 25^0 -28^0 C.Within a week or two the media was covered with mycelial growth. On the 13^{th} day the culture was filtered using Whiteman filter paper and mycelium was filtered for biomass determination (fresh mycelial weight) and then the mycelium was placed in hot air oven and weighed after 24 and 48 hours.

2.3 Determination of Biological Efficiency

The experiment was conducted at Mushroom hut of DKSCARS, Bhatapara in CRD design with five replication and observation was observed in various yield parameters Spawn Run days, Pin head initiation days, Days taken for first harvest, Fruit body/bed Size of fruit body (cm), Average weight of body (gm), Average weight of body (gm), Mean yield, (kg), B.E(%).

2.4 Spawn production

2.4.1 Grain spawn

The500gm of grains was taken and soaked in 1ltr of water for 6-7hours, cooked for 10min and the excess water was removed followed by spreading it under shade for 2:30-3hours then mixed with calcium carbonate and calcium sulphate @ 2% i.e. 20gms mixture is filled in conical flask, glucose bottle and polypropylene bag in $2/3^{rd}$ proportion then closed it using cotton plugs followed by sterilizing ofglucose bottle, flask and polypropylene bags containing spawn substrate at 126^0 C and 22psi pressure for 2 hours and then cooled it under laminar air flow.After a couple of days, sub-culture was inoculated and spawn substrate was incubated at 25^0 -28^0 C for 2 weeks.

2.5Method of Cultivation

The cultivation method of *Pleurotus spp.* has been done on locally available substrates. The various steps involved in the cultivation of oyster mushroom are as follows: -

2.5.1 Sterilization of area – Proper sterilization is required for the proper growth and maintenance of oyster mushroom of the room and stand. The mushroom bags were placed with 4% formalin solution with the help of spray.

2.5.2 Substrate preparation –Insect and disease-free chopped paddy/rice straw was soaked in water. It is mixed with a fungicide (7.5gm) and formalin (125ml) for about 24 hrs.The treated straw was then spread over to drain off the excess moisture till its content becomes 65-70%.

2.5.3 Spawning -Mix spawning method was used for this step. In this, spawn is mixed with substrate in a proportion and then filled in the bag. The neck of the bag is then tied with nylon thread.

2.5.4 Cropping –The string tied bags are then hanged through threads and 8-10 holes are made in each bag to allow free passage of air. The temperature and humidity of the room where the mushrooms are hanged, is maintained by regular sprinkling of water three times aday as per requirement of the bags. This also helps to profuse proper growth of fruiting bodies. The water is not only sprayed on bags but also on walls and floor frequently. After complete colonization of mushroom bed, the polythene bags were cut off and removed.

2.5.5 Harvesting –The right stage for picking of mushroom can be judged based on the shape and size of fruiting bodies. It was handpicked before spraying water. As the mushroom begin fruiting, it'simportant to the humidity high. Temperature can now be higher for the initial picking stage. Harvesting is done after 20-25 days when oyster mushroom mature. Simultaneously, four crops of mushroom are harvested at an interval of 4-5 days.

P. florida. ***P.djamorP.sajorcaju***

Fig 1. Different species of *Pleurotus*.

3.RESULT AND DISCUSSION

3.1 Radial growth

Among the 3 isolates of *Pleurotus spp, P. sajorcaju*is found to be majorly significant than the other two isolates with a radial growth of 78 mm followed by*P.florida* marking 70 mm and then *P.djamor*with lowest radial growth of 62 mm.

3.2 Biomass

3.2.1 Fresh mycelial weight

Out of 3 varieties of *Pleurotus spp. P.sajorcaju*was found to be supreme than the rest of the two species with 4.884 gm accounting in term of fresh mycelial weight followed by*P.florida* with 3.872 gm and lowest mycelial weight was recorded in *P.djamor*with 2.420 gm respectively.

3.2.2 Dry mycelial weight

Out of 3 isolates of *Pleurotus spp, P.sajorcaju*was found to be significantly superior with 0.480 gm of dry mycelial weight followed by*P.florida* with 0.302 gm and lowest DMW was of *P.djamor* i.e. 0.240 gm respectively.

Table 1. Radial growth of the three species of *Pleurotus*

Treatment	Day 3	Day 9	Day 13
P.sajorcaju (Black)	17.0	39.33	75
P.florida (White)	12.53	32.35	70.55

P.djamor (Pink)	9.8	27.53	57.53
CD	1.296	0.980	0.672
CV	4.313	1.283	0.429

Radial growth – Among the 3 isolates of *Pleurotus* minimum number of days were required by *P.sajorcaju*to cover the petriplate. Observation was noted on Day 3, Day 9 & Day 13. In *P.sajorcaju*on Day 3 (17.0 mm), Day 9 (39.33 mm) and on Day 13 (75 mm) radial growth followed by*P.florida*which covered (12.53 mm) of petriplate on Day 3 (32.35 mm) on Day 9 and (70.55 mm) on Day 13 and very less mycelium growth was observed in *P.djamor*i.e (9.8 mm) on Day 3 , on Day 9 it covered (27.33 mm) and in Day 13 it covered (57.53mm) respectively.

Fig.2. *P.sajorcajuP.florida. P.djamor*

Table 2. Radial growth and Biomassof the three species of *Pleurotus.*

Treatment	Radial growth (mm)	Fresh mycelium weight(gm.)	Dry mycelium weight (gm.)
P.sajorcaju (Black)	78	4.884	0.480
P.florida (White)	70	3.872	0.302
P.djamor (Pink)	62	2.420	0.240
CD	4.720	0.491	0.055
CV	2.906	5.985	7.079

Radial growth and Biomass-

Radial growth –

Among the 3 isolates *P.sajorcaju*was found to be the fastest growing and has taken less days to cover 90 mm of petriplate in comparison with the other isolates of *Pleurotus* on Day 15 *P.sajorcaju*covered (78 mm) of petriplatefollowed by*P.florida*with growth of (70 mm) and slowest growth was recorded in *P.djamor*with (62 mm) respectively.

Biomass – In liquid medium, Fresh mycelia weight(FMW) was differing in isolates of *Pleurotus* in which *P.sajorcaju*has significantly higher FMW i.e. (4.884 gm) followed by*P.florida*with (3.872 gm) and lowest FMW was reported in *P.djamor*i.e. (2.420 gm). The Dry mycelia weight (DMW) of *P. sajorcaju*was similar with that of FMW in which it was leading with (0.480 gm) followed by *P.florida*with (0.302 gm) and poorest DMW was observed in *P. djamor*i.e. (0.240 gm) respectively

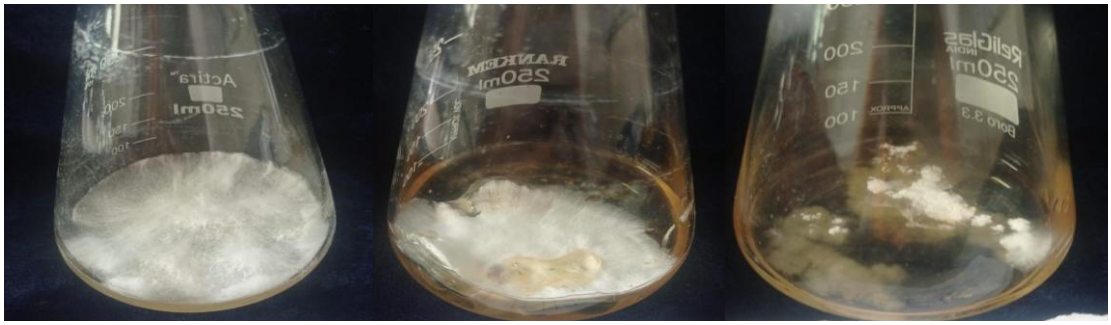

Fig 3.*P.sajorcajuP.florida.* *P.djamor*

Morphological characterization of the mycelium of *Pleurotus spp.*

The characters shown by different strains of *Pleurotus spp.* were found to be cottony. The density ranging from high to medium-low. The growth pattern was noticed to be uniformly radiated. The colour varied from white to whitish.

Colony morphology, texture & growth pattern –

Among the 3 isolates of *Pleutotus*species presence of high density uniformly radiating, cottony whitish mycelium was observed in *P.sajorcaju. P.florida* shown moderately high uniformly radiating mycelium which has white colour and cotton like texture while medium to low density of mycelium was noticed in *P.djamor*with uniformly radiating mycelium and cottony texture with whitish colour.

Table 3 Morphological characteristics

Treatment	Texture	Density	Growth	Colour
P.sajorcaju (Black)	Cottony	High	Uniformly radiated	Whitish
P.florida (White)	Cottony	Moderately high	Uniformly radiated	White
P.djamor (Pink)	Cottony	Medium to low	Uniformly radiated	Whitish

List 1 : Comparison of different species of *Pleurotus* for growth behaviour

Pleurotus spp.	Spawn run period (day)	Pin head initiation (day)	1st Harvesting (day)	2nd Harvesting (day)	3rd Harvesting (day)	4th Harvesting (day)
P. sajorcaju	17	22	26	32.67	43.67	54
P.florida	19.33	24	27	35	46.67	58.33
P.djamor	20.41	26	29	38.67	49.33	0
CD	1.657	1.820	2.199	3.196	2.053	N/A
CV	3.887	3.383	3.471	3.970	1.922	3.602

A). Growth behaviour of *Pleurotus* species

As shown in table 1 in comparison is depicted on the basis of spawn run period, pin head initiation, days of harvesting that is 1st ,2nd ,3rd and 4th.

Spawn run period - The difference of 17 to 20.41 was recorded in the different species of oyster mushroom i.e. *P.florida, P.djamor and P.sajorcaju*and*P.sajorcaju*is found to be superior with a period of 17 days followed by *P.florida*i.e. 19.33 days and maximum days for spawn run was taken by *P.djamor*(20.41 days).

Pin head initiation – Time required for the pin head initiation ranged between 22-26 days from which again *P.sajorcaju*has taken significantly minimum no. of days i.e. 22 followed by*P.florida* with 24 daysand maximum no. of days required by*P.djamor*i.e 26 days respectively.

Harvesting – Total 4 flushes were recorded in *Pleurotus spp.* during crop period. The first harvesting days ranged from (26-29 days) in which minimum was *P.sajorcaju* with (26 days)&*P.florida and P.djamor* with (27 and 29) days, respectively. The second flush varied from (32.67-38.67 days) in which less period of time was taken by *P.sajorcaju*i.e. (32.67 days) Followed by*P.florida*with (35days) *and P.djamor*(38.67 days). The result pertaining to third harvesting ranges from (43.67-49.33 days) in which minimum no of days i.e(43.67 days) required by *P.sajorcaju*followed by*P.florida* with (46.67 days)and*P.djamor* has taken maximum no. of days i.e.(49.33 days). Days (54-58.33) were noted in fourth flush of crop period in which again *P.sajorcaju* was found to be significantly superior with less no. of days i.e(54 days) followed by *P.florida*with (58.33 days) and no fruit bodies were seen in bags of *P. djamor*, respectively.

List 2 : Comparison of different species of *Pleurotus* for yield potential

Pleurotus spp.	**1st flush (gm)**	**2nd flush (gm)**	**3rd flush (gm)**	**4th flush (gm)**	**Yield (gm)**	**B.E (%)**
P. sajorcaju	425	205	102	72	804	80.4%
P.florida	378	184	89	67	718	71.%
P.djamor	320	170	82	0	572	57.2%
CD	26.346	28.697	8.320	4.085	20.703	
CV	3.074	6.628	3.933	1.573	1.306	

B). Comparative study of yield potential of different species of *Pleurotus.*

Yield potential – It is observed from the data presented in table 4..*P.sajorcaju*is the best species among the three with highest yield and biological efficiency as recorded in 4 flushes and found that yield got decreased in every flush and it was lowest in the fourth one. In first flush *P.sajorcaju* was leading with (425 gm) fresh weight of mushroom followed by *P.florida* with (378 gm) and lowest yield was noted in *P.djamor*i.e (320 gm). In second flush, maximum yield of (205 gm) was shown by *P.sajorcaju*followed by *P.florida*with (184 gm) of and minimum yield seen was (170 gm) of *P.djamor.* 102-82gm variation in yield was noticed in third flush with highest yield in *P.sajorcaju*i.e (102 gm) followed by *P.florida*with (82 gm). and minimum yield was observed in *P.djamor*i.e(82 gm). Lowest yield observed in fourth flush which ranged from (72-67 gm) in which *P.sajorcaju*followed by *P.florida*with (72 gm) and (67 gm) respectively. Maximum yield reported in *P.sajorcaju*i.e(804 gm)followed by *P.florida* with (719 gm) and the least one was *P.djamor*with(572 gm).

The effectiveness of mushroom strains was determined by biological efficiency (B.E) which can be measured by the ratio between fresh weights of mushroom fruit body to the dry weight of substrate multiplied by 100, expressed in %. *P.sajorcaju* was found to have highest B.E of 80.4% which was statistically at par with *P.florida*with 75.6% and least B.E was recorded in *P.djamor*i.e 57.2% respectively.

List 3 : Comparison of different species of *Pleurotus* for growthparameter

Pleurotu	Total no.	Max.	Min.	Avg.	Avg,.	Avg. Dia.	Total length

s spp.	of fruiting bodies	Weight of fruiting body	weight of fruiting body	Length of stalk (cm.)	Width of stalk (cm.)	Of mushroom (cm.)	of mushroom (cm.)
P.sajorcaju	148	27.33	7.98	6.23	2.48	7.70	13.03
P.florida	102	25.30	6.29	5.94	2.24	6.92	12.12
P.djamor	85	18.85	4.05	5.05	1.81	5.23	9.28
CD	13.258	2.798	0.766	0.939	0.392	0.801	0.656
CV	5.152	5.251	5.396	7.693	8.416	5.239	2.475

Growth parameter – In growth parameters, maximum no. of fruiting body was observed highest in *P.sajorcaju*(148) marked followed by *P.florida*(102) and the minimum no. of fruiting body was recorded lowest in*P.djamor*(85). The maximum weight of fruiting body in *P.sajorcaju*was (27.33gm) and minimum was (7.98 gm) followed by *P.florida*withmaximum weight (25.30gm) and minimum was (6.29gm) followed by *P.djamor* with maximum weight (18.85 gm) and minimum weight was (4.05 gm) respectively.

Length and width of stalk - In*P.sajorcaju*thelength and width of stalk was measured (6.23cm) and (2.48cm) followed by *P.florida*whose stalk length was (5.94cm) and width was (2.24cm). The shortest stalk length (5.05cm) and average width of (1.81cm) was reported in *P.djamor.*

Average diameter of mushroom - Remarkable difference was noticed in average diameter of mushroom ranging from (5.23–7.70cm) in which *P.sajorcaju* had (7.70cm) followed by *P.florida* measuring (6.92cm) and shortest diameter was that of *P.djamor*i.e. (5.23cm).

Total length of mushroom – Ranging from (9.28-13.03cm) total length of mushroom fruit body was determined in which *P.sajorcaju* was significantly superior with (13.03cm) followed by *P.florida* (12.12cm) and lowest length was observed in fruiting body of *P.djamor*i.e (9.28cm) respectively.

The findings can be correlated with the experiment in which 2 strains of *Pleurotus* i.e.*P. ostreatus*(P-11) and *P. djamor* (P-19) for studying growth rate and biomass. They found that in media EML + BF media, the growth of P-11 isolate was significantly superior with specific growth rate of 0.403 [h.sup.-1] and in (P-19) its 0.196 [h.sup.-1] while in buffer of liquid phosphates added with cereals media (BFL + C) it was of 0.233[h.sup.-1] for strain P-19 and 0.395 [h.sup.-1] for strain P-11. Highest mycelial biomass was recorded in BFL + C media with 0.70 g/l in P-11 and 0.49 g/l in EML + BF media [9].This is also in conformity with the report in which 2 species of oyster mushroom i.e. *P. florida&P. sajorcaju*were studiedand *P.florida*was found superior isolate with yield of 1363 gm. and that of *P. sajorcaju* with 940 gm. with biological efficiency of 136.3% and 94.0% in *P. sajorcaju*respectively [10].Similar experiment also reported, five strains (R-13-13-03, R-13-13-04, R-13-13-05, R-13-13-07 and R-51) of oystermushroom (Pleurotus spp.) were studied and R13-13-5 was the superior isolate with yield of (0.483gm.) and 96.6% biological efficiency followed by R13-13-7, R13-13-4,R-51 and minimum yield recorded in R13-13-4 respectively [11].The finding can be correlated with the research in which 5 species of oyster mushroom i.e, *P.florida, P.sajorcaju, P.flabellatus, P.eryngii and P.osteratus*were studied for various growth parameters including growth behaviour, sporophore parameter & yield potential and found that *P.florida* has taken minimum days for spawn run i.e. 16 days, highest yield was also recorded in *P.florida* (982.33 g) with maximum no. of fruit bodies and highest biological efficiency of 98.23% [12].

4.CONCLUSION

The mentioned research was carried out to determine the best variety of oyster mushroom among the three variants of *Pleurotus* i.e. *P.sajorcaju, P.florida and P.djamor*that were taken from the mushroomunit farm for radial growth, biomass and yield parameters. From the three isolates of oyster mushroom *P. sajorcaju*was found to be superior with (4.884gm)fresh mycelial weight, dry mycelial weight of (0.480gm), and radial growth of (78mm)followed by with *P.florida* followed by *P.djamor*respectively. From the above experiment, it is concluded that *P.sajorcaju*is significantly supreme among the three strains in all aspects and can be cultivated majorly looking at its qualities

for a better yield. The yield was reported to be found highest in *P.sajorcaju*weighingabout(804gm) observed at par with *P.florida*(719gm) and lowest yield was granted by *P.djamor*(572gm).The mycelium taken from *P.sajorcaju*was faster to spread for spawn production therefore, this variety again proves its superiority among the three isolates of oyster mushroom.Thus, the results obtained from the present study can help the mushroom growers in selecting superior isolate for cultivation in commercial scale, leading to enhanced quality and quantity of spawn there by increasing the yield of this nutritious & medicinal crop and moving towards sustainable and progressive farming practices.

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