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RESEARCH ARTICLE

STABILIZATION OF BIOLOGICAL EFFICIENCY OF ISOLATES OF *VOLVARIELLA VOLVACEA (*BULL EX. FR.) SINGER THROUGH SINGLE SPORE CULTURES

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Abstract: An experiment was conducted to stabilize the variable biological efficiency of *Volvariella volvacea* through single spore culture. Pure culture of *Volvariella volvacea* raised from tissue culture, multispore culture and monospore culture are studied for the radial growth, Biomass & Chlamydospore production, also studied for growth and yield parameters significantly highest yield (1.530 kg) & biological efficiency (21.86%) in single spore culture (SSI 3) found statistically at par with tissue culture of BYT VV-05 isolate with yield (1.410 kg) and biological efficiency (20.14%) significantly lowest Yield in multispore culture (0.900 kg/ bed) and (12.14%) biological efficiency was observed. Similar trends also observed on radial growth & biomass production culture of monosporous isolates (SSI 3)was found significantly highest radial growth (88.00 mm) and biomass production (0.420 gram) found statistically at par with tissue culture raised from JSP2 highest radial growth (84.40 mm) and biomass production (0.410 gram) isolate of *V. volvacea.*

Keywords: Radial growth, Biomass, Yield potential, Biological efficiency

INTRODUCTION

***Volvariella volvacea* Bull Ex. Fr. singer** commonly known as Paddy straw mushroom or straw mushroom belonging to the family Pluteaceae of basidiomycetes. It is a very delicious and nutritious mushroom with many medicinal properties. Healthy and best quality i.e., productive spawn is an essential part for achieving high yield and quality mushroom crops. The yield potential of spawn selected and the techniques of its preparation determine the superior quality crop. In the world China is leading with 6,00,000 tons annually production and in India Odisha is leading with 8129 tons per annum with viability, high biological efficiency, yield potential and large-scale production. Although Odisha is a supplier of all over India but due to inadequate research to improve quality of spawn production and lack of knowledge spawn quality has compromised a lot.

Very little research has been done to improve the quality parameter of spawn of straw mushroom except few. On other hand lots of work has been done to standardize the method of spawn production of different edible mushrooms.

One of the major constraints with paddy straw mushroom production is yield of *V. volvacea* isolates is variable and deteriorating year after year stabilization of biological efficiency can be possible only by single spore isolation and its maintenance with objective of stabilization of yield of *V. volvacea* an investigation was undertaken to study and comparing the growth and yield parameters of spawn prepared from pure cultures of Single spore, Multispore and tissue cultures isolates of *V. volvacea*.

MATERIALS AND METHODS

Site of experiment

Experiment was conducted in plant pathology Laboratory at Dau Kalyan Singh College of Agriculture and Research station, Alesur, Bhatapara (C.G.)

Statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with eight treatments with three replications. The data was analyzed by the statistical procedure given by Gomez and Gomez1984.

Isolation of pure culture

For tissue culture preparation, a fruiting body at egg stage was selected. It was then surface sterilized with 70% ethanol followed by rinsing with sterile water with the help of sterilized blade, the fruit body was cut into 2 equal halves without touching inner surface small pieces of tissue from the junction between Stalk and Pileus were taken and transferred

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aseptically to freshly prepared PDA media and incubated at 32°-34°C.

Measurement of radial growth and Biomass production

In vitro studies for radial growth were conducted on potato dextrose agar medium (PDA). To avoid bacterial contamination, streptomycin sulphate was incorporated in the medium (One g of streptomycin sulphate was dissolved in 100 ml of sterile distilled water and added at the rate of 0.3 ml/100 ml of autoclaved medium and then it was cooled to 45°C). Then the medium was poured into sterile Petri plates and allowed to solidify. Mycelial discs of 5 mm diameter were cut from seven day old actively growing cultures of *V. volvacea*. The disc was aseptically placed exactly at the center of Petri plate containing PDA and incubated at temperature (30 ± 2°C). Observations for radial growth were recorded when any plates showed full mycelial growth in any treatment. Each treatment had 05 replications. The observations were also carefully done for the formation of chlamydospores. The colonies were studied by characterizing the mycelia colour, texture, density and the chlamydospore formation. The strains were grouped according to the criteria established by Chang *et al*. (1981). The hyphal diameter, chlamydospore diameter were measured micro metrically.

The biomass production of different isolates was determined by inoculating mycelial discs of 8 mm diameter separately in different flasks containing PDA broth. The flasks were incubated at temperature (30 ± 2°C) observations for biomass were recorded when any flask showed full mycelial growth in any treatment. Each treatment had 05 replications. The contents of the flasks were harvested by filtering them through pre-weighed Whatman No.l filter papers and the mycelial mat was dried in hot air oven at 60°C for 2 hrs. The final dry weight was taken and weight of the mycelial mat was calculated by difference from the original and dry weight.

Collection of Basidiospores

Collected from mushroom as spore print, the pileuslay directly in sterilized petri plate keeping moist cotton above it and placed it in an incubator at 32°-34° C in the BOD incubator. for 24 hrs. spore print can be seen exactly like radial symmetry of the gills.

Multi spore culture

To get pure culture sterilize scalpel to red hot in a burning flame for 8-9 sec. under aseptic condition spore mass was scraped from the spore print followed by streaking in freshly prepared PDA media and then incubation of petri plates at 32°-34°c in BOD incubator. After 3-4 days pure multi spore culture was prepared.

Single spore culture

Single spore culture was prepared in same way as the multispore culture, In the multispore culture at 2nd day of incubation some single spore germination can be observed that germination was cut and transferred aseptically to another PDA media plate and incubation was done at 35•C for 5-6 days.

Comparative evaluation of growth and yield parameters

The experiment was laid out in Completely Randomized Design (CRD) with eight treatments i.e., four single spore isolates, two multispore isolates and two tissue culture with four replications. The data was recorded on spawn run days, pinhead initiation days, days taken for first harvest, number of fruit body/bag, biological efficiency.

RESULTS AND DISCUSSION

A data presented in table 1.1 shows radial growth and biomass of isolates in which single spore isolates have the significantly highest (88.30mm) radial growth, fresh mycelium weight (4.50 g) and dry mycelium weighs (0.420 g) found statistically at par with tissue culture of (JSP-1) junction between Stalk and Pileus radial growth was (86.50mm.), fresh mycelium weighs (4.20 g) and dry mycelium weighs (0.400g) and while significantly lowest mycelium growth was observed in multispore culture with radial growth (68.80 mm), fresh mycelium weight (2.95 g) and dry mycelium weighs (0.250g) findings are supported by researchers they found that spawn prepared from monospore culture shows highest yield, mycelia growth as well as biological efficiency somewhat similar result was seen in tissue culture also and lowest yield, mycelia growth and biological efficiency was of multispore culture.

Microscopic and Morphological characterization of isolates-

Colony morphology and aerial hyphae

Colony morphology and aerial hyphae of single spore isolates (SSI-3) were found to be best with thick fluffy colonies and high density of aerial hyphae followed by JSP-1 & JSP-2 tissue culture i.e. junction between Stalk and Pileus with thick uniformly projecting mycelium colony and moderate density of aerial hyphae in both BYT-5 and BYT -2 and very less aerial hyphae is seen in multispore culture in both BYT-5 and BYT-2 with thin fluffy transparent irregularly projecting mycelium.

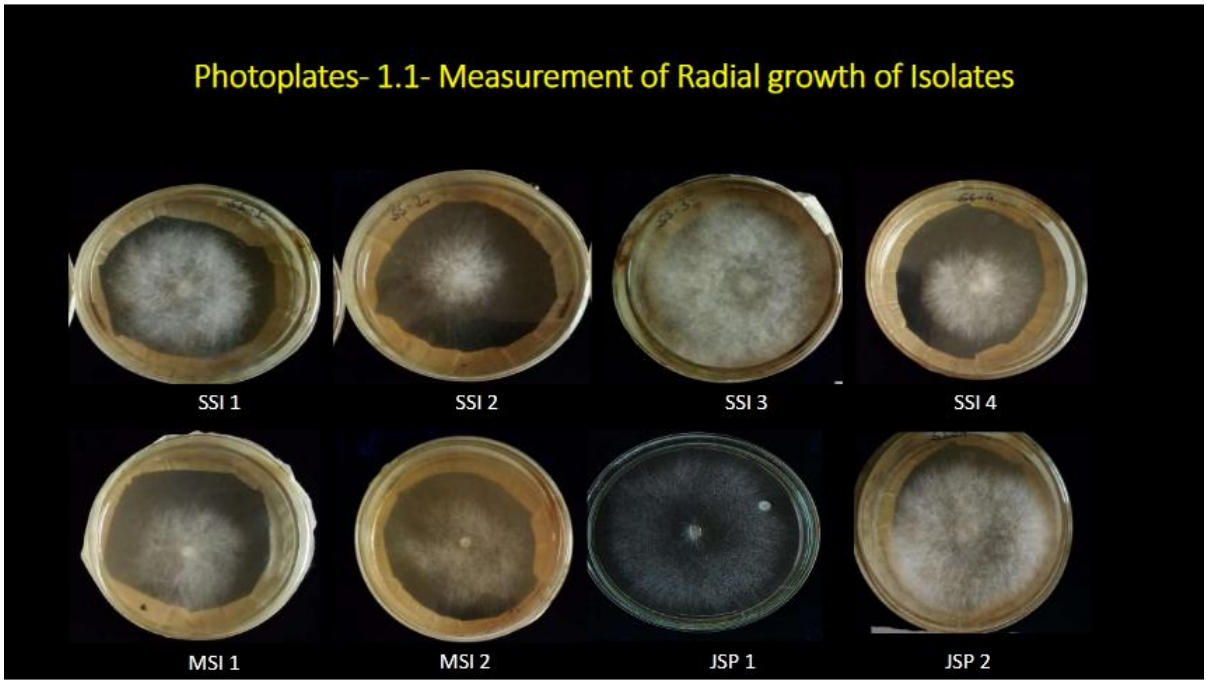
Table 1. Measurement of Radial growth and Biomass of isolates.

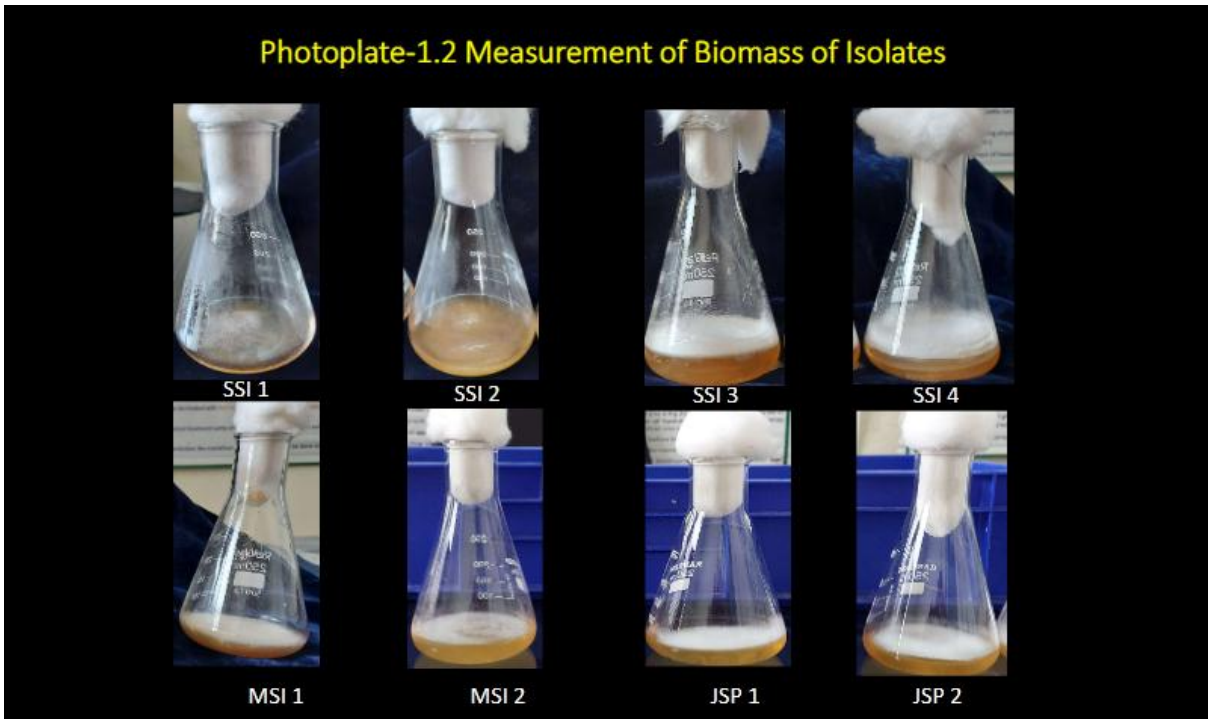
S. No.	Particular	Radial growth	Biomass Production	
			Fresh mycelia weight	Dry mycelia weight
1	Single spore isolates (SS1)	72.20 mm	3.20 gm.	0.305 gm

2	Single spore isolates (SS2)	71.60 mm	3.10 gm	0.209 gm
3	Single spore isolates (SS3)	**88.00mm**	**4.50 gm**	**0.420 gm**
4	Single spore isolates (SS4)	78.50 mm	3.45 gm	0.330 gm
5	Multi spore culture (MS1)	68.80 mm	2.95 gm	0.250 gm
6	Multi spore culture (MS2)	69.70 mm	3.10 gm	0.280 gm
7	Junction between stalk and pileus (JSP1)	**84.40 mm**	**4.10 gm**	**0.390 gm**
8	Junction between stalk and pileus (JSP2)	**86.50 mm**	**4.20 gm**	**0.400 gm**
	CD 0.05%	5.688	0.439	0.035

Table2. Microscopic and morphological characterization of *Volvariellavolvacea* isolates

S. No.	Isolates	Colony morphology	Aerial hyphae	DTT CPP	No. of septa/ Microscopic field	Septa to septa distance/ microscopic field	Hyphal diameter (µm)	No. of days to prepare spawn
1.	SSI 1	Thick fluffy	++	7.60	4.40	15.60	6.50	14.50
2.	SSI 2	Thick fluffy	++	6.60	4.50	15.20	5.80	15.60
3.	SSI 3	Thick fluffy	++++	**4.60**	**6.40**	**9.20**	**8.60**	**12.30**
4.	SSI 4	Thick fluffy	++	7.80	5.60	12.80	7.30	13.40
5.	MSI 1	Thin fluffy transparent irregularly projecting	+	8.80	4.80	18.20	5.90	16.40
6.	MSI 2	Thin fluffy transparent irregularly projecting	+	9.20	3.60	10.25	5.95	16.25
7.	JSP 1	Thick uniformly projecting	+++	**4.70**	**5.50**	**14.25**	**7.25**	**13.30**
8.	JSP 2	Thick uniformly projecting	+++	**4.95**	**5.80**	**9.80**	**8.20**	**14.50**
	CDP=0.05			1.080	0.529	1.570	0.640	1.432





Days taken to cover 90 mm of petri plate

Single spore isolates SSI-3 isolate of *V. volvacea* takes significantly minimum(4.60days) to cover 90 mm of petri plate found statistically at par with JSP-1 and JSP-2 tissue culture i.e. junction between stalk and pileus JSP-1 (BYT-2) has taken (4.70) days and JSP2 (BYT-5) has taken (4.00) days to cover petri plate followed by SSI-2 (BYT-2) has taken (6.60 days), SSI-1 has taken (7.60days) and SSI-4 has taken (7.80days) while significantly more time taken by multispore culture MSI-2 (9.20 days) and MSI-1(8.80 days).

No. of septa per microscopic field

Considerable difference was found in no. of septa per microscopic field in all isolates shown in table-1.2 it varied from (3.60 to 6.40). Significantly maximum (6.40) no. of septa reported in SSI-3 followed by JSP-2(5.80), SSI-4- (5.60), JSP1-(5.50), MSI-1(4.80), SSI-2- (4.50) and SSI-1(4.40) while significantly least (3.60) no. of septa was reported in multispore culture MSI-2.

Septa to septa distance/microscopic field

Distance between septa (table 4.2) was varied from (9.20 µm to 18.20 µm). In single spore isolation SSI-3 significantly minimum (9.20 µm) septa to septa distance wasreportedfound statistically at par with JSP -2 (9.80µm) and MSP-1 (10.25 µm) followed by SSI-4 (12.80 µm), (14.25 µm) SSI-2 (15.20 µm) and SSI-1(15.60 µm) significantly maximum (18.20 µm) distance reported in MSI-1.

Hyphal diameter (µm)

Significantly maximum (8.60µm) hyphal diameter was reported in SSI-3 found statistically at par with JSP-2 (8.20 µm)followed by SSI-4 (7.30µm), JSP-1 (7.25µm), SSI-1 (6.50µm), MSI-2 (5.95µm) and MSI-1 (5.90 µm) and while significantly minimum diameter was observed in SSI-2 (5.80 µm).

No. of days to prepare spawn

Significantly less (12.30days) for the preparation of spawn was reported in SSI-3 found statistically at par with JSP-01 (13.30 days) and SSI-3 (13.40 days) followed by SSI-1 (14.50days), JSP-2 (14.50 days), SSI-2 (15.60 days), MSI-1 (16.25 days) while significantly maximum (16.40 days) was reported in multispore culture MSI-1.

Microscopic characterization of chlamydospore of *Volvariella volvacea* isolates.

Days taken for chlamydospore production

Significantly less (11.45 days) was observed in single spore isolates SSI-3 found statistically at par with JSP-1 (12.00 days), SSI-4 (12.10 days) and JSP-2 (12.60 days) followed by SSI-4 (13.30 days) SSI-1 (14.15 days) and MSI-1 (14.20 days) while multi spore isolates MSI-1 required significantly maximum (15.60) days for chlamydospore production.

Chlamydospore color and density

All isolates had light orange color chlamydospore without dark ring with highest density in single spore isolates SSI-3 and least density in multispore isolates (MSI-1 andMSI-2). Tissue culture (JSI-1 and JSI-2) and single spore isolates (SSI-4 & SSI-2) have moderate density and (SSI-1) was very less density of Chlamydospores were observed.

Diameter of chlamydospore

Significantly maximum chlamydospore diameter was reported in single spore isolates SSI-3 (26.40 µm) followed by SSI-4 (25.60 µm), SSI-1 (24.10 µm) and SSI-2 (23.20 µm) followed by tissue culture JSP-1 (24.60 µm) and JSP-2 (25.60 µm) and significantly minimum in multi spore culture isolates MSI-1

(23.00 µm) and MSI-2 (22.60 µm) was reported.

Comparison of Growth and Yield parameters of SSI (Single spore isolates) with multispore and tissue culture isolates.

Spawn run days

Significantly less (7.20 days) was observed in single spore isolate SSI 3 among other isolates found statistically at par with JSP 1 (7.80) days, JSP 2 (7.50days) and SSI 4 (7.30 days) followed by SSI 2 (8.75days), SSI 1 (9.35 days) for complete spawn run days while multispore isolates takes significantly more (9.50 days) by MSI- 1 followed by MSI-2 (10.30 days).

Pinhead initiation

Significantly less (7.00 days) was observed in single spore isolate SSI 3 among other isolates found statistically at par with SSI 4 (7.10 days), JSP 1 (7.50 days), JSP 2 (7.20) days are followed by MSI-01 (9.00 days), SSI 2 (9.50days), SSI 1 (9.50 days), MSI-2 (9.50 days) for complete spawn run days while multispore isolates takes significantly more (10.35 days) by SSI- 1.

Days taken for 1st harvest

Mushroom tissue culture JSP 2 was the first to be ready for harvest with (9.10) days found statistically at par with. SSI 3 (9.35 days), SSI-04 (9.75 days) and JSP 1 (9.60) days followed by SSI-2 (11.20 days), MSI-1 (11.25 days) and significantly more time (12.10 days) was reported in MSI-2.

Fruit body/bed

Significantly maximum number of fruit body per bed was observed in Single spore isolate (SSI 3) (51.20) found statistically at par with JSP 2 (48.45), SSI-4 (45.60) and JSP-1 (44.50) are followed by SSI 1 (32.35), SSI 2 (31.10) and MSI 1(30.30) while significantly lowest number of fruit body/ bed MSI 2 (28.50).

Mean yield/ bed

Significantly maximum mean yield was reported in isolate SSI-3 with (1.530 kg /bed) found statistically at par with JSP-2 (1.410 Kg/bed) and SSI-4 (1.320 kg/bed) followed by JSP-1 (1.300 kg /bed), MSI-1 (0.900 kg/bed), MSI-2 (0.875 kg/bed), SSI-1 (0.850 Kg/bed) while lowest yield (0.780 kg/bed) reported in SSI-2.

Biological efficiency

Highest biological efficiency was recorded in SSI3 (21.86%) and JSP 2 (20.14 %) followed by SSI 4 (18.86 %), JSP1 (18.57%), MSI 1 (12.86%), MSI 2 (12.40%) and lowest recorded was SSI 1 and SSI 2 with biological efficiencies (12.14 %) and (11.14%) respectively.

Table 3. Microscopic characterizations of chlamydospore of *Volvariella volvacea* isolates

S.No.	Isolates	Days taken for chlamydospore production (DTFCP)	Chlamydospore density	Colour of chlamydospore	Diameter of chlamydospore (µm)
1.	SSI 1	14.15	+	Light orange colour Without dark ring	24.10
2.	SSI 2	13.30	++	Light orange colour Without dark ring	23.20
3.	SSI 3	11.45	++++	Light orange colour Without dark ring	26.40
4.	SSI 4	12.10	+++	Light orange colour Without dark ring	25.60
5.	MSI 1	15.60	+	Light orange colour Without dark ring	23.00
6.	MSI 2	14.20	+	Light orange colour Without dark ring	22.60
7.	JSP 1	12.00	+++	Light orange colour Without dark ring	24.60
8.	JSP 2	12.60	+++	Light orange colour Without dark ring	25.40
	CDP=0.05	1.5554			2.452

Table 4. Comparative Yield Evaluation of Single spore isolates.

S. No.	Treatments strains	Spawn run days	Pin head initiation (days)	Days taken for 1st harvest	Fruit body/bed	Mean yield ((kg.)	B.E (%)
1.	SSI 1 (BYT-2)	9.35	10.35	12.30	32.35	0.850	12.14
2.	SSI 2 (BYT-2)	8.75	9.50	11.20	31.10	0.780	11.14
3.	SSI 3 (BYT-5)	7.20	7.00	09.35	51.20	1.530	21.86
4.	SSI 4 (BYT-5)	7.30	7.10	09.75	45.60	1.320	18.86
5.	MSI 1 (BYT-2)	9.50	9.00	11.25	30.30	0.900	12.86
6.	MSI 2 (BYT-5)	10.30	9.50	12.10	28.50	0.875	12.50

7.	JSP 1 (BYT-5)	7.80	7.50	09.60	44.50	1.300	18.57
8.	JSP 2 (BYT-2)	7.50	7.20	09.10	48.45	1.410	20.14
	CD 5%	1.180	1.080	1.1670	8.120	0.210	

They found that spawn prepared from monospore culture had higher yield (15.7%) than tissue culture (14.6%) and the multispore culture (13.0%) Biological efficiency. This finding corroborates with earlier reports of Kalra and Phutela (1991) they reported biological efficiency and mycelia character of Straw mushroom. They found higher yield (1105.5) grams as well as highest biological efficiency (15.7%) was recorded in spawn prepared from monospore culture as compared to tissue culture or multispore culture. The findings can be correlated with researchers. They reported that spawn prepared from Tissue culture sustained higher yield (14.6%) B.E from that of multispore culture (13.0%) but significantly lowers as compared to monospore culture yield.

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