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Studies on Different Cultural Parameters on Vegetative Growth of Straw Mushroom (*Volvariella volvacea*)

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Abstract

Volvariella volvacea is an edible straw mushroom of tropics and subtropics. The optimum temperature and moisture for the growth of this mushroom are 35 °C and 57-60%, respectively. Mushrooms grow on natural or semi synthetic compost and absorb nutrients for their survival. The maintenance and revival of pure culture mycelium with magnificent quality is the first critical stage towards the success of spawn preparation. To maintain any microorganism in artificial conditions, the former has to be cultured on a suitable nutrient medium. All microbes require a set of conditions under which they can grow and sporulate best and culture medium is the major factor influencing fungal cultivation. Five different culture media both in liquid and solid phase, five different pH levels and five temperature regimes were evaluated for the vegetative growth of four different strains of *Volvariella volvacea* i.e. DMR-484, DMR-463, DMR-819 and DMR-820. Among five liquid media studied, malt extract was found to be the best medium for the growth of all the strains of *V. volvacea* both in solid as well as in liquid phase. Out of five different pH levels evaluated for the growth of *V. volvacea* pH7.0 was observed to be the best pH for the growth of all the strains of the test fungus. Out of five different temperature regimes evaluated, 30 °C was observed to be the ideal temperature for the growth of *V. volvacea.*

Keywords: *Volvariella volvacea,* semi-synthetic, revival, microbes, sporulate, strains, regimes

1. Introduction

Mushrooms are fleshy, spore bearing reproductive structures of fungi. For a long time, wild edible mushrooms have played an important role as a human food. However, empirical methods for their cultivation are relatively recent (Martinez-Carrera, 2000). The consumption of mushrooms probably occurred during prehistory, in the hunting and gathering period. Unlike plants, mushrooms could not be cultivated at first and were collected for a long period of time. Even today, relatively few species of mushrooms can be cultivated compared to the number of edible species. Mushrooms were thought to be special and supernatural in origin as 4600 years ago, the Egyptians believed mushrooms to be the plant immortal; the Pharaohs decreed that only they could eat mushrooms (Goldstein and Goldstein, 2010). The Romans thought mushrooms were the food of the Gods (Valverde et al., 2015). Many people collect mushrooms for the purpose of consumption, but lots of

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myths and false concepts still survive today (Rogers, 2015).

V. volvacea is an edible mushroom of tropics and subtropics. The optimum temperature and moisture for the growth of this mushroom are 35 °C and 57-60%, respectively. It can be cultivated in North Indian plains from July to September and in peninsular India from March to November. However, in the hilly areas during the November to July months artificial heating is necessary to raise the bed temperature but in the plains, artificial heating can be minimized by the incorporation of *Melia azadirachta* and *Tamarindus indicus* leaves in alternate layers (Singh et al*.,* 2011).

Mushrooms grow on natural or semi synthetic compost and absorb nutrients for their survival. The maintenance and revival of pure culture mycelium with magnificent quality is the first critical stage towards the success of spawn preparation (Kumar et al., 2018). To maintain any microorganism in artificial conditions, the former has to be cultured on a suitable nutrient medium. All microbes require a set of conditions under which they can grow and sporulate best and culture medium is the major factor influencing fungal cultivation (Dhingra and Sinclair, 2014). Much literature is not available on this aspect of *V. volvacea*, however there are few reports on the cultural studies of *Volvariella* species (Gupta et al.,1970;Ukoima et al., 2009;Tudses, 2016 and Kapoor,2004). Keeping in view the importance of cultural studies in the cultivation process of a particular mushroom and paucity of literature on this aspect of *V. volvacea*, the present investigations were conducted with an objective to study cultural parameters of different strains of *V. volvacea.*

2. Materials and Methods

2.1. Screening of liquid medium

Five different nutrient media viz., malt extract broth, potato dextrose broth, oat meal broth, Czapek's dox broth, sweet potato dextrose broth were evaluated for mycelial growth of different strains of *V. volvacea.* A 5 mm bit of different strains of *V. volvacea* (DMR-484, DMR-463, DMR-819, DMR-820) was taken with the help of sterilized cork borer from the pure culture plate and inoculated in respective broth (150 ml capacity Erlenmeyer's flasks). After inoculation, the flasks were incubated in a BOD incubator at 30 °C. Each treatments were replicated thrice and the data were recorded after 7, 14 and 21 days of the inoculation in terms of dry mycelial weight (mg). For the calculation of fungal biomass in liquid media, mycelial mats of the test fungus were filtered through Whatmann no. 1 filter paper disc having 11 cm diameter and dried at 50 °C overnight. The dry weight of the fungus was calculated by using the following formulae:

Dry weight=(weight of filter paper+mycelium)–(weight of filter paper)

2.2. Screening of solid media

Five different solid media viz*.,* malt extract agar (MEA), potato dextrose agar (PDA), oatmeal agar (OMA), Czapek's dox agar (CA) and sweet potato dextrose agar (SDA) were evaluated for mycelial growth of different strains of *V. volvacea.* A 5 mm bit of test fungus was taken with the help of sterilized cork borer from the pure culture plate and inoculated in the centre of petriplate and then incubated in BOD at 30°C. Each treatments were replicated thrice and data were recorded in terms of average diametric growth (mm) and type of growth (fluffy/ strandy/ sparse) after 48, 72 and 96 h of incubation. Based on these studies best nutrient medium was selected for further experiments.

2.3. Effect of different pH level on growth of V. volvacea

To see the effect of different pH levels on the mycelial growth of different strains of *V. volvacea,* the best nutrient medium selected in 3.4.2 was adjusted to different pH levels viz*.,* 5.0, 6.0, 7.0, 8.0, and 9.0. Then each of the media with different pH levels was poured in petriplates and after solidifying of the media a culture bit of 5 mm was placed on each of the petriplates. These petriplates were then incubated at 30°C for 48 hrs to record the data. Each treatments were replicated thrice and the data were recorded in terms of average diametric growth (mm) and type of growth (fluffy/ strandy/ sparse) after 48, 72 and 96 h of inoculation.

2.4. Effect of different temperature regimes

To study the effect of different temperature regimes, petriplates containing MEA as a basal medium along with culture bit of 5 mm diameter of the test fungus were subjected to different temperatures viz*.,* 25, 30, 35, 40 and 45 °C in different incubators for 48 h. Each treatments were replicated thrice and data were recorded in terms of average diametric growth (mm) and type of growth (fluffy/ strand/ sparse) after 48, 72 and 96 h of inoculation.

3. Results and Discussion

3.1. Screening of liquid media

Data presented in Table 1 clearly depict that on an average malt extract broth was recorded to support significantly maximum (2625.42 mg) dry weight of the test fungus followed by potato dextrose broth (1667.22 mg) and sweet potato dextrose broth (604.17 mg) while, average minimum (246.94 mg) dry weight of the fungus was observed in Czapek's dox broth followed by oat meal broth (400.83 mg), irrespective of the different strains used and days after inoculation. As far as the growth behaviours of different strains of *V. volvacea* was concerned, strain DMR-484 exhibited significantly maximum (1780.67 mg) average dry weight of the fungus followed by strain DMR-463 (1124.00 mg) while, average minimum dry weight of the fungus was recorded in strain DMR-820 (713.00 mg) followed by the strain DMR-819 (818.00 mg), irrespective of different media used and time after inoculation. Irrespective of nutrient media and different strains of *V. volvacea* under investigations, significantly maximum average dry weight (2231.25 mg) was recorded

Table 1: Effect of different liquid media on the growth of different strains of *V. volvacea*

Nutrient medium	Strain	Growth after intervals (mg)			Overall mean	Overall mean
		7th day	14th day	21st day		
MEB	DMR-484	1483.33 (3.17)	2523.33 (3.40)	8250.00 (3.91)	2625.42 (3.41)	1780.67 (3.25)
	DMR-463	1370.00 (3.13)	2280.00 (3.35)	3863.33 (3.58)		1124.00 (3.05)
	DMR-819	1126.67 (3.05)	1953.33 (3.29)	3320.00 (3.50)		818.00 (2.91)
	DMR-820	1213.33 (3.08)	1900.00 (3.27)	2221.67 (3.34)		713.00 (2.85)
PDB	DMR-484	183.33 (2.25)	643.33 (2.80)	7850.00 (3.89)	1667.22 (3.22)	
	DMR-463	160.00 (2.20)	650.00 (2.81)	3480.00 (3.54)		
	DMR-819	156.67 (2.19)	690.00 (2.83)	2860.00 (3.45)		
	DMR-820	173.33 (2.24)	526.67 (2.72)	2633.33 (3.42)		
OMB	DMR-484	90.00 (1.95)	493.33 (2.69)	990.00 (2.99)	400.83 (2.60)	
	DMR-463	83.33 (1.92)	463.33 (2.66)	876.67 (2.94)		
	DMR-819	70.00 (1.84)	380.00 (2.58)	483.33 (2.68)		
	DMR-820	63.33 (1.80)	353.33 (2.54)	463.33 (2.66)		
SDB	DMR-484	86.67 (1.94)	1000.00 (3.00)	1500.00 (3.17)	604.17 (2.78)	
	DMR-463	86.67 (1.94)	833.33 (2.92)	1366.67 (3.13)		
	DMR-819	73.33 (1.87)	126.67 (2.10)	1030.00 (3.01)		
	DMR-820	53.33 (1.73)	110.00 (2.04)	983.33 (2.99)		
CDB	DMR-484	76.67 (1.89)	183.33 (2.26)	1356.67 (3.13)	246.94 (2.39)	
	DMR-463	76.67 (1.89)	173.33 (2.24)	1096.67 (3.04)		
	DMR-819	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)		
	DMR-820	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)		
Overall Mean		331.33 (2.52)	764.17 (2.88)	2231.25 (3.34)		
		CD (p=0.05)	SE			
Media		0.03	0.01			
Strain		0.03	0.01			
Interval		0.02	0.01			
Interaction		0.01	0.04			

Figures in parentheses are log (x+1) transformed values

after 21 days of inoculation followed by 14 (764.17 mg) and 7days of the inoculation (331.33 mg). The body of table reveals that strain DMR-484 exhibited significantly maximum average dry weight (8250.00 mg) in malt extract broth after 21 days of inoculation. However, minimum (53.33 mg) average dry weight was recorded in strain DMR-820 grown in sweet potato dextrose broth after 7 days of inoculation. Rest of the strains exhibited intermediate range of average dry weight in different media under studies after 7, 14 and 21 days of the inoculation except strain DMR-819 and DMR-820 grown in Czapek's dox broth, where no growth was recorded even after 21 days of incubation.

3.2. Screening of solid media

It is evident from the data presented in Table 2 that on an average malt extract agar medium was observed to support the maximum average diametric growth (38.92 mm) of the fungus significantly followed by sweet potato dextrose agar (36.14 mm) and oat meal agar (33.64 mm) while, the minimum average diametric growth (26.86 mm) was recorded in Czapek's dox agar followed by potato dextrose agar (28.69 mm), irrespective of different strains and time of inoculation under study. Irrespective of different media and hours of incubation, strain DMR-484 was observed to exhibit maximum average diametric growth (41.38 mm) followed significantly by DMR-463 (33.40 mm), DMR-820 (29.04 mm) and DMR-819 (27.58 mm). As far as time of incubation was concerned, the maximum average diametric growth (50.63 mm) was recorded after 96 h of incubation while, the minimum average diametric growth (16.42 mm) was recorded after 48 h of inoculation.

Table 2: Effect of different solid media on the growth of different strains of *V. volvacea*

Nutrient medium	Strain	Average diametric growth (mm)			Overall mean	Overall mean
		48 h	72 h	96 h		
MEA	DMR-484	30.33	46.00	79.00	38.92	41.38
	DMR-463	20.33	38.67	58.67		33.40
	DMR-819	15.33	30.33	47.33		27.58
	DMR-820	15.67	34.33	51.00		29.04
PDA	DMR-484	18.00	30.33	52.67	28.69	
	DMR-463	14.67	28.67	48.67		
	DMR-819	10.33	14.67	45.00		
	DMR-820	14.33	22.33	44.67		
OMA	DMR-484	25.67	39.33	69.67	33.64	
	DMR-463	15.00	38.00	42.33		
	DMR-819	12.33	36.00	40.00		
	DMR-820	11.67	33.67	40.00		
SDA	DMR-484	29.00	41.00	64.00	36.14	
	DMR-463	18.33	39.33	50.33		
	DMR-819	13.33	37.00	46.00		
	DMR-820	13.67	31.67	50.00		
CDA	DMR-484	16.33	29.00	50.33	26.86	
	DMR-463	14.00	27.00	47.00		
	DMR-819	9.00	13.00	44.00		
	DMR-820	11.00	19.67	42.00		
Overall mean		16.42	31.50	50.63		
		CD (p=0.05)	SE			
Media		0.51	0.18			
Strain		0.46	0.16			
Interval		0.39	0.14			
Interaction		1.77	0.63			

The growth of the test fungus depicted significant increase after each duration of incubation. The body of the table reveals that significantly maximum average diametric growth (79.00 mm) was recorded in the strain DMR-484 grown in malt extract agar after 96 h of inoculation while, minimum average diametric growth (9.00 mm) was recorded in strain DMR-819 after 48 h of incubation grown in Czapek's dox agar. Rest of the strains exhibited intermediate range of average diametric growth grown on various solid media after different hours of incubation.

The growth characteristics of the different strains of *V. volvacea* varied differently in various liquid as well as solid media under study. The present investigations reveal malt extract broth to be the best nutrient medium for the growth of all four strains of *V. volvacea* while, Czapek's dox broth was least supportive for the growth of test fungus. These results are in conformity with the findings of Gupta (2015) who reported that malt extract broth supported maximum mycelial growth while, Czapek's dox broth supported the minimum mycelial growth of *V. volvacea* both in liquid and solid phase.

Among five different solid media studied, malt extract agar was found to support the maximum mycelial growth followed by sweet potato dextrose agar. These results are in accordance with the findings of Nasim et al. (2001) who reported that malt extract agar supported the maximum mycelial growth of *V. volvacea* and potato dextrose agar was found to support least mycelial growth. Further the present findings are in conformity with the results of Nie et al. (2016) who reported that malt extract agar supported faster mycelial growth of *V. volvacea* than potato dextrose agar. On the contrary, Kumar et al. (2016b) reported potato dextrose agar to support the maximum mycelial growth followed by malt extract agar.

3.3. Evaluation of different pH regimes

It is clear from the Table 3 that maximum average diametric growth (30.75 mm) was recorded at pH 7.0 significantly followed by pH level 8.0 (25.25 mm) which was statistically at par with the growth at pH 9.0 (25.11 mm) and pH 6.0 (24.19 mm) while, significantly minimum average diametric growth (13.75 mm) was recorded at pH 5.0, irrespective of different strains and hours of incubation. Evaluating the performance of different strains of the test fungus, strain DMR-484 was observed to exhibit maximum average diametric growth (29.64 mm) of fungus significantly followed by strain DMR-463 (24.42 mm) while, minimum average diametric growth (20.27 mm) was recorded in DMR-819 which was statistically at par with DMR-820 (20.91 mm), irrespective of the different pH level and hours of incubation. As far as the average diametric growth after different intervals is concerned, significantly maximum average diametric growth (37.70 mm) was recorded after 96 h of incubation while, minimum average diametric growth (9.58 mm) was recorded after 48 h of incubation, irrespective of the different pH levels and strains under evaluation. It was interesting to note that on an average, all the strains grew faster between 48 to 72 h of incubation while, after 72 to 96 h of incubation the increase in mycelial growth was comparatively lesser. The body of table reveals that average diametric growth was significantly maximum (62.00 mm) in strain DMR-484 grown at pH 7.0 after 96 h of incubation while, minimum (4.67 mm) growth was recorded in strain DMR-819 grown at pH 5.0 being statistically at par with rest of the three strains grown at same pH level; DMR-463, DMR-819, DMR-820 grown at pH 6.0; DMR-819, DMR-820 grown at pH 8.0 and DMR-819, DMR-820 grown at pH 9.0 after 48 h of incubation. Intermediate range of average growth of

Table 3: Effect of different pH levels on mycelial growth of different strains of V. volvacea

pH	Strain	Average diametric growth (mm)			Overall Mean	Overall Mean
		48 h	72 h	96 h		
MEA	DMR-484	8.33	15.33	26.33	13.75	29.64
	DMR-463	7.33	15.00	21.67		24.42
	DMR-819	4.67	10.33	13.00		20.27
	DMR-820	5.00	14.33	23.67		20.91
PDA	DMR-484	9.67	29.33	51.67	24.19	
	DMR-463	7.67	24.67	40.33		
	DMR-819	5.33	21.00	38.67		
	DMR-820	5.33	24.67	32.00		
OMA	DMR-484	23.00	38.67	62.00	30.75	
	DMR-463	15.33	33.00	45.33		
	DMR-819	13.33	25.67	37.67		
	DMR-820	10.67	27.00	37.33		
SDA	DMR-484	11.00	32.67	47.33	25.25	
	DMR-463	10.67	25.00	42.33		
	DMR-819	7.33	21.67	39.33		
	DMR-820	7.33	25.67	32.67		
CDA	DMR-484	11.33	31.33	46.67	25.11	
	DMR-463	10.67	23.67	43.67		
	DMR-819	9.33	21.00	35.67		
	DMR-820	8.33	22.00	37.67		
Overall Mean		9.58	24.10	37.70		
		CD (p=0.05)	SE			
pH		1.46	0.52			
Strain		1.30	0.47			
Interval		1.13	0.40			
Interaction		5.06	1.80			

different strains was recorded when grown at different pH levels after 42, 72 and 96 h of incubation. It was interesting to note that at pH level 5.0, strain DMR-819 did not exhibit significant increase in growth from 72 to 96 h of incubation.

In the present investigations, pH 7.0 was found to be the best pH for the mycelial growth of *V. volvacea* as it supported the maximum growth of the test fungus followed by pH 8.0. The present findings regarding the optimum pH levels are in accordance with the findings of Kumar et al. (2016a) who reported that the best pH is 7.0 for the growth of *V. volvacea* however, Kapoor (2004) reported that pH between 6.0 and 7.0 was more suitable for the growth of straw mushroom. On the contrary, Ahlawat and Tewari (2007) reported pH 9.0 to be the best supportive pH for the growth of *V. volvacea.*

3.4. Effect of different temperature regimes

Data presented in Table 4 clearly depict that significantly maximum average diametric growth (41.78 mm) was recorded at 30 °C followed by growth at 35 °C (39.17 mm), 40°C (27.22 mm) and 25 °C (17.44 mm), irrespective of the strains and time of incubation under study. However, no growth of any of the four strains under study was recorded at 45°C even after 96 h of incubation. Irrespective of different temperature regimes and time of incubation, significantly maximum average diametric growth (37.00 mm) was recorded in DMR-484 followed by strain DMR-463 (30.81 mm) while, minimum average diametric growth (27.83 mm) was recorded in DMR-819 which was significantly followed by strain DMR-820 (29.97 mm). Irrespective of different temperature regimes and strains under study significantly maximum diametric growth

Table 4: Effect of different temperature regimes on the mycilial growth of different strains of *V. volvacea*

Temperature (°C)	Strain	Average diametric growth (mm)			Overall Mean	Overall Mean
		48 h	72 h	96 h		
MEA	DMR-484	11.67	15.00	30.33	17.44	37.00
	DMR-463	10.67	12.67	24.00		30.81
	DMR-819	11.67	17.00	22.33		27.83
	DMR-820	14.00	16.33	23.67		29.97
PDA	DMR-484	35.67	46.67	71.33	41.78	
	DMR-463	27.00	43.00	63.67		
	DMR-819	15.33	32.00	52.67		
	DMR-820	19.67	36.33	58.00		
OMA	DMR-484	33.33	37.00	64.33	39.17	
	DMR-463	26.00	41.33	56.67		
	DMR-819	20.00	31.00	50.33		
	DMR-820	19.00	37.67	53.33		
SDA	DMR-484	20.67	35.67	42.33	27.22	
	DMR-463	13.33	21.33	30.00		
	DMR-819	17.00	22.00	42.67		
	DMR-820	16.00	24.33	41.33		
CDA	DMR-484	-	-	-	-	
	DMR-463	-	-	-		
	DMR-819	-	-	-		
	DMR-820	-	-	-		
Overall mean		19.44	29.33	45.44		
		CD (p=0.05)	SE			
Temperature		1.54	0.55			
Strain		1.54	0.55			
Interval		1.34	0.47			
Interaction		5.36	1.91			

*No growth recorded at this temperature regime

(45.44 mm) was recorded after 96 h of incubation significantly followed by growth after 72 (29.33 mm) and 48 (19.44 mm) h of incubation. It was interesting to note that the growth of all strains was faster between 72 to 96 h of incubation as compare to growth between 48 to 72 h of incubation. Interaction of different temperature regimes, strains and time of incubation reveals that strain DMR-484 exhibited significantly maximum average diametric growth (71.33 mm) at 30 °C after 96 h of incubation while, strain DMR-463 exhibited minimum average diametric growth (10.67 mm) at 25°C after 48 h of incubation which was statistically at par with growth of rest three strains at 25 °C, strain DMR-819 at 30 °C and DMR-463 at 40 °C after 48 h of incubation as well as growth of strain DMR-463 and strain DMR-484 at 25 °C after 72 h of incubation. During the present investigations, the growth of the test fungus was best supported at a temperature of 30°C followed significantly by 35 °C. These results are in accordance with the findings of Akinyele and Adetuyi (2005) who reported 30 °C to be optimum temperature for the growth of *V. volvacea*. The present investigations are further supported by Kumar et al. (2016a) who reported that optimum range of temperature of *V. volvacea* for its best growth lies between 30-35 °C. Also, Deshpande and Tamhane (1982) reported that the best yield of *V. volvacea* was obtained at 27-30 °C. Chang and Steinkraus (1981) also reported a temperature of 35 °C to be optimum for the growth of *V. volvacea.*

3.5. Type of mycelial growth V. volvacea strains under different cultural conditions

Under cultural studies of different strains of *V. volvacea*, type of growth and colour of mycelium was recorded. It is clear from the Table 5 that out of five nutrient media studied, malt extract agar medium supported the best type of growth i.e. thick strandy in all strains under investigations while, Czapek's dox broth supported only sparse growth of different strains of *V. volvacea.* However, very sparse growth was observed in case of strain DMR-463 grown in oat meal agar and sweet potato dextrose agar. As far as the growth of different strains of *V. volvacea* at different pH levels was concerned, very sparse growth of all the strains was observed at pH 5.0 while, thick strand mycelium was observed at pH level 7.0. However, at pH 8.0 strain DMR-484 and DMR-463 exhibited thin strandy growth and DMR-819 as well as DMR-820 exhibited sparse growth.

Table 5: Morphological characters of different strains of *V. volvacea* recorded in lab conditions

Strain	Type of growth														
	Media					pH					Temperature (°C)				
	MEA	PDA	OMA	SDA	CA	5.0	6.0	7.0	8.0	9.0	25	30	35	40	45
DMR-484	TS	TS	S	S	S	VS	S	TS	ThS	ThS	VS	TS	TS	S	NG
DMR-463	TS	TS	VS	VS	VS	VS	S	TS	ThS	S	VS	TS	TS	S	NG
DMR-819	TS	S	S	S	S	VS	S	TS	S	S	VS	TS	TS	S	NG
DMR-820	TS	S	S	S	S	VS	S	TS	S	S	VS	TS	TS	S	NG

TS: Thick Strand; ThS: Thin Strand; S: Sparse; VS: Very Sparse; NG: No Growth

4. Conclusion

V. volvacea being a very fast growing fungus grows best at malt extract medium both in solid and liquid phase, at pH 7.0 and temperature of 30 °C. Out of four different strains under investigations of *V. volvacea*strain DMR-484 grew best in all the media, pH levels and temperature regimes studied. From these results we can say that it could easily be cultivated in subtropical regions having temperature range of temperature between 30-35 °C and substrates of neutral to slightly alkaline pH**.**

5. Further Research

Further detailed studies on the biochemical properties of *V. volvacea* should be studied for the complete knowledge of its hidden therapeutic properties and also for improving its post-harvest life so that it can be stored for long term use.

6. Acknowledgement

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