Biological Forum – An International Journal **14(2a): 32-37(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Cultural and Nutritional Requirements for the Growth of Medicinal Mushroom *Schizophyllum commune* Fr.

M. Roja[1], G. Thiribhuvanamala[2]*, K. Angappan[3], P. Geetha[4] and D. Amirtham[5]
[1]M.Sc. Scholar, Department of Plant Pathology, TNAU, Coimbatore (Tamil Nadu), India.
[2]Associate Professor, Department of Plant Pathology, TNAU, Coimbatore (Tamil Nadu), India.
[3]Professor, Department of Plant Pathology, TNAU, Coimbatore (Tamil Nadu), India.
[4]Associate Professor, Food Science and Nutrition, Center For Post-Harvest Technology, TNAU, Coimbatore (Tamil Nadu), India.
[5]Assistant Professor, Department of Biochemistry, TNAU, Coimbatore (Tamil Nadu), India.

(Corresponding author: G. Thiribhuvanamala)*
(Received 27 April 2022, Accepted 15 June, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: *Schizophyllum commune* Fr. is a medicinal mushroom renowned for its potential pharmacological and nutraceutical properties. To enhance the yield and explore its potential values it is necessary to investigate the cultural and nutritional conditions for the growth in detail. The investigation on the cultural characters of all isolates in potato dextrose agar medium showed the maximum colony diameter in isolate 1 (90.00mm) followed by isolate 8 (89.00mm) and isolate 2 (86.66mm). The maximum mycelial mat dry weight was recorded in the isolate 5 (2.33g/100mL) followed by isolate 2 (2.20g/100mL) and isolate 1 (2.00g/100mL). Of the different growth media tested, mushroom complete media (MCM) showed the maximum mycelial growth in all the isolates. Among the different carbon sources tested, sorbitol and mannitol supported the maximum mycelial growth in most of the selected isolates such as isolate 1 and isolate 4 (90.00mm) compared to other carbon sources. Similarly, among the different nitrogen sources tested peptone recorded maximum mycelial growth of (90.00mm) in isolate 2 when compared to all other nitrogen sources. The pH requirement for *S. commune* isolate 1 for its maximum mycelial growth (89.60mm) recorded in pH 5 and pH 6. And optimum temperature of 25 to 30°C was quite suitable for the mycelial growth of *S. commune* where isolate 4 and isolate 8 recorded maximum mycelial growth (90.00mm).

Keywords: *Schizophyllum commune*, Different media, Carbon source, Nitrogen source, pH, Temperature, Colony diameter.

INTRODUCTION

Schizophyllum commune Fr. is a medicinal mushroom that grows naturally on decaying woods, thus the name white rot fungus. This mushroom is also known as Split gill mushroom, and the name *Schizophyllum commune* is derived from the Greek terms *Schiza,* which means "split," and *commune*, which means "common" (Mahajan 2022). The fruiting body of *S. commune* is tiny flabellilform (fan shaped) white stipeless cap with hairs. It is consumed as food and medicine in number of nations, including Korea, Malaysia, China, Thailand, Vietnam, and North East India due to its high medicinal properties. *S. commune* mushroom possess a storey of potential compounds that have antimicrobial, anticancerous, antidiabetic activities against many human diseases (Chandrawanshi *et al.,* 2017). Extracellular melanin by *S. commune* showed antibacterial, antifungal activity and anti cell proliferation activity against human epidermoid larynx carcinoma cell lines (Arun *et al.,* 2015). The polysaccharide schizophyllan from *S.commune* is known for its high medicinal value. Chandrawanshi *et al.* (2019) reported that different solvent extracts of *S. commune* possess antidiabetic activity. The bioactive compounds from the mushroom *S. commune* is also reported to have antimicrobial property against plant pathogens. Dutta *et al*. (2019) reported that the active compound schizostatin from *S. commune* is responsible for the antifungal activity against the plant pathogens of pepper. Considering its importance, the cultivation technique is required for the large scale production and for its other industrial use. Thiribhuvanamala *et al.* (2020) reported that *S. commune* was a good source of lignin degrading fungus that has high lignocellulolytic

machinery and scope in industrial applications. Thus the cultivation could be done on substrates that are rich in lignin content. Good substrates for the growth of the mushroom include paddy straw, wheat straw, saw dust etc., (Singh *et al.,* 2021; Dasanayaka and Wijeyaratne 2017). All the fungi require good cultural and nutritional conditions for their growth. Limited studies were carried out in the field of cultural and nutritional requirements of *S. commune*. *S. commune* have little gastronomic appeal due to its rough texture and small fruiting body size. In order to increase the yield and tap its bioactive compounds from *S. commune* which could be in pharmaceutical and nutraceutical wide applications, the cultural and nutritional requirements is investigated in detail.

MATERIALS AND METHODS

The cultures of *S. commune* - Isolate 1, Isolate 2, Isolate 3, Isolate 4, Isolate 5, Isolate 6 and Isolate 7 obtained from Directorate of Mushroom Research, Solan; Isolate 8 and Isolate 9 from Department of Plant Pathology, TNAU, Coimbatore and Isolate 10 from Indira Gandhi Krishi Vishwavidyalaya, Raipur were used in this study.

A. Colony diameter measurement

Under *in vitro* conditions, the cultural characteristics of ten different *S. commune* mushroom isolates were investigated. In a Petri plate, 15mL of sterilized Potato Dextrose Agar media was poured and allowed to solidify. A 9 mm mycelial disc was taken from a 7-day old culture of different isolates and placed in the centre of a Petri plate aseptically. The plates were incubated at room temperature for further observations. On 3rd, 5th and 7th days after inoculation, growth characters such as colony diameter, colony colour and morphology were recorded.

B. Estimation of Biomass production.

To estimate the biomass production (fresh and dry weight) of different *S. commune* isolates, a 9 mm mycelial disc of *S. commune* mushroom isolates from a 7-day old culture was inoculated in Potato dextrose broth and incubated at room temperature. After 10 days of inoculation, the mycelial mat was separated from the broth using Whatman No. 1 filter paper and oven dried at 50 to 55°C to determine the amount of biomass produced. Fresh and dry weights of mycelial mats were recorded for each isolate.

C. Effect of different media for the growth of S. commune

Six different culture media (Czapek Dox (CPZ), Malt extract agar (MEA), Mushroom complete media (MCM), Oat meal agar (OMA), Sabouraud dextrose agar (SDA) and Yeast-malt extract (YME) were evaluated for the growth of *S. commune* isolates as described by Imtiaj *et al*. (2008). These media was prepared with their respective composition and sterilized in an autoclave. After sterilization the media poured into the sterilized petriplate and 9 mm diameter disc of culture was taken from a 7 days old culture grown on PDA medium and placed in the centre of each plate of six different culture media. After 7 days of incubation period at room temperature, mycelial growth was recorded.

D. Effect of different carbon and nitrogen sources for the growth of S. commune

Based on the results of the cultural growth characters of *S. commune* in PDA media, the five *S. commune* isolates (Isolate 1, Isolate 2, Isolate 4, Isolate 5 and Isolate 8) were chosen for further tests. The effect of various carbon and nitrogen sources on the growth of selected *S. commune* isolates was tested. Different carbon source viz., fructose, lactose, sorbitol, mannitol and dextrin was added to the basal medium at the rate of 4% and different nitrogen sources such as sodium nitrtate, calcium nitrate, potassium nitrate, glycine and petone was added to PDA medium at the rate of 1%. The medium containing different carbon and nitrogen sources was sterilized in an autoclave at 121°C for 15 minutes. A 9 mm mycelial disc of 7 days old culture of selected isolates was placed separately in the centre of the Petri plate. The plates were incubated at room temperature for colony diameter measurement and morphology (Adejoye *et al*., 2007).

E. Effect of different Temperature and pH for the growth of S. commune

Based on the results of the cultural growth characters of *S. commune* in PDA media, the five *S. commune* isolates (Isolate 1, Isolate 2, Isolate 4, Isolate 5 and Isolate 8) were chosen for further tests. The effects of different temperature and pH for the selected *S. commune* isolates were tested. PDA media was prepared and it was sterilized in an autoclave at 121°C for 15 minutes. A 9 mm mycelial disc of 7 days old culture of selected isolates was placed separately in the centre of the Petri plate. The plates were incubated at 20°C, 25°C, 30°C, 35°C and 40°C temperatures to observe the suitable temperature for the mycelial growth. Similarly the PDA media was prepared and adjusted to different pH *viz*., pH 5 to 9 to test its effect on mycelial growth of *S. commune* (Kumar *et al.,* 2017).

RESULT AND DISCUSSION

A. Colony diameter measurement

The colony diameter was measured at 3, 5 and 7 days after inoculation. The maximum mycelial diameter at 5 DAI was recorded in isolate 1 (90.00mm) followed by isolate 8 (89.00mm), isolate 2 (88.66mm), isolate 5 (87.33mm) and isolate 4 (86.66mm). The colony morphology of the *S. commune* was pure white mycelium with varying texture such as radiating, cottony, fluffy, flat mycelium depending upon the isolates. Conclusively all the cultures attained the full

plate mycelial coverage at 7DAI. This shows that the full plate mycelial coverage could be attained after 5 to 7 DAI. The data on colony diameter is included in the Table 1.

Table 1: Morphological and cultural characters of *S. commune* mushroom.

Colony diameter and biomass production							
Isolates	**Colony diameter (mm)**			**DTFG (Days)**	**Cultural Characters**	**Biomass production in liquid medium (g/100 ml)**	
	3d	**5d**	**7d**			**Fw**	**Dw**
Isolate 1	49.66[a]	90.00[a]	90	5.00	Pure white cottony mycelium	4.86[b]	2.00[c]
Isolate 2	46.66[b]	88.66[c]	90	6.00	Pure white radiating dense mycelium	4.70[d]	2.20[b]
Isolate 3	36.33[g]	86.00[f]	90	6.00	Pure white radiating mycelium	3.90[f]	0.60[g]
Isolate 4	44.66[d]	86.66[e]	90	6.00	Moderately dense mycelium	4.80[c]	1.03[e]
Isolate 5	42.33[e]	87.33[d]	90	6.00	Pure white dense mycelium	4.96[a]	2.33[a]
Isolate 6	34.33[h]	81.33[g]	90	6.00	Pure white fluffy mycelium	3.30[g]	0.90[f]
Isolate 7	31.33[j]	80.33[h]	90	6.00	Pure white mycelium	2.00[j]	0.30[h]
Isolate 8	46.00[c]	89.00[b]	90	6.00	Pure white flat mycelium	2.06[i]	0.16[j]
Isolate 9	33.00[i]	71.66[j]	90	7.00	Pure white flat mycelium	2.80[h]	0.20[i]
Isolate 10	38.66[f]	79.66[i]	90	7.00	Pure white moderately dense mycelium	4.10[e]	1.96[d]
SEd	**0.85**	**0.47**	**0.00**			**0.45**	**0.12**
CD (0.05)	**1.80**	**0.98**	**0.00**			**0.95**	**0.27**

Values are means of three replications. The means followed by the same letter are not significantly different from each other by DMRT (P=0.05). DTFG= Days taken for full plate growth Fw= Fresh weight Dw= Dry weight

B. Estimation of Biomass Production

The maximum mycelial dry weight was recorded from isolate 5 (2.33g/100mL) followed by isolate 2 (2.20g /100mL), isolate1 (2.00g /100mL), isolate 10 (1.96g /100mL) and isolate 4 (1.033g /100mL). The reduced mycelia dry weight was recorded in isolate 8 (0.16 g / 100mL), isolate 9 (0.20 g / 100mL) and isolate 7 (0.30 g / 100mL). The fresh and dry weight recorded for all the isolates is included in the Table 1.

C. Effect of different culture media for the growth of S. commune

Among the tested culture media, Mushroom complete media supported the growth of *S. commune* followed by Malt extract agar, Oat meal agar, Sabouraud dextrose agar, Yeast malt extract agar medium. CPZ medium was found to be poor performing medium. The mycelial growth was dense in MCM and Oat meal agar medium whereas sparse mycelial growth was observed in Malt extract agar medium and SDA medium and moderately thin mycelial growth in Yeast extract agar medium and poor growth in CPZ medium. All the tested isolates recorded maximum colony diameter (90.00mm) at 7DAI in MCM. Whereas in MEA the maximum colony diameter was observed in isolate 5 (90.00mm) and isolate 4 (89.66mm). The maximum colony diameter (89.33mm) in OMA was recorded in isolate 1 and isolate 10. The colony diameter in SDA was maximum in isolate 1 (87.33mm) and isolate 10 (85.33mm). YME recorded the maximum colony diameter in isolate 8 (78.33mm) and isolate 4 (76.66mm).

Table 2: Effect of different Media for the growth of *S. commune* mushroom.

Strain/ isolate	**Mycelial growth (mm)**											
	MCM		**MEA**		**OMA**		**SDA**		**YME**		**CPZ**	
	7 DAI	**DTFG**	**7 DAI**	**DTFG**	**7 DAI**	**DTFG**	**7 DAI**	**DTFG**	**7 DAI**	**DTFG**	**7 DAI**	**DTFG**
Isolate 1	90.00	7.0	87.66[c]	8.0	89.33[a]	7.0	74.66[j]	9.0	76.33[d]	9.0	20.66[a]	-
Isolate 2	90.00	7.0	77.33[h]	9.0	84.33[d]	8.0	81.33[d]	8.0	71.66[f]	9.0	14.66[d]	-
Isolate 3	90.00	7.0	80.60[f]	8.0	76.33[h]	9.0	80.66[e]	8.0	71.33[g]	9.0	10.00[e]	-
Isolate 4	90.00	7.0	89.66[b]	7.0	86.60[c]	8.0	87.33[a]	8.0	76.66[b]	9.0	6.93[h]	-
Isolate 5	90.00	7.0	90.00[a]	7.0	74.33[j]	9.0	82.00[c]	9.0	66.33[i]	10.0	6.90[i]	-
Isolate 6	90.00	7.0	87.00[d]	8.0	83.33[e]	8.0	76.33[i]	9.0	71.33[e]	9.0	18.86[b]	-
Isolate 7	90.00	7.0	86.66[e]	8.0	76.33[i]	9.0	76.66[h]	9.0	76.66[b]	9.0	17.66[c]	-
Isolate 8	90.00	7.0	71.66[i]	9.0	76.66[g]	9.0	79.33[f]	9.0	78.33[a]	9.0	9.83[f]	-
Isolate 9	90.00	7.0	70.33[j]	10.0	77.33[f]	8.0	78.00[g]	8.0	68.33[h]	9.0	9.83[f]	-
Isolate 10	90.00	7.0	80.00[g]	9.0	89.33[a]	7.0	86.33[b]	8.0	76.66[b]	9.0	9.93[f]	-
SEd	**0.00**		**1.91**		**1.87**		**3.49**		**3.09**		**0.47**	
CD (0.05)	**0.00**		**4.01**		**3.92**		**7.33**		**6.49**		**1.00**	

Values are means of three replications. The means followed by the same letter are not significantly different from each other by DMRT (P=0.05). MCM= Mushroom complete media MEA= Malt extract Agar media OMA= Oat meal agar media SDA= Sabouraud dextrose agar media YME= Yeast Malt Extract Agar media CPZ= Czapex Dox media. DTFG= Days taken for full plate growth.

Observations showed that there was no growth even after the 15 days of incubation in CPZ medium after a certain growth of the mycelium. Conclusively MCM was found to be the best media for *S. commune* next to PDA medium based on the mycelial growth. This is similar to the report by Imtiaj *et al*. (2008) where they found that MCM supported compact mycelial density. Kumar *et al*. (2017) reported that *S. commune* strains grow moderately in MEA medium which is similar to this report. Czapek Dox did not support the mycelial growth of *Macrolepiota procera* (Shim *et al.,* 2005) and *Phellinus* spp. Hur *et al*. (2008) which is similar to our study. The colony diameter of different isolates in different media is mentioned in the Table 2.

D. Effect of different carbon and nitrogen sources for the growth of S. commune

Investigation on different carbon sources revealed that sorbitol, fructose, mannitol were most suitable carbon sources for the growth of the selected *S. commune* isolates and recorded the maximum colony diameter in most of the isolates which is similar to the report by (Alam *et al*. (2010). Sorbitol and mannitol recorded the maximum colony diameter in isolate 1 and isolate 4 (90.00mm). Contrarily, lactose showed maximum mycelial growth and dextrin showed the moderate mycelial growth compared to other carbon sources as reported by Imtiaj *et al.* (2008). Different nitrogen sources like peptone, sodium nitrate and calcium nitrate showed maximum mycelial growth as compared to glycine and potassium nitrate which recorded the moderate mycelial growth. The peptone as nitrogen source recorded the maximum colony diameter in isolate 2 (90.00) and isolate 4 (88.33mm). Similar to our study, Niederpruem *et al*. (1964) reported peptone as good nitrogen source for the growth of *S. commune.* Deshaware *et al*. (2021) indicated that peptone and sodium nitrate was suitable nitrogen sources for the growth of *Cantharellus cibarius*. In our study, calcium nitrate was also suitable for the growth of mycelium which is similar to the report by Alam *et al*. (2010); Imtiaj *et al*. (2008) where they mentioned that calcium nitrate was the most suitable nitrogen sources for the growth of *S. commune*. The colony growth diameter for carbon and nitrogen sources after 5 days of inoculation is included in the Table 3.

Table 3: Effect different carbon and nitrogen sources for the mycelial growth of *S. commune.*

Colony diameter (mm) 5DAI					
Carbon souces	**Isolate 1**	**Isolate 2**	**Isolate 4**	**Isolate 5**	**Isolate 8**
Fructose	90.00[a]	84.00[c]	88.00[d]	83.66[b]	84.66[d]
Sorbitol	90.00[a]	85.30[b]	90.00[a]	88.33[a]	89.00[a]
Lcatose	90.00[a]	88.66[a]	74.00[e]	81.33[c]	89.00[a]
Mannitol	90.00[a]	83.66[d]	90.00[a]	76.00[e]	82.30[e]
Dextrin	63.33[e]	65.33[e]	90.00[a]	78.66[d]	87.66[c]
	0.55	2.26	0.51	1.64	0.51
CD (0.05)	1.24	5.03	1.15	3.78	1.15
Colony diameter (mm) 5DAI					
Nitrogen sources	**Isolate 1**	**Isolate 2**	**Isolate 4**	**Isolate 5**	**Isolate 8**
$NaNO_3$	78.66b	87.00b	89.33a	88.66a	84.00c
$CaNO_3$	80.00a	63.33c	87.33c	65.00e	79.33d
Glycine	74.00d	45.00d	73.66e	70.33d	79.33d
KNO_3	78.00c	42.66e	75.33d	80.66c	88.66a
Peptone	65.33e	90.00a	88.33b	81.00b	86.33b
SEd	**3.61**	**2.82**	**0.91**	**2.66**	**0.90**
CD (0.05)	**8.33**	**6.51**	**2.11**	**6.13**	**2.07**

Values are means of three replications. The means followed by the same letter are not significantly different from each other by DMRT (P=0.05). DAI= Days after inoculation.

E. Effect of different Temperature and pH for the growth of S. commune

Based on the investigation on different temperatures, 25˚C and 30˚C was found to be optimum temperatures for the good mycelial growth. The maximum colony diameter at 25˚C was recorded in the isolate 4 (90.00mm) and isolate 8 (90.00mm). The maximum colony diameter at 30˚C was recorded in the isolate 4 (90.00mm) and isolate 8 (90.00mm).These observations clear that 25˚C to 30˚C serves as an optimum temperature for the growth of *S. commune*. This was analog to the findings by Adejoye *et al*. (2007); Kumar *et al*. (2017) as they reported that 25˚C to 30˚C serves as a suitable temperature for the growth of *S. commune*. Whereas moderate mycelial growth was observed in case of 20˚C. Very slow and poor growth was observed in case of 35˚C and 40˚C. The investigation on the different pH showed that pH 5 and pH6 was most suitable for the growth of *S. commune* and the maximum colony diameter was observed in the isolate 1 (89.60mm) and isolate 4 (89.60mm) at pH 5. In case of pH 6 the maximum colony diameter was recorded in isolate 1 (89.60mm) and isolate 5 (89.60mm). The rest of the pH also showed good mycelial growth. Emayavarman *et al*. (2021) reported that the maximum mycelial growth was observed at pH 8 and pH 6 in elm oyster mushroom. The colony growth diameter of

different temperature and pH was included in the Table 4.

Statistical Analysis: The design of experiments i.e. CRD and statistical analyses were followed as suggested by Gomez and Gomez (1984). Statistical software used for the analysis of data is AGRES (Developed by the Department of Physical science, TNAU, Coimbatore).

Table 4: Effect different pH and temperature for the mycelial growth of *S. commune*.

Colony diameter (mm) 5DAI					
pH	**Isolate 1**	**Isolate 2**	**Isolate 4**	**Isolate 5**	**Isolate 8**
pH 5	89.60[a]	79.00[b]	89.60[a]	77.30[d]	85.00[b]
pH 6	89.60[a]	83.00[a]	88.33[b]	89.60[a]	67.00[e]
pH 7	88.33[c]	43.66[e]	82.00[c]	76.33[e]	78.60[c]
pH 8	87.66[d]	74.66[c]	87.00[c]	88.33[b]	78.00[d]
pH 9	87.00[e]	68.00[d]	86.00[d]	86.00[c]	88.66[a]
SEd	**0.69**	**1.03**	**0.39**	**1.03**	**1.13**
CD (0.05)	**1.55**	**2.30**	**0.90**	**2.30**	**2.52**
Colony diameter (mm) 5DAI					
Temperature	**Isolate 1**	**Isolate 2**	**Isolate 4**	**Isolate 5**	**Isolate 8**
20˚C	78.33[c]	68.66[c]	80.33[c]	72.33[c]	81.33[c]
25˚C	81.00[b]	77.33[b]	90.00[a]	88.66[b]	90.00[a]
30˚C	89.33[a]	79.33[a]	90.00[a]	89.60[a]	90.00[a]
35˚C	56.00[d]	47.33[d]	38.66[d]	53.00[d]	63.00[d]
40˚C	32.33[e]	30.33[e]	24.66[e]	19.00[e]	30.66[e]
SEd	**1.98**	**2.52**	**0.63**	**1.87**	**1.02**
CD (0.05)	**4.57**	**5.82**	**1.40**	**4.32**	**2.35**

Values are means of three replications. The means followed by the same letter are not significantly different from each other by DMRT (P=0.05). DAI= Days after inoculation.

CONCLUSION

Schizophyllum commune is an edible medicinal mushroom that is furnished with lot of medicinal values related to human health including the antioxidant, antidiabetic, anticancerous potential compounds. Development of cultivation technology is indeed important to tap its ample potential values. Hence, the results emanated from the *in vitro* investigations on the nutritional requirements like different culture media, carbon nitrogen sources and cultural requirements like different pH and temperature studied will be useful for selection of substrates to take up cultivation and tap more yield.

Acknowledgment. Authors thank the ICAR- AICRP scheme on mushroom, Directorate of Mushroom Research, Solon for the funds to carry out this work.

Conflict of Interest: Nil.

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How to cite this article: M. Roja, G. Thiribhuvanamala, K. Angappan, P. Geetha and D. Amirtham (2022). Cultural and Nutritional Requirements for the Growth of Medicinal Mushroom *Schizophyllum commune* Fr.. *Biological Forum – An International Journal*, *14*(2a): 32-37.