

1 Original Research Article

2 Assessment of grain extract media on mycelial growth of *Pleurotus* spp. (*P. sapidus* and 3 *P. flabellatus*)

4 Abstract

5 Present investigation pertains to study the impact of different culture media on the mycelial
6 growth and dry weight of *Pleurotus* spp. The study was conducted at Mushroom Laboratory
7 Department Plant Pathology, S. V. P. University of Agriculture and Technology, Meerut, UP,
8 India. In the present research effect of grains against *Pleurotus* spp. was investigated.
9 Experiment was conducted in seven treatments with four replications. All the extracts
10 observed varied mycelial growth of *Pleurotus* spp. After nine days maximum radial growth
11 (90.00 mm) in both species (*P. sapidus* and *P. flabellatus*) was observed in Barley extract
12 media. The maximum growth rate (10.00 mm/day) of mycelium in *P. sapidus* and *P.*
13 *flabellatus* was recorded in barley extract agar. Maximum dry mycelium weight (7.98 mg/100
14 ml & 8.35 mg/100 ml) of *P. sapidus* and *P. flabellatus* was observed in barley extract broth,
15 respectively.

16

17 **Key words:** *Cereals, Media, Mycelial growth and Pleurotus.*

18 Introduction

19 Mushrooms are the macro fungi which possess fleshy, sub fleshy fruiting bodies of
20 fungi. Mushroom has been defined as “a macrofungus with a distinctive fruiting body which
21 can be either epigeous or hypogeous and large enough to be seen with naked eyes and picked
22 by hand” (Chang and Miles, 1993). Mushroom is being widely used as food and food
23 supplements from ancient times.

24 The word mushroom is derived from the Greek word for sponge- ‘Sphonggos’ or
25 ‘Sphoggas’ referring to the sponge like structure of some species. ‘Ksumpa’ was found to be
26 the earliest word in Sanskrit for mushroom. In the present day, it has turned into ‘Khumbi’
27 and other words are ‘Kukurmutta’, ‘Kawak’, ‘Bhoomikawak’ and ‘Bhustrna’. The cultivated
28 edible mushrooms are a group of large macroscopic fleshy fungi, generally belong to
29 Basidiomycetes but some are Ascomycetes. Oyster mushroom (*Pleurotus* spp.) cultivation
30 has increased tremendously throughout the world during the last few decades (Chang, 1999;
31 Royse, 2002). The name *Pleurotus* has its origin from Greek word, ‘Pleuro’ that means
32 formed laterally or lateral position of the stalk or stem. Oyster mushroom commonly referred
33 as ‘Dhingri’ in India, is a Basidiomycetes and belongs to the genus ‘*Pleurotus*’. It is
34 lignocellulolytic fungus that grows naturally in the temperate and tropical forests on dead,
35 decaying wood logs, sometimes on drying trunks of deciduous or coniferous trees. It can also
36 grow on decaying organic matter. The fruiting bodies of this mushroom are distinctly shell,
37 fan or spatula shaped with different shades of white, cream, grey, yellow, pink or light brown
38 depending upon the species. However, the colour of the sporophores is extremely variable
39 character influenced by the temperature, light intensity and nutrients present in the substrate.
40 The avocation of mushroom farming will become a very important cottage industry in the
41 integrated rural development programmes, which will lead to the economic betterment of not

42 only small farmers but also of landless labourers and other weak sections of communities.
43 About 385 million tonnes of agricultural wastes are available annually in India and about half
44 of this residue remains unused. If even 1% of this crop residue is used to produce mushroom,
45 India will become a major mushroom producing country in the world. Edible mushroom
46 production represented an attractive method of improving the nutritional quality of ligno-
47 celluloid wastes for use as an animal feed stock. Among the various physical, chemical and
48 biological methods used for upgrading the digestibility and nutritive value of agricultural
49 wastes, biodegradation by using white rot fungi including mushrooms have been found
50 promising. Mushroom production represents one of the most commercially important steps
51 towards diversification of agriculture based on microbial technology for large- scale
52 recycling of agro-wastes in an agricultural country like India.

53 MATERIALS AND METHODS

54 Experimental site

55 For the present investigations, experiments were conducted at Mushroom Laboratory,
56 Department of Plant Pathology, College of Agriculture, Sardar Vallabhbhai Patel University
57 of Agriculture & Technology, Modipuram, Meerut, Uttar Pradesh- 250110 situated on the
58 Western side of the Delhi - Dehradun high way NH-58 at a distance of 10.0 km away in the
59 north of Meerut city. The district Meerut is situated between 29° 01'N latitude and 77° 45'E
60 longitude at an altitude of 237 meters above the mean sea level.

61 Establishment and maintenance of pure culture

62 The culture of *P. sapidus* and *P. flabellatus*, used in the present investigations were
63 collected from Directorate of Mushroom Research Centre, Solan, Himanchal Pradesh and
64 Mushroom Research and Training Centre, G. B. Pant University of Agriculture and
65 Technology, Pantnagar. The cultures of *Pleurotus* species were further purified by single
66 hyphal tip method. For this purpose, the cultures were grown in sterilized petri plates on
67 potato dextrose agar (PDA) medium for 8 days. Single branched hyphae from the periphery
68 of the growing colony were marked under low power (10x) of compound microscope and
69 transferred to PDA slants for maintenance. These culture tubes were incubated at 24±1°C for
70 about a week and again sub-cultured on PDA medium and then stored in a refrigerator at
71 05^o±1C for further use.

72 Effect of Media

73 For the effect of different media studies, seven media (*i.e.* wheat extract agar, rice extract
74 agar, barley extract agar, oat extract agar, sorghum extract agar, pearl millet extract agar and
75 potato dextrose agar) were used for the radial growth. The ingredients and methods of their
76 preparation are given below:

77 Wheat Extract Agar (WEA) Medium

78	Wheat grain	200g
79	Agar-agar	20g
80	Distilled Water	1000ml

81 Two hundred gram grains were washed with water 2-3 times and then boiled with 500
82 ml distilled water for 20 minutes, allowed to cool, the grains were then separated and the
83 liquid suspension passed through a muslin cloth. The volume of the extract so obtained was
84 made up to 500 ml by adding distilled water. Twenty gram agar-agar was melted separately in
85 500 ml of distilled water and mixed with grains extract. The total volume was made up to
86 1000 ml by adding distilled water.

87 Rice extract agar, barley extract agar, oat extract agar, sorghum extract agar, pearl
88 millet extract agar and potato dextrose agar were also prepared by same methods as described
89 above for Wheat extract agar medium. All the seven prepared media were sterilized by
90 autoclaving at 1.1 kg/cm² pressure (121⁰C) for 20 minutes. The test media were poured to
91 Petri plates and culture tubes then inoculated with culture of *Pleurotus* spp. under aseptic
92 conditions. The plates (90 mm @ 20 ml/plate) were inoculated with culture of *Pleurotus* spp.
93 centrally and incubated at 27±1°C. Radial growth and growth rate were determined at each 48
94 hrs till the colony covered the full plate.

95 **Statistical Analysis**

96 The suitable statistical design (CRD) was applied and the data thus obtained were analysed
97 statistically. Analysis of variance (ANOVA) technique and critical difference (CD) was
98 calculated at five per cent level of significance for comparison with other treatment.

99

100 **Result and Discussion**

101 This experiment was conducted for the study of effect of different cereal grains
102 extract on mycelial growth of *P. sapidus* and *P. flabellatus* *in-vitro* condition. In the
103 experiment six different types of cereals extract media *viz.* wheat extract agar, rice extract
104 agar, barley extract agar, oat extract agar, sorghum extract agar, pearl millet extract agar were
105 used, while potato dextrose agar media was use as control with four replications. The
106 observations of mycelial growth were recorded on 3rd, 6th and 9th days after inoculation as
107 shown in Table-.

108 On 3rd day, maximum radial growth (19.75 mm) of *P. sapidus* was recorded in barley
109 extract agar followed by oat extract agar (17.00 mm) and minimum radial growth (11.25 mm)
110 in potato dextrose agar. While in case of *P. flabellatus* maximum radial growth (29.00 mm)
111 was recorded in barley extract agar followed by oat extract agar (28.50 mm) and minimum
112 radial growth (20.75 mm) was observed in potato dextrose agar.

113 On 6th day in case of *P. sapidus* maximum radial growth (59.00 mm) was recorded in
114 barley extract agar and statically similar with oat extract agar followed by pearl millet extract
115 agar (57.75 mm). Minimum radial growth (49.50 mm) was recorded in potato dextrose agar
116 which was statistically lower than all treatment. While in case of *P. flabellatus* maximum
117 radial growth (72.50 mm) was recorded in barley extract agar followed by oat extract agar
118 (65.50 mm) which was significantly higher than all treatments. Minimum radial growth
119 (56.00 mm) was recorded in potato dextrose agar (control).

120 On 9th day in case *P. sapidus* maximum radial growth (90.00 mm) was recorded in
121 barley extract agar followed by oat extract agar (88.00 mm), which was significantly higher
122 than all treatments. Minimum radial growth (76.50 mm) was recorded in potato dextrose
123 agar. While in case of *P. flabellatus* maximum radial growth (90.00 mm) was recorded in

124 barley extract agar followed by oat extract agar (87.75 mm). Minimum radial growth (75.50
125 mm) was recorded in potato dextrose agar (control).

126 On 9th day, growth rate (mm/day) was recorded in *P. sapidus* and *P. flabellatus*.
127 Maximum growth rate (10.00 mm/day) of *P. sapidus* was recorded in barley extract agar
128 followed by oat extract agar (9.77 mm/day) and minimum growth rate (8.50 mm/day) was
129 recorded in potato dextrose agar. While in case of *P. flabellatus* maximum growth rate (10.00
130 mm/day) was recorded in barley extract agar followed by oat extract agar (9.75 mm/day) and
131 minimum growth rate (8.38 mm/day) was recorded in potato dextrose agar which was
132 significantly lower than all other treatments.

133 The results were in accordance with the findings of Hussain and Hussain, (2004) who
134 reported that *Pleurotus* spp. showed fastest growth of mycelium on potato dextrose agar
135 among different media used. Baliyan (2008) studied the mycelial growth of *Pleurotus* spp.
136 (*i.e.* *P. florida*, *P. flabellatus*, *P. sajor-caju*, *P. fossulatus* and *P. sapidus*) rates which were
137 higher on MEA (Malt Extract agar) medium than on PDA or WSEA. Zubair (2012) also
138 observed maximum radial growth (9.00 cm) of *P. sapidus* in potato dextrose agar medium.
139 Bhadana (2014) also reported maximum radial growth was found in oat extract agar medium
140 and potato dextrose agar medium and pearl millet extract agar medium of *P. djamore*, *P.*
141 *florida*, *P. eryngii* and *P. flabellatus* respectively. Yadav (2014) radial growth of *P.*
142 *flabellatus* was found maximum in barley extract agar followed by oat agar medium and
143 minimum radial growth recoded in potato dextrose agar medium. Sardar *et al.*, (2015) also
144 reported the effects of various growth conditions on growth and development of *Pleurotus*
145 species six different *Pleurotus* strains were cultured on different agar media *viz.* PDA (Potato
146 dextrose agar), MEA (Malt extract agar) and WEA (Wheat extract agar). Among these media
147 Potato dextrose agar medium (PDA) was found to be the best medium than malt extract agar
148 (MEA) and wheat extract agar (WEA) for the growth of mycelium of all *Pleurotus* species.

149 **Dry mycelial weight of *Pleurotus* spp.**

150 This experiment was conducted for the study of dry mycelial weight of *P. sapidus* and
151 *P. flabellatus in-vitro* condition. In the experiment seven different types of broth media *viz.*
152 wheat extract broth, rice extract broth, barley extract broth, oat extract broth, sorghum extract
153 broth, pearl millet extract broth and potato dextrose broth were taken with four replications as
154 shown in Table-2.

155 In case of *P. sapidus* maximum dry mycelium weight (7.98 mg/100ml) was observed
156 in barley extract broth significantly higher than all treatments followed by oat extract broth
157 (6.60 mg/100ml). Minimum dry weight of mycelium (5.06 m/100ml) was observed in potato
158 dextrose broth. While in case of *P. flabellatus* maximum dry weight (8.35 mg/100ml) was
159 observed in barley extract broth significantly higher than all treatments followed by oat
160 extract broth (7.58 mg/100ml). Minimum dry weight (5.26 mg/100ml)) was observed in
161 potato dextrose broth.

162 Dry matter growth rate (mg/day) of *P. sapidus*, maximum dried mycelial growth rate
163 (0.53 mg/day) was observed in barley extract broth followed by oat extract broth (0.44
164 mg/day). The minimum dried mycelial growth rate (0.33 mg/day) was observed in potato
165 dextrose broth. While in case of *P. flabellatus* maximum dried mycelial growth rate (0.55
166 mg/day) was observed in barley extract broth followed by oat extract broth (0.50 mg/day).
167 The minimum dried mycelial growth rate (0.35 mg/day). The results were in accordance with

168 the findings of Potato Dextrose Broth has been reported to be supporting maximum mycelial
 169 growth by earlier workers Suharban and Nair (1991). Kumar (2015) also revealed that
 170 maximum radial growth was recorded on pigeon pea extract agar medium (90.00 mm) and
 171 minimum radial growth was found in control (69.50 mm) in *P. flabellatus*. Maximum radial
 172 growth rate was observed in pigeon pea extract agar medium (11.25 mm/day) in *P.*
 173 *flabellatus*. Maximum dried mycelial weight was in pigeon pea extract broth medium (5.86
 174 mg/50ml) in *P. flabellatus*.

S. No	Media	Radial Growth (mm)						9 th days Growth rate (mm/day)	
		3 rd day		6 th day		9 th day		<i>P. sapidus</i>	<i>P. flabellatus</i>
		<i>P. sapidus</i>	<i>P. flabellatus</i>	<i>P. sapidus</i>	<i>P. flabellatus</i>	<i>P. sapidus</i>	<i>P. flabellatus</i>		
1.	Wheat Extract media	11.50	23.50	50.50	59.50	80.50	80.00	8.94	8.88
2.	Rice Extract media	12.75	24.50	51.00	61.00	81.25	82.25	9.02	9.13
3.	Barley Extract media	19.75	29.00	59.00	72.50	90.00	90.00	10	10
4.	Oat Extract media	17.00	28.50	59.00	65.50	88.00	87.75	9.77	9.75
5.	Sorghum Extract media	15.75	26.25	56.50	60.25	83.75	84.25	9.30	9.36

175 **Table-01: Effect of different cereals grain extracts media on mycelial growth of**
 176 ***Pleurotus* spp. (*P. sapidus* and *flabellatus*).**

177

6.	Pearl Millet Extract media	16.00	27.00	57.75	63.25	85.00	87.00	9.44	9.66
7.	Potato Dextrose media (Control)	11.25	20.75	49.50	56.00	76.50	75.50	8.50	8.38
CD at 5%		3.06	3.00	4.57	3.54	3.09	3.58	-	-
SE(m)		1.03	1.01	1.54	1.19	1.04	1.21	-	-

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179

180 **Table-02: Effect of different cereals grain extracts broth media on dry mycelial weight**
181 **of *Pleurotus* spp.**

S. No.	Broth Media	<i>P. sapidus</i>		<i>P. flabellatus</i>	
		Dry matter weight (mg/100ml)	Dry matter growth rate (mg/day)	Dry matter weight (mg/100ml)	Dry matter growth rate (mg/day)
1.	Wheat Extract broth media	5.76	0.38	6.74	0.44
2.	Rice Extract broth media	5.88	0.39	6.80	0.45
3.	Barley Extract broth media	7.98	0.53	8.35	0.55
4.	Oat Extract broth media	6.60	0.44	7.58	0.50
5.	Sorghum Extract broth media	5.90	0.39	7.20	0.48
6.	Pearl Millet Extract broth media	6.41	0.42	7.33	0.48
7.	Potato Dextrose broth media (Control)	5.06	0.33	5.26	0.35
CD at 5%		0.42	-	0.33	-
SE(m)		0.14	-	0.11	-

182

183 **Conclusion:** In this article effect of grains was observed more or less effect on mycelial
184 growth against *Pleurotus* spp. Radial growth was observed in Barley extract media.
185 Maximum dry mycelium weight (7.98 mg/100ml & 8.35 mg/100ml) of *P. sapidus* and *P.*
186 *flabellatus* was observed in barley extract broth respectively. This might enhance the
187 mushroom production in the coming future.

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Fig 1 *P. sapidus*



Fig 2 *P. flabellat*

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