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Effect of some Abiotic Factors on the Growth and Development of Different *Pleurotus* spp.

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A B S T R A C T

Mushrooms need carbon and nitrogen for structural and functional purposes in addition to trace elements, growth regulators and vitamins. Therefore, evaluation of their role in influencing the growth of the mushroom is a necessary aspect to be studied. The influences of sugar, temperature and growth regulators were investigated during this experiment on the mycelium growth of different species of oyster mushroom *viz*., *P. florida*, *P. flabellatus*, *P. djamor* and *P. eryngii*. The results of the present experiment indicated that maximum radial growth was found in glucose followed by maltose. *P. djamor* and *P. eryngi* exhibited highest radial growth (90.00 mm) at the temperature of 29°C followed by *P. florida* (83.50 mm). Whereas *P. flabellatus* (78.00 mm) showed maximum radial growth at 26ºC. Maximum radial growth of *Pleurotus* spp. was recorded at 5 ppm and 10 ppm concentration of gibberellic acid and indolbutaric acid. So, it was concluded that we can increase the vegetative growth of *Pleurotus* spp. by adding glucose, maltose as sugar along with growth regulators like gibberellic acid and indolbutaric acid.

Introduction

Pleurotus spp. has a great commercial potential being an edible and wood decaying fungus (Kaur and Atri, 2016). Mushrooms that are edible known as food of Gods also used delicacy or garnish and eaten routinely as in human diet generally known as healthy food (Memon *et al.*, 2017; Singh *et al.*, 2018). Mushrooms considered as a good source of vitamins, which are essential for human diet including vitamin C, niacin, and riboflavin. Dhingri (*Pleurotus* spp.) Oyster mushrooms are mostly found in India (Ahmed *et al.*, 2009).

Pleurotus genus is one of most extensively studied white-rot fungi due to its exceptional

ligninolytic properties. It is an edible mushroom and it also has several biological effects, as it contains important bioactive molecules. In basidiomycete fungi, lignocellulolytic enzymes are affected by many typical fermentation factors, such as medium composition, ratio of carbon to nitrogen, pH, temperature, air composition, etc (Kadiri and Kehinde, 1999). The survival and multiplication of mushrooms is related to a number of factors, which may act separately or have interactive effects among them (Bellettini *et al.*, 2019).

Mushroom production has been limited throughout the world due to incompetence; incapability and lack of technical knowledge to culture edible mushrooms. Spawn (active mycelium) production is one of the major limiting factors to mushroom cultivation all over the world (Stanley, 2010). The high temperatures between 27ºC and 35ºC during the day has been a major problem for the farmers of tropical regions who wish to grow mushrooms (Gaitán and Salmones, 2008).

So, in the present experiment, influences of different sugars, temperature range and growth regulators were investigated on the mycelium growth of different species of oyster mushroom *viz*., *P. florida*, *P. flabellatus*, *P. djamor* and *P. eryngii*.

Materials and Methods

The experiments were conducted in Mushroom Laboratory of Department Plant Pathology, S. V. P. University of Agriculture & Technology, Meerut, U.P. on the Western side of the Delhi-Dehradun high way at a distance of 10.0 km away in the north of Meerut city.

Establishment of pure culture

The culture of different species of *Pleurotus viz*., *P. florida*, *P. flabellatus*, *P. djamor* and *P. eryngii* included in the present investigation were collected from Mushroom Research and Training Centre, G. B. P. U. A. & T, Pantnagar and C.S.A.U.A &T, Kanpur. The cultures of *Pleurotus* species obtained were further purified by single hyphal tip method. For this purpose, the cultures were grown in sterilized petri plate on potato dextrose agar (PDA) for 4-5 days. Single branched hyphae from the periphery of the growing colony were marked under low power (10X) in the compound microscope and transferred to PDA slants for maintenance. These tubes were incubated at 25-27^{0}C for about a week, again subculture on PDA and then stored in a refrigerator at 2-10^{0}C for further use.

Effect of sugar solutions on radial growth and dry mycelial weight

Different sugar solutions were used to study their effect on radial and dry mycelial growth. One percent solutions of glucose, sucrose, maltose, fructose and starch were mixed in sterilized media. The pH of the solutions was adjusted to 6.5-7.0 and spore suspension made in each solution was incubated at 25±1^{0}C. The 20 ml medium was poured in each sterilized petri plate and subsequently inoculated with 5 mm disc of 10 days old culture. Inoculated plates were incubated at 25±1^{0}C and observation for radial growth was recorded on eight day when the colony covered the first full plate. Observations on radial and dry mycelial growth were recorded.

Effect of different temperature on radial growth

For studies on variability, the culture of four *Pleurotus* species (i.e. *P. djamor, P. florida, P. flabellatus* and *P.eryngii*) were incubated at five different temperature viz. 20, 23, 26, 29, 32 and 35^{0}C. Petri plates containing 20 ml of sterilized PDA medium were inoculated at the centre with 9 mm diameter disc from 10

days old actively growing mycelium under aseptic conditions. Four replications for each treatment were maintained. Observations on radial growth were taken at each 72 hrs till the colony covered the first full plate.

Effect of growth regulator on radial mycelial growth

For the present investigation two growth regulators viz. Gibbrellic Acid (GA) and Indole Butaric Acid (IBA) were carried out at different concentrations i.e. 05 ppm, 10ppm, 15ppm, and 20ppm on potato dextrose agar (PDA) medium for radial growth. PDA medium was prepared and required amount of growth regulators were added in the medium after sterilization. The 25 ml medium was poured in each sterilized Petri plate and subsequently inoculated with 9 mm disc of seven days old culture of *Pleurotus spp. like P florida, P djamor, P flabllatus, P. eryngii*. Inoculated plates were incubated at 25±1^{0}C. The observations of radial growth were taken at each 72 hrs till the colony covered the first full plate.

Statistical analysis of data

Data with appropriate transformations were analyzed with the help of analysis of variance table wherever required. The F value was tested and critical difference (CD) was calculated at 5 per cent of significance for comparing treatment means (Gomez and Gomez, 1996; Chandel, 2002).

Results and Discussion

Effect of different sugar solution on radial growth

Four species of *Pleurotus* spp. (i.e. *P. Florida P. djamor, P. flabllatus* and *P. eryngii*) were grown in five sugar solution viz. fructose, maltose, sucrose, starch and glucose, as shown in Table 1. The results revealed that maximum dry mycelial growth was found in glucose (90.00mm) followed by maltose *P. djamor*. While in *P. florida* maximum radial growth in glucose (90.00mm) followed in sucrose (87.00mm). In case of *P. flabellatus* maximum radial growth in glucose (88.30mm) followed in fructose (88.00mm).in *P*. eryngii maximum radial growth was observed in glucose (90.00mm) and followed in maltose sugar (83.00mm). Minimum mycelial growth of each species varied significantly in all the sugar. The least growth was recorded in starch on which mycelial growth (78.00 mm) obtained in *P. florida. In P. djamor* (78.66mm sucrose) *P. florida* (78.00 mm starch), *P.flabllatus (80.00 mm* starch*) and P. eryngii* (80.66 mm fructose).

Sastre-Ahuatzi *et al.,* (2007) studied that the radial growth rate was evaluated in five strains of *Pleurotus ostreatus*, grown on starch-based and glucose-based agar media containing different concentrations. The radial growth rate of some strains showed a positive and a negative correlation with the productivity.

Effect of different sugar solution on dry mycelial growth

Four species of *Pleurotus* spp. (*i.e. P. florida, P. djamor, P. flabllatus* and *P. eryngii*) were grown in five sugar solution with PDB *viz*., fructose, maltose, sucrose, starch and glucose, as shown in Table 2. Maximum dry mycelial growth was found in glucose (5.70 mg.) in *P. flabellatus*. In case of *Pleurotus djamor* maximum dry mycelial growth in glucose (5.59 mg.) followed by fructose (5.36mg.). In *P. florida* maximum mycelial growth in glucose (4.77mg.) followed in maltose (4.31mg.). In case of *P. eryngii* maximum mycelial growth was observed in sucrose (4.95mg.) followed in glucose sugar (4.45mg.). The least growth of the *Pleurotus*

spp. were recorded in starch on which mycelial growth (2.28mg.) obtained in P. *djamor, P. florida* (2.34mg.starch), *P. flabllatus* (3.74mgmaltose*)* and *P. eryngii* (3.13mg. fructose).

Gbolagade *et al.,* (2006) Revealed that the greatest biomass Among the monosaccharide, glucose stimulated the best biomass production (186.7 mg/30 cm3) followed in order by fructose mannose, and sorbose (P<=0.05). In the series of complex sugars and sugar alcohols, mannitol supported the highest biomass yield with mycelial dry weight of 130.0 mg/30 cm3. The maximum biomass yield (330.0 mg/100 cm3) was obtained when 7.0 cm3of *P. florida* inoculum was inoculated into a submerged medium.

Effect of temperature on radial growth

Experiment was conducted to study the effect of range of temperatures (20-35^{0}C) on the radial growth of four *Pleurotus* species (*i.e. P. florida, P. flabellatus, P. eryngii* and *P djamor*). As shown in Table 3, *P. djamor* and *P eryngi* exhibited highest radial growth (90.00 mm) at the temperature of 29^{0}C followed by *P. florida* (83.50 mm) which is at par with each other. Whereas P. *flabellatus* (78.00 mm) showed maximum radial growth at 26^{0}C. At 35^{0}C the radial growth of *P. djamor, P. eryngi and P florida* were 69.25mm , 59.75 mm, and 65.25 mm, respectively while *P. flabellatus* could not grow at 35^{0}C.While *P.flabellatus* exhibited minimum radial growth (40.50 mm) at the temperature of 32^{0}C followed by *P. djamor* (42.00 mm) at the temperature 20^{0}C.

Baliyan (2008) found also a range of temperature (25-30^{0}C) to be suitable for mycelial growth of five *Pleurotus* spp. Radial growth of *P. florida* and *P. sapidus* were found maximum at 30^{0}C where as the growth of *P. sajor caju, P. fossulatus* and *P. flabellatus* was maximum at 25^{0}C. Zharare *et al.,* (2010) studied the sensitivity of Pleurotus mycelium to different temperature range. Eight *Pleurotus* spp., which included *P. sajor caju* and *P. eryngii* were cultured aseptically on agar at 25, 30 and or 35^{0}C. Mycelial growth was maximum at 25-30^{0}C whereas a temperature of 35^{0}C was detrimental to mycelial growth except in one strain. At the highest temperature tested (35^{0}C), the relative mycelial growth rate ranged from 6 to 91%, indicating marked differences in tolerance of the strains to high temperature. Sardar *et al.,* (2015) also reported that *Pleurotus* species were cultured aseptically on PDA at different temperature ranges and found mostly Pleurotus species grows best at 25°C.

Effect of growth regulators on radial growth

As shown in data presented in (Table 4) maximum radial growth (89.00 mm) in *P. djamor* was recorded on 8th day of observation at Gibberellic acid10 ppm concentration followed by 5 ppm concentration (85.66 mm). In case of Indol butyric acid at 5 ppm concentration (45.66 mm) followed by 10 ppm concentration (33.33 mm). Similarly, in case of Gibberellic acid maximum radial growth of *P. florida* on 8th day observed at 5 ppm concentration (90.00 mm) followed by 10 ppm concentration (84.33 mm) and in case of Indol butaric acid at 5 ppm concentration (63.00 mm) followed by 10 ppm concentration (42.66 mm). In case of Gibberellic acid maximum radial growth of *P. flabllatus* on 8th day observation at 10 ppm concentration (90.00 mm) followed by the 20 ppm concentration (85.66 mm) and in case of Indol butaric acid maximum radial growth at 5ppm concentration (48.66 mm) followed by 10ppm concentration.

Table.1 Effect of sugar solution on radial growth (mm) of different *Pleurotus* species on PDA

S. No.	Sugar solution	*P. djamor*		*P. florida*		*P. flabellatus*		*P. eryngii*	
		Radial growth (mm)	**Radial growth rate (mm/day)**	**Radial growth (mm)**	**Radial growth rate (mm/day)**	**Radial growth (mm)**	**Radial growth rate (mm/day)**	**Radial growth (mm)**	**Radial growth rate (mm/day)**
1	Fructose	82.00	10.25	78.66	9.83	88.00	11.00	80.66	10.08
2	Maltose	83.66	10.45	79.33	9.91	85.33	10.66	83.00	10.37
3	Sucrose	78.66	9.83	87.00	10.87	87.33	10.91	81.66	10.20
4	Starch	80.66	10.08	78.00	9.75	80.00	10.00	82.00	10.25
5	Glucose	90.00	11.25	90.00	11.25	88.30	11.04	90.00	11.25
6	control	78.00	9.75	77.66	9.70	82.33	10.29	80.00	10.00
C.Dspecies1.0511 sugar 1.2873 speciesxsugar2.575									

Average of three replications

Values in each vertical column followed by same letter(s) do not differ significantly

Table.2 Effect of different sugar solution on weight of dried mycelium (mg/50 ml) of *Pleurotus* species in PDA broth

S. No.	Sugar Solution	*P. djamor*	*P. florida*	*P. flabellatus*	*P. eryngii*
1.	Fructose	5.36	3.95	4.10	3.13
2.	Maltose	4.94	4.31	3.74	4.36
3.	Sucrose	4.09	3.84	4.31	4.95
4.	Starch	2.28	2.34	5.31	4.03
5.	Glucose	5.59	4.77	5.70	4.45
6.	Control	4.05	4.06	3.96	4.04
CD at5%species0.1192 sugar 0.1460 speciesxsugar0.292					

Average of four replicate

Value in each vertical column followed by same letter(s) do not differ significantly

Table.3 Effect of different temperature on radial growth (mm) of different *Pleurotus* species

S.No.	Temp. (^{0}C)	*P. djamor*		*P. florida*		*P. flabellatus*		*P. eryngii*	
		Radial growth (mm)	**Radial growth rate (mm/day)**	**Radial growth (mm)**	**Radial growth rate (mm/day)**	**Radial growth (mm)**	**Radial growth rate (mm/day)**	**Radial growth (mm)**	**Radial growth rate (mm/day)**
1.	20	42.00	5.25	67.75	8.46	61.75	7.71	70.00	8.75
2.	23	58.00	7.25	71.75	8.96	71.75	8.96	75.75	9.46
3.	26	64.00	8.00	83.50	10.43	78.00	9.75	81.25	10.15
4.	29	90.00	11.25	88.50	11.06	65.75	8.21	88.50	11.06
5.	32	75.50	9.43	79.50	9.93	40.50	5.68	78.50	9.81
6	35	69.25	8.65	65.25	8.15	0	0	59.75	7.46
CD at5%species		**0.9263**							
Temp.		**1.1345**							
Temp x species		**2.269**							

Average of four replications
Values in each vertical column followed by same letter(s) do not differ significantly

Table.4 Effect of growth regulator on radial growth (mm) of different *Pleurotus* species on PDA

S. No.	Growth regulator	*P. djamor*		*P. florida*		*P. flabellatus*		*P. eryngii*	
		Radial growth (mm)	**Radial growth rate (mm/day)**	**Radial growth (mm)**	**Radial growth rate (mm/day)**	**Radial growth (mm)**	**Radial growth rate (mm/day)**	**Radial growth (mm)**	**Radial growth rate (mm/day)**
1	GA@ 5PPM	85.66	10.70	90.00	11.25	82.33	10.29	90.00	11.25
2	GA @0PPM	89.00	11.12	84.33	10.54	90.00	11.25	82.00	10.25
3	GA @ 15PPM	84.66	10.58	83.66	10.45	85.33	10.66	71.66	8.95
4	GA @0PPM	83.33	10.41	84.00	10.50	85.66	10.70	69.00	8.62
5	IBA @ 5PPM	45.66	57.07	63.00	7.87	48.66	6.08	54.33	6.79
6	IBA @ 10PPM	33.33	4.16	42.66	5.33	35.00	4.37	42.00	5.25
7	IBA @ 15PPM	21.00	2.62	34.33	4.29	19.00	2.37	31.33	3.91
8	IBA @ 20PPM	0	0	0	0	0	0	0	0
9	Control	80.00	10	81.33	10.16	78.66	9.83	63.00	7.87
CDspecies	**0.8240**								
regulator	**1.2360**								
speciesxregulator	**2.472**								

repliAverage of four replications

Values in each vertical column followed by same letter(s) do not differ significantly

In case of Gibberellic acid maximum radial growth of *P. eryngii* on at 5ppm concentration(90.00 mm) and in case of Indol butaric acid maximum radial growth at 5ppm concentration (54.33 mm) followed by 10 ppm concentration(42.00 mm). The poorest growth was recorded on Gibberellic acid in *P. djamor* (83.33 mm), *P. florida* (83.66mm), *P. flabllatus* (82.33mm) and *P.eryngii* (69.00 mm) and in Indol butaric acid *P. djamor* (21.00 mm), *P florida* (34.33mm), *P. flabllatus* (19.00mm) and *P.eryngii* (31.33 mm).

The results are in accordance to Guanxi *et al.*, (2004) studied the effect of growth regulators gibberellic acid, kinetin, 2,4- D and 6- BA (6 benzyladenine) on mycelial growth of *P. eryngii*. The best combination for mycelia growth was found 2x10-6 gm/liter GA+ 20x10-6 g /liter 2,4-D+ 1x10-6 g/liter 6-BA. This combination increased the activities of cellulose, polyphenol oxidase, amylase and catalase. This combination was mixed with the culture substrate, hyphal growth and fruit bodies appeared earlier and mushroom yield and biological efficiency of *P. eryngii* increased. Mukhopadhyay *et al.*, (2005) evaluate the plant growth hormone *viz*. indole-3-acetic acid (IAA), gibberellic acid (GA3) and kinetin for biomass production of *P. sajor-caju*. The hormone, at different concentrations, increased the biomass of *P. sajor-caju* by 15-26%. Maximum enhancement was observed with IAA.

In conclusion, the mycelium growth of oyster mushroom was affected by different sugars, temperature conditions and growth regulators. The results obtained in the present study revealed that gibberellic acid and IAA at 5 ppm and 10 ppm concentration supported the maximum vegetative growth of different species of oyster mushroom. The present findings have helped us to understand the biochemical and temperature requirements of *Pleurotus* spp. for enhancing the vegetative growth of this mushroom in the basal medium.

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