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In-vitro evaluation of mycelial growth of *Pleurotus sapidus* against different botanicals

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Abstract
The aim of present study was evaluation of mycelial growth of *Pleurotus sapidus* against different botanicals extract in laboratory. The study was conducted during September 2018 at Mushroom Laboratory Department Plant Pathology, S. V. P. University of Agriculture and Technology, Meerut, UP, India. In-vitro evaluation of *Pleurotus* spp. were conducted seven treatment with four replication. The Petri plates were incubated at 23±2°C for 9 days and observations were recorded at each 72 hrs. All plant extracts were founded more or less mycelial growth of mushroom. Maximum growth was observed after nine days lantana leaf extract @ 4% (88.25 mm) followed by lantana leaf extract @ 2% (87.25 mm) and 82.25 mm in control without extract. The *Eucalyptus* was recorded less or inhibited the mycelial growth of *Pleurotus* (15.75 and 46.00 mm) @ 4 and 2 percent of *Eucalyptus* extract respectively. The lantana leaf extract was found very fast growth of *P. sapidus* mycelium.

Keywords: Evaluation, botanicals and *Pleurotus* mycelial growth

Introduction
The cultivation of edible mushrooms has become an attractive economic alternative over past few years, mainly due to increase in its demand and market value (Chang, 2006) [4]. Oyster mushrooms are the easiest and least expensive commercial mushrooms to grow because they are well known for conversion of crop residues to food protein (Banik and Nandi, 2004) [1]. Oyster mushroom (*Pleurotus* spp.) cultivation has increased tremendously throughout the world during the last few decades (Chang, 1999; Royse, 2002) [3]. The name *Pleurotus* has its origin from Greek word, 'Pleuro' that means formed laterally or lateral position of the stalk or stem.

Cultivation of oyster mushroom having ability to convert lingo cellulosic waste material in to high quality food material, The fresh sporophores of this mushroom contain moisture (90.8%), whereas fresh as well as dry oyster mushrooms are rich in proteins (30.4%), carbohydrates (57.6%), and fiber (8.7%) with 345 K (cal) energy value on 100 g dry weight basis; (Pandey & Ghosh, 1996) [6]. Rambelli & Menini (1985) [8] reported that this mushroom is reputed to be anti-tumoural because of its chemical composition. It is lignocellulolytic fungus that grows naturally in the temperate and tropical forests on dead, decaying wood logs, sometimes on drying trunks of deciduous or coniferous trees. It can also grow on decaying organic matter. Mushrooms are now getting significant importance due to their nutritional and medicinal value and today their cultivation is being done in all over the world. Though 20 mushroom varieties are domesticated about half a dozen varieties *viz.,* button, shiitake, oyster, wood ear and paddy straw mushrooms contribute 99% of the total world production. Mushroom offers prospects for converting lignocellulosic residues from agricultural fields, forests into protein rich biomass. Such processing of agro waste not only reduces environmental pollution but the by product of mushroom cultivation is also a good source of manure, animal feed and soil.

Materials and Methods
The present study is mycelial growth of *Pleurotus sapidus* against different botanicals extract in laboratory. The study were conducted during September 2018 at Mushroom Laboratory Department Plant Pathology, S. V. P. University of Agriculture and Technology, Meerut, UP, India, which is situated on the Western side of the Delhi-Dehradun high way (NH-58) at a distance of 10.0 km away in the north of Meerut city. The district Meerut is situated between 29°01'N latitude and 77° 45'E longitude at an altitude of 237 meters above the mean sea level.

Establishment of pure culture
Culture of *Pleurotus sapidus* were purified and maintained by single hyphal tip method. For this purpose, the culture was grown in sterilized Petri plates on Potato Dextrose Agar Medium (PDA) for 8-10 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. These tubes were incubated at 21-24°C for about a week, again sub cultured on PDA and then stored in a refrigerator at 5-10°C for further use. Optimization of culture conditions on redial growth of *Pleurotus sapidus* given below:

Preparation of extracts
For preparation of extracts, 50 gram plant products were collected, wash in tap water, air dried and homogenized with equal amount of distilled water (50mL) by crashing them with electric grinder machine. The extract was filtered through double - layered muslin cloth and filtered through Whatman No.1 filter paper which was considered as standard solution.

***In-Vitro* study**
In this study, extract of 3 botanicals were evaluated in the laboratory against *Pleurotus sapidus* mycelium. The plant extracts were evaluated *in vitro* through Poison food technique (Nene and Thapliyal 2000) [5]. Botanical are doses 2% and 4% extract (standard solution) was incorporated in 100 mL of potato dextrose agar medium (PDA) sterilized by autoclaving at 121°C (15 lbs pressure) for 20 minutes. The molten media were poured into four sterilized glass petriplates (90 mm) considering each as a replication. After solidification of the agar plates were inoculated with 5 mm diameter mycelial cut from 6 days old culture of *P. sapidus.* The media without the botanical extract served as check. The plates were incubated at 27±1°C till the complete growth was observed in control plates. The observation was collected mycelial growth (mm) in three days interval.

Statistical analysis
The suitable statistical design (CRD) was applied and the data thus obtained were analyzed statistically. Analysis of variance (ANOVA) technique and critical difference (CD) was calculated at five percent level of significance for comparison with other treatment.

Result and Discussion
In the study of different botanical extract were obtained mycelial growth of *P. sapidus*. The *P. sapidus* was inoculated in PDA medium Petri plates. The Petri plates were incubated at 23±2°C for 9 days and observations were recorded at each 72 hrs. The result was presented in Table 1. All plant extracts were founded more or less mycelial growth of mushroom. Maximum growth was observed in lantana leaf extract @ 4% (88.25 mm) followed by lantana leaf extract @ 2% (87.25 mm) and 82.25 mm in control without extract. The *Eucalyptus* was recorded less or inhibited the mycelial growth of *Pleurotus* (15.75 and 46.00 mm) @ 4 and 2 percent of *Eucalyptus* extract respectively.
In after three days observation maximum radial growth in T4 (25.25 mm) and radial growth rate 8.41 mm/day, followed by in T3 (22.00 mm) growth rate 7.33 mm/day and T7 (21.75 mm) growth rate (7.25 mm/day). Minimum growth was observed after three days in T6 (11.00 mm) growth rate 3.66 mm/day followed by T5 (14.75 mm) growth rate 4.91 mm/day. After six days observation was founded maximum in T4 (70.25 mm) growth rate 11.70 mm/day followed by T3 (63.75 mm) growth rate 10.62 mm/day and T7 (63.00 mm) growth rate 10.50 mm/day. Minimum growth was observed after six days in T6 (12.75 mm) growth rate 2.12 mm/day followed by T5 (29.00 mm) growth rate 4.83 mm/day. After nine days observation was founded maximum in T4 (88.75 mm) growth rate 9.86 mm/day followed by T3 (87.25 mm) growth rate 9.69 mm/day and T7 (82.25 mm) growth rate 9.13 mm/day. Minimum growth was observed after nine days in T6 (15.75 mm) growth rate 1.75 mm/day followed by T5 (46.00 mm) growth rate 5.11 mm/day.
Pervez *et al.* (2012) were similarly observed mycelial growth in lantana extract 51.25% and neem extract (47.75%) in 5 and 10% concentration. Among the botanicals, *A. indica* (neem) showed found less effective against the mycelium growth of *P. ostreatus* (4.4%). This was followed by extracts of *Pongamia pinnata* (karanja) 6.7% (*P. ostreatus*), *Clerodendron indicum* (clerodendron) 8.9% and *Eucalyptus* (11.10%) mycelium growth of *P. ostreatus* respectively reported by Biswas (2015) [1].

Table 1: Effect of different botanicals leaf extract on radial growth of *P. sapidus*.

Treatment	Dose	3rd days		6th days		9th days	
		Radial growth (mm)	Radial growth rate (mm/Day)	Radial growth (mm)	Radial growth rate (mm/Day)	Radial growth (mm)	Radial growth rate (mm/Day)
Neem leaf extract	2%	19.50	6.50	48.00	8.00	81.25	9.02
Neem leaf extract	4%	17.75	5.91	47.50	7.91	81.00	9.00
Lantana leaf extract	2%	22.00	7.33	63.75	10.62	87.25	9.69
Lantana leaf extract	4%	25.25	8.41	70.25	11.70	88.75	9.86
Eucalyptus leaf extract	2%	14.75	4.91	29.00	4.83	46.00	5.11
Eucalyptus leaf extract	4%	11.00	3.66	12.75	2.12	15.75	1.75
Control (PDA)		21.75	7.25	63.00	10.50	82.25	9.13
CD at 5%		4.33	-	3.67	-	4.91	-
SE (m)		1.46	-	1.24	-	1.66	-

Conclusion
Thus it can be concluded that maximal mycelial growth and growth rate/day of *Pleurotus sapidus* can be achieved by lantana leaf extract @ 4% maximal mycelial growth and growth rate obtained. The minimum mycelial growth was founded in *Eucalyptus* leaf extract. *Eucalyptus* is a highly inhibited the growth of mycelium.

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