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Effect of different medicinal plant leaves powder on the mycelial growth and spawn production of oyster mushroom (*Pleurotus* spp.)

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Abstract
The effect of different medicinal plants leaves powders was mycelia growth and spawn production of Oyster mushroom was observed in the present investigation. *Pleurotus* spp. (PL-20-201) and leaves powders of three medicinal plants [*viz.*, Eucalyptus (*Eucalyptus globulus*), Jamun (*Syzygium cumini*) and Neem (*Azadirachta indica*)] in two doses (1.5% and 3.0%) were used for this investigation. Maximum mycelial growth, mycelial growth rate, dry mycelia weight, dry mycelia weight growth rate, spawn growth and spawn growth rate of *Pleurotus* spp. (PL-20-201) were observed in control followed by 1.5% Jamun leaves powder. While, minimum mycelial growth, mycelial growth rate, dry mycelia weight, dry mycelia weight growth rate, spawn growth and spawn growth rate were observed in 3.0% eucalyptus leaves powder.

Keywords: Mycelium, spawn, medicinal plant, eucalyptus, jamun, neem

1. Introduction
Taxonomically Oyster mushroom (*Pleurotus* spp.) belong to the Phyllum Basidiomycota, Class Basidiomycetes, Order Agaricales and Family Pleurotaceae (Tricholomataceae) (Kirk *et al.*, 2008) [8]. Oyster mushroom is an easiest and least expensive commercial mushroom because they are well known for conversion of crop residues to food protein. Oyster mushrooms has immune-modulating properties, limit tumour development and inflammation, have hypoglycaemic and antithrombotic effects, reduce blood cholesterol levels, prevent high blood pressure and atherosclerosis, and have antibacterial and other properties (Gunde, 1999) [5]. Oyster mushrooms are the third largest commercially produced mushroom in the world (Obodai *et al.*, 2003) [12]. However, (Sánchez *et al.*, 2010) [14] reported that *P. ostreatus* is the second largest next to *Agaricus bisporus* in the world market. In India, the oyster mushroom (*Pleurotus* spp.) is known as 'dhingri' and grows in temperate and tropical forests on dead and rotting wooden logs, as well as on dying deciduous and coniferous tree trunks. In India, mushroom cultivation is started just a few decades old, and it has begun to exhibit an upward trend.

2. Materials and Methods
Present investigation has been carried outduring September to October 2021 on Mushroom Laboratory, Department of Plant Pathology, S.V.P. University of Agriculture & Technology, Meerut (U.P.) 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.

2.1 Establishment of pure culture
In this study strain of *Pleurotus* spp., (PL-20-201) were procured from Directorate of Mushroom Research, Solan, Himachal Pradesh, India. This strain was purified for further use with the help of single hyphal tip culture technique. The cultures were incubated for nine days in sterilized Petri plates containing potato dextrose agar (PDA) medium in BOD incubator at 24±1 ^{0}C.

2.2 Preparation of powders
For preparation of leaves powders, leaves of medicinal plants were dried in sunshine to

remove excess moisture. After that they were grinded in electric grinder and finally make fine powder.

2.3 Evaluation of mycelial growth

Three medicinal plants leaves powders were evaluated *in vitro*. Medicinal plants leaves powders incorporated in both, potato dextrose agar medium (PDA) and potato dextrose broth, in 1.5% and 3.0% doses respectively, sterilized by autoclaving at 121°C (15 psi) for 20 minutes. The molten media were poured into sterilized Petri plates (90 mm) considering each as a replication. After solidification of medium the poured Petri plates were inoculated with 9 mm diameter mycelium bit cut from 7-10 days old culture. The medium without any medicinal plant leaves powder served as control. The inoculated Petri-plates were incubated at 24±1°C temperature until the complete growth was observed in any one Petri-plate. The observations were recorded *viz.*, mycelial growth (mm) and mycelial growth rate at three days interval. Similarly, flasks containing 100 ml potato dextrose broth were inoculated with 9 mm diameter mycelium bit cut from 7-10 days old culture of *Pleurotus* spp., (PL-20-201). The inoculated flasks were incubated until the complete growths were observed in any one flask. Finally observations were recorded *viz.*, dry mycelial weight and dry mycelial weight growth rate.

2.4 Evaluation of spawn growth

For to evaluate, the effect of three medicinal plants leaves powders on spawn production of Oyster mushroom wheat grains were soaked overnight and excess water was drained out the next day. After this, grains were dried on a sterilized flat surface to bring down the moisture content up to 60-65%. Before filling the bag of spawn calcium carbonate and calcium sulphate were mixed in the grains @ 0.3% and 1.2% (dry grains weight basis) respectively. The medicinal plants leaves powders were also mixed @ 1.5% and 3.0% doses (dry grains weight basis) respectively in the wheat grains, these grains were then filled in saline glass bottles and autoclaved them for two times at 121ºC and 15 psi for two hours. These sterilization bottles were inoculated with 9 mm diameter mycelium cut from 7-10 days old culture of *Pleurotus* spp., (PL-20-201) and kept inside the BOD incubator after inoculation for complete mycelium run. The observations were recorded at 3 days interval until the any once saline glass bottles got complete mycelial growth.

2.5 Data analysis using statistics

The collected data from present investigation was analysed using Completely Randomized Block (CRD) design in case of evaluation of mycelial growth, dry mycelial growth and spawn growth. Analysis of variance (ANOVA) technique and critical difference (CD) was calculated at five percent level of significance for comparison with other treatment (Gomez and Gomez, 1984; Kumar *et al*., 2019 and Singh *et al.*, 2021)[4] [10] [15].

3. Results and Discussion

Regarding observations on mycelial growth, maximum mycelial growth (31.12 mm) of *Pleurotus* spp., (PL-20-201) was found in control followed by 1.5% Jamun leaves powder (29.50 mm). While, minimum mycelial growth was recorded in 3.0% Eucalyptus leaves powder (10.75 mm) on 3rd day observation. On 6th day observation, maximum mycelial growth (64.87 mm) was found in control followed by 1.5% Jamun leaves powder (63.87 mm). While, minimum mycelial growth (12.75 mm) was observed in 3.0% Eucalyptus leaves powder respectively. In 9th day observation the maximum mycelial growth (89.50 mm) with (9.94 mm/day) growth rate was recorded in control followed by 1.5% Jamun leaves powder (82.37 mm) with (9.15 mm/day) growth rate. While, minimum mycelial growth (25.87 mm) with (2.87 mm/day) was noted in 3.0% Eucalyptus leaves powder (Table 1, Fig. 1).

The seven different treatments including the control as shown the maximum dry mycelial weight were observed as shown Table -1, Fig. 1&2 (7.31 mg/100 ml) was observed in control which was significantly higher than all other treatments followed by 1.5% Jamun leaves powder (5.80 mg/100 ml). While, No dried mycelial weight (0.00 mg/100 ml) was found in 3.0% Eucalyptus leaves powder which was significantly lower than all the other treatments including control. Maximum dry mycelial weight growth rate (0.49 mg/day) was observed in control followed by 1.5% Jamun leaves powder (0.39 mg/day). Results are in accordance with Kumar *et al*., (2019) in which maximum mycelial growth of *Pleurotus sapidus* after nine days in treatment containing lantana leaf extract @ 4% (88.25 mm) followed by lantana leaf extract @ 2% (87.25 mm) and 82.25 mm in control. Less mycelial growth of *Pleurotus* (15.75 and 46.00 mm) was observed with eucalyptus @ 4% and 2% extract respectively. (Mousumi *et al*., 2017) [11] also found that, extracts of neem, lantana and datura are compatible with *P. ostreatus* in compatibility test of different plant extracts at 5% concentration. (Biswas, 2015) [1] noted that extract of *Pongamia pinnata* is less effective against the mycelium of *P. ostreatus* (6.7%) as compared to mould fungi (42.4 to 61.3%). Leaf extracts of *Aloe vera, Ocimum sanctum and Curcuma longa* were less effective on *Pleurotus* mycelium as compare to *Trichoderma harzianum* (Pervez *et al.*, 2012) [13].

Regarding the experiment on spawn production as shown in the Table 2 and Fig. 2, the maximum spawn growth (16.25 mm) was found in control followed by 1.5% Jamun leaves powder (14.25 mm). While, minimum spawn growth were observed in 3.0% Eucalyptus leaves powder (5.12 mm) on 3rd day observations. On 6th day, the maximum spawn growth (25.37 mm) was found in control followed by 1.5% Jamun leaves powder (23.62 mm). While minimum spawn growths were recorded in 3.0% Eucalyptus leaves powder (13.62 mm). On 9th day, maximum spawn growth (43.00 mm) was observed in control followed by 1.5% Jamun leaves powder (40.25 mm). While, minimum spawn growth were recorded in 3.0% Eucalyptus leaves powder (26.25 mm). On 12th day maximum spawn growth (75.87 mm) was found in control followed by 1.5% Jamun leaves powder (71.25 mm). While, minimum spawn growth was recorded in 3.0% Eucalyptus leaves powder (38.00 mm) On 15th day observations the maximum spawn growth (89.12 mm) was observed in control followed by 1.5% Jamun leaves powder (82.75 mm). However, minimum mycelium growth was recorded in 3.0% Eucalyptus leaves powder (48.87 mm).

Regarding spawn growth rate (mm/day) of *Pleurotus* spp., (PL-20-201), maximum spawn growth rate was found in control (5.94 mm/day) followed by 1.5% Jamun leaves powder (5.52 mm/day). While, minimum growth rate was found in 3.0% Eucalyptus leaves powder (3.26 mm/day). (Pervez *et al*. 2012) [13] reported that the average number of

the days required for spawn run in *P. ostreatus* was significantly less (15.23 days) in *Lantana camara*. It was followed by *Allium cepa* (15.7 days) and *Azadirachta indica* (16.03 days). The average number of the days for spawn-run was significantly more (18 days) in control. (Mousumi *et al*., 2017) [11] found that the minimum number of contamination (5.0%) was found in Neem treated spawn packets which were followed by 7.0%, 8.0% and 10.0% in case of Lantana, Aloe-vera and Garlic respectively. While, highest percent contaminated spawn packets (25.0%) were found in control treatment. The maximum spawn growth (83.33 mm) in onion leaf 4% followed by garlic leaf 4% (82.66 mm) and minimum spawn growth (48.33 mm) in control in case of *P. djamor*. While in case of *P. sajor-caju*, maximum spawn growth (87.66 mm) was recorded in garlic leaf 4% and followed by onion leaf 4% (81.66 mm). However minimum spawn growth (69.55 mm) was recorded in control (Kannaujia, 2019) [7].

Table 1: Effect of different medicinal plants leaves powders on mycelia growth and dry mycelial weight of *Pleurotus* spp. (PL-20-201)

Sr.	Treatments	Radial Growth			Growth	PL-20-201	PL-20-201
		3 Days	6 Days	9 Days	Rate/Day	Dry mycelial weight (mg/100m)	Dry matter growth rate (mg/day)
		PL-20-201	PL-20-201	PL-20-201	PL-20-201		
1.	PDA + 1.5% Eucalyptus leaf powder	13.00	21.37	44.62	4.96	2.09	0.14
2.	PDA + 3.0% Eucalyptus leaf powder	10.75	12.75	25.87	2.87	0.00	0.00
3.	PDA + 1.5% Neem leaf powder	18.00	47.00	75.00	8.33	3.62	0.24
4.	PDA + 3.0% Neem leaf powder	14.87	44.12	70.62	7.85	2.98	0.20
5.	PDA + 1.5% Jamun leaf powder	29.50	63.87	82.37	9.15	5.80	0.39
6.	PDA + 3.0% Jamun leaf powder	18.75	49.50	78.00	8.67	5.45	0.36
7.	Control	31.12	64.87	89.50	9.94	7.31	0.49
CD at 5%		1.61	3.01	4.19	-	0.34	-
SE (m)		0.54	1.02	1.42	-	0.11	-

Average of four replications

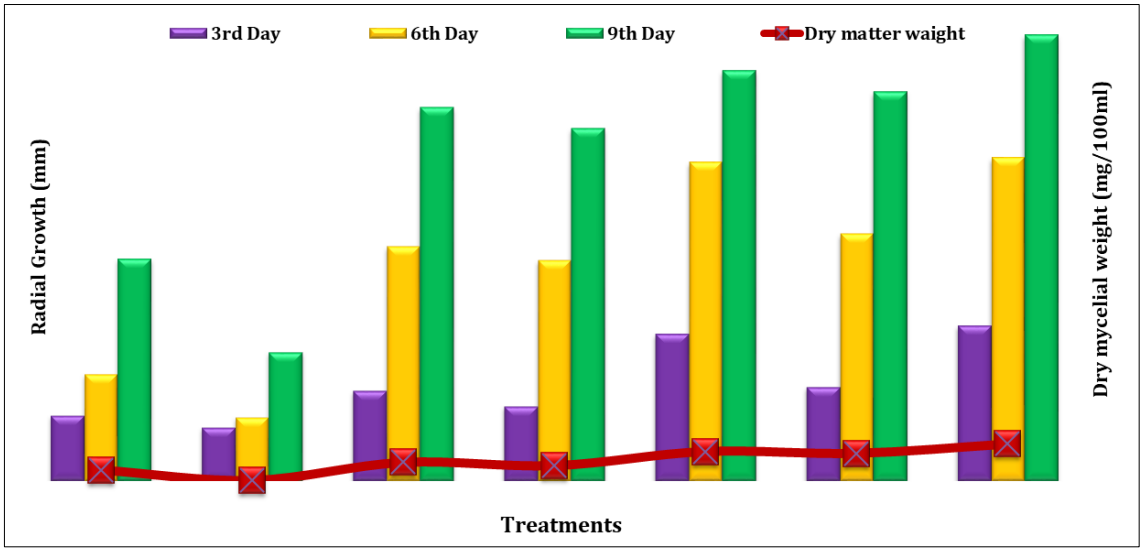

Fig 1: Effect of different medicinal plants leaves powder on mycelial growth and dry mycelial weight of Pleurotus spp., (PL-20-201)

Table 2: Effect of different medicinal plants leaves powders on the spawn growth of *Pleurotus* spp., (PL-20201)

Sr.	Treatments	Spawn Growth					15th day Growth Rate (mm/Day)
		3 Days	6 Days	9 Days	12th Day	15th Day	
		PL-20-201	PL-20-201	PL-20-201	PL-20-201	PL-20-201	PL-20-201
1.	Wheat grains + 1.5% Eucalyptus leaf powder	8.25	15.50	29.37	43.37	52.62	3.51
2.	Wheat grains + 3.0% Eucalyptus leaf powder	5.12	13.62	26.25	38.00	48.87	3.26
3.	Wheat grains + 1.5% Neem leaf powder	12.00	19.87	35.37	56.87	71.37	4.76
4.	Wheat grains + 3.0% Neem leaf powder	10.12	15.87	34.50	50.62	66.87	4.46
5.	Wheat grains + 1.5% Jamun leaf powder	14.25	23.62	40.25	71.25	82.75	5.52
6.	Wheat grains + 3.0% Jamun leaf powder	13.00	22.12	37.87	63.12	77.37	5.16
7.	Control	16.25	25.37	43.00	75.87	89.12	5.94
CD at 5%		1.24	1.20	2.47	1.96	2.49	-
SE (m)		0.42	0.41	0.84	0.66	0.84	-

Average of four replications

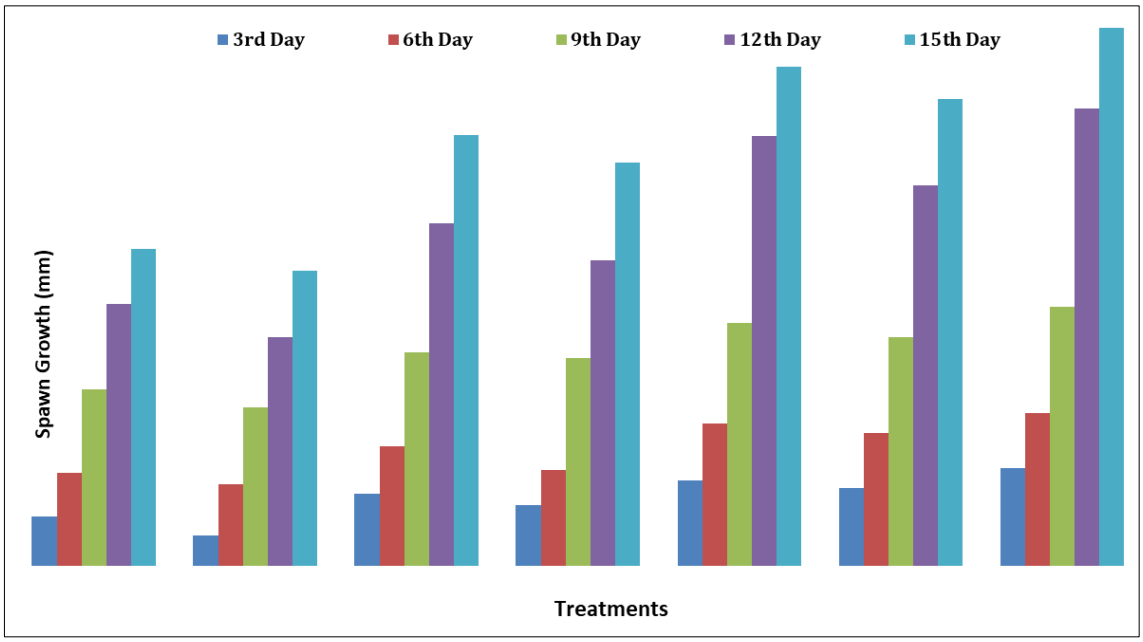

Fig 2: Effect of different medicinal plants leaves powders on the spawn growth of Pleurotus spp., (PL-20-201)

4. Conclusion

In present investigations the maximum mycelial growth, mycelial growth rate, dry mycelia weight, dry mycelia weight growth rate, spawn growth and spawn growth rate of *Pleurotus* spp. (PL-20-201) were observed in control. While, minimum mycelial growth, mycelial growth rate, dry mycelia weight, dry mycelia weight growth rate, spawn growth and spawn growth rate were observed in 3.0% eucalyptus leaves powder. On the basis of present investigations, we concluded that different medicinal plants leaves powders does not influent to the mycelial growth, mycelial growth rate, dry mycelial weight and dry mycelial weight growth rate of Oyster mushroom in the regard of, to enhance the above mentioned all these growth parameters of this study positively.

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