



RESEARCH ARTICLE

Influence of botanical extracts and powders on radial growth, spawn growth and production of sporophores of oyster mushroom (*Pleurotus* spp.)

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Abstract

The aim of evaluating the mycelial development and production of *Pleurotus* spp. to various botanical extracts in the laboratory. The effects of the botanicals evaluated on Petri plates on *Pleurotus sapidus* and *Pleurotus flabellatus* fruiting body formation and spawn growth. All plant extracts were found to be supportive of mushroom mycelial growth. *P. sapidus* showed the highest yield, 861.25 g/kg dry substrate at 86.12 % biological efficiency (BE) with minimum days for spawn run were observed (14.25 days), minimum days for first harvesting (21.25 days) and maximum numbers of fruiting bodies (65.75) were observed in wheat straw + *Lantana camara* foliage powder at 4.0 %. In case of *P. flabellatus*, the highest yield of 878.25 g/kg dry matter at 87.82 % BE with the minimum number of days for spawn run was recorded in 13.50 days. The minimum days for first harvesting were 20.50 days and the highest number of fruiting bodies (65.50) was noticed in the medium of wheat straw + *L. camara* leaf powder at 4.0 %. Eucalyptus was found to be comparatively less effective for *Pleurotus* spp. The *L. camara* foliage extract and powder were the highest growth promoters of mycelium and increasing BE and can be adopted to enhance the production of oyster mushroom cultivation. The botanicals used in the study are easily available for the growers and directly impact to their economy.

Keywords: botanicals; mushroom; mycelial growth; *Pleurotus* spp.; production

Introduction

As major decomposers, oyster mushrooms thrive in their natural habitat on tree stumps or dead, woody tree branches (1). The simplest to grow and requiring the least amount of production technology are oyster mushrooms (2). Oyster mushrooms are widely used primarily for their taste, nutrition, strong anti-inflammatory, immune-modulatory and other medicinal properties. As more individuals become aware of these mushrooms' nutritional potential to help with protein deficiencies and their potential antibacterial properties, their popularity is growing (1). The fruiting bodies of this fungus vary in color from white to cream to grey to yellow to pink to light brown and depending on the species, they have a characteristic shell, fan or spatula shape. An appealing way to enhance the nutritional value of ligno-celluloid wastes for use as animal feedstock was to produce edible mushrooms. In India, the cultivation of mushrooms is one of the most profitable approaches towards agricultural diversification on the basis of microbial

techniques for the purpose of mass recycling of agricultural wastes.

The botanical extracts are mostly employed to prevent competitive molds because they include antifungal and antibacterial chemicals like azadirachtin, limonoid and terpenoids (3, 4). *Azadirachta indica* leaf extracts containing azadirachtin and meliantriol, have antifungal qualities against *Aspergillus parasiticus*, which produces aflatoxin (5). The biochemical processes are still mostly unclear, though. Additionally, *Pleurotus* contains beta-1, 3/1 and 6-glucan, which are immune-enhancing stimulants, along with the chemical mevinolin, which decreases cholesterol. These are also known to prolong life, prevent high blood pressure and aid in tiredness recovery (6). *Pleurotus djamor* (Rumph. ex Fr.) Boedijn is a common oyster mushroom that is valued more economically and is regarded as food worldwide. Because this species has several phytochemical compounds that are familiar to *Pleurotus ostreatus*, *florida*, *pulmonarius* and *P. sajorajju*, researchers are quite interested in it (7, 8). The biological activities of *Allium sativum* and

Allium cepa, which include antibacterial, antioxidant, anti-carcinogenic, anti-asthmatic, immunomodulatory and prebiotic attributes, have long been recognized for their therapeutic benefits. Garlic extraction was also reported to effectively suppress the growth of *Trichoderma harzianum* (9). And biologically active phyto-compounds have been used to cure many health complications, such as cholesterol, thrombus, diabetes, hypertension, etc. (10). These plant extracts offer a good option that is safer to use and eco-friendly in nature. Given the aforementioned, an effort was made to develop a suitable method in an environmentally friendly way under agro-ecological conditions to combat the competing fungi and molds of *P. djamor*.

Materials and Methods

Establishment of pure culture

The cultures of *P. sapidus* and *P. flabellatus* used in this study, conducted in 2019, were obtained from the Mushroom Research and Training Center at Govind Ballabh Pant University of Agriculture and Technology in Pantnagar, as well as from the Directorate of Mushroom Research Center in Solan, Himachal Pradesh. The single hyphal tip approach was used to further purify the cultures of *Pleurotus* species. The cultures were cultivated for 8 days on potato dextrose agar (PDA) medium on sterile petri dishes for this purpose. Under a low magnification (10x) compound microscope, single-branched hyphae from the colony's periphery were identified and moved to PDA slants for maintenance. After around a week of incubation at 24±1 °C, these culture tubes were once more sub-cultured on PDA media and kept in a refrigerator at 5±1 °C for further use.

Aqueous extraction of plants

Three commonly available plants i.e. eucalyptus (*Eucalyptus globulus*), lantana (*Lantana camara*) and neem (*Azadirachta indica*) were used for evaluation of their aqueous extract and leaf powder on 2 species of oyster mushroom. A blender was used to combine the 100 g of sterilized leaves with 50 mL of sterilized distilled water (11, 12). Four layers of muslin cloth were used to filter the leaf extracts. The leaf extracts were passed through 4 layers of muslin cloth. The plant extract was extracted by maceration of leaves in water (2:1, w/v). PDA was prepared and 100 mL of the medium was taken in each 250 mL flasks and sterilized. To the molten cooled sterilized medium requisite quantity of the plant extracts is added separately and thoroughly mixed so as to get the required concentrations.

Each 250 mL conical flask held 100 mL of the agar-agar-free medium, which was autoclave sterilized for 20 min at 15 lbs pressure and 121 °C temperature. In each broth medium, 2 % and 4 % of the botanicals mentioned above were introduced to the medium. Then, it was inoculated into the culture with 5 mm disc of *Pleurotus* species. For 15 days, the flasks were incubated at 22±1 °C. After that, the culture was filtered through Whatman filter paper No. 1 and its mycelium was dried in an oven set to 60 °C for a period of 48 hr before determining the dry weight of mycelia.

Evaluation of botanical powders on oyster mushrooms

This study was performed to find out the mycelial growth (mm) of spawn against botanical powder. Three botanicals (neem, lantana and eucalyptus leaves powder) commonly available were used for experiments at 2 % and 4 % were added as an additive with wheat grain. The spawn was made in accordance with 3.8. In 3 duplicates,

the grains were stuffed into the bottle to a depth of 90 mm. A 9 mm disc was used to inoculate each of the 2 *Pleurotus* species (*P. sapidus* and *P. flabellatus*) in a separate bottle. The mycelial growth was observed on 3rd, 6th, 9th and 12th days after the spawn bottles were incubated in a biological oxygen demand (BOD) at 24±1 °C without shaking.

Evaluation of botanical extracts on *Pleurotus* spp.

For this experiment, 3 botanicals in powdered form, such as neem, lantana and eucalyptus leaves at 2.0 % and 4.0 % doses were used with a control without any additives. These additives were sun-dried, powdered and sterilized in an autoclave at 121 °C (15 lbs pressure) for 20 min. Before spawning, these compounds were separately combined with wheat straw at 2.0 % and 4.0 % on a dry weight basis. Seven treatments with 4 replications were used to produce sporophores in the crop room using the inoculated bags. *Pleurotus* species, namely *P. sapidus* and *P. flabellatus*, were observed for their yield (g/Kg dry substrate), average weight of fruiting bodies (g/FB), number of fruiting bodies, stipe length, pilus width, days for spawn run, days for initial harvesting and cropping time. BE of the substrate was determined using the following formula (13).

$$BE\% = (W_f/W_d) \times 100$$

Where, BE = Biological efficiency, W_f = Fresh weight of fruit body, W_d = Dry weight of substrate

Statistical analysis

The experimental data thus obtained were analyzed through statistical analysis using the appropriate statistical design (CRD). The significant difference among various treatment groups was determined by Analysis of Variance (ANOVA) and the critical difference (CD) was computed at 5 % level of significance (14).

Results

Effect of botanical leaf extracts on mycelial growth and dry matter weight

Significant differences were recorded in the effects of several botanical leaf extracts on *P. sapidus* and *P. flabellatus* mycelial growth and dry matter accumulation. The highest radial growth by the 9th day was promoted by lantana leaf extract (LE) at a concentration of 4.0 % for both *P. sapidus* (89.00 mm) and *P. flabellatus* (89.50 mm), with daily growth rates of 9.88 mm and 9.94 mm respectively, among the treatments (Table 1, Fig. 1). Further, this treatment produced the highest rates of dry matter accumulation and development, with *P. sapidus* reaching 7.15 mg/100 mL (0.47 mg/day) and *P. flabellatus* reaching 8.66 mg/100 mL (0.57 mg/day).

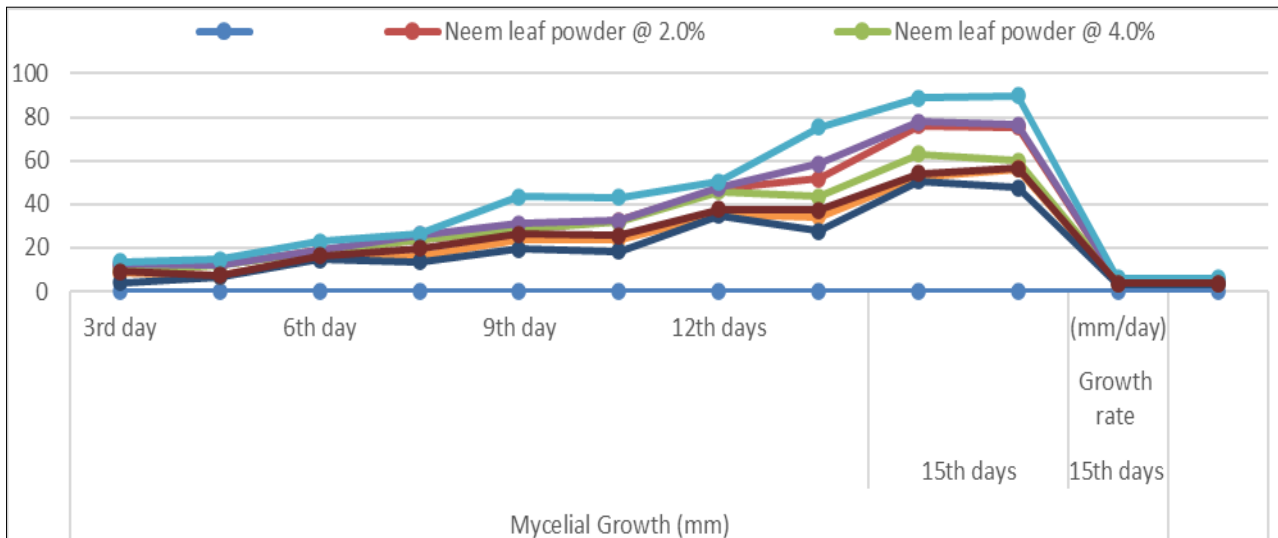
On the other hand, eucalyptus LE at 4.0 % exhibited the strongest inhibitory impact, completely suppressing the production of dry matter in both species and exhibiting the lowest radial growth (25.00 mm and 26.00 mm). While lantana extracts outperformed the control (PDA medium), neem extracts at both doses promoted modest growth and biomass buildup. These findings suggest that lantana LE, especially at 4.0 %, considerably increases the tested oyster mushroom species' biomass and radial growth.

Effect of botanical leaf powders on spawn growth

The results presented in Table 2 and Fig. 2 indicate that the type and concentration of botanical leaf powder (LP) significantly influenced

Table 1. Effect of botanical leaf extracts on mycelial growth and dry matter weight of *P. sapidus* and *P. flabellatus* species of oyster mushroom

S. No.	Media	Radial growth (mm)						9 th day growth rate (mm/day)		<i>P. sapidus</i>		<i>P. flabellatus</i>	
		3 rd day		6 th day		9 th day		<i>P. sapidus</i>	<i>P. flabellatus</i>	Dry matter growth (mg/100 mL)	Dry matter growth rate (mg/day)	Dry matter growth (mg/100 mL)	Dry matter growth rate (mg/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>						
1.	Neem LE at 2.0 %	21.50	18.75	52.50	49.50	77.25	77.50	8.58	8.61	3.72	0.24	3.83	0.25
2.	Neem LE at 4.0 %	16.00	15.25	41.00	46.75	70.25	70.75	7.80	7.86	3.06	0.20	3.27	0.21
3.	Lantana LE at 2.0 %	22.00	30.00	62.00	64.00	86.25	82.75	9.16	9.19	5.79	0.38	6.80	0.45
4.	Lantana LE at 4.0 %	25.25	32.25	66.50	65.50	89.00	89.50	9.88	9.94	7.15	0.47	8.66	0.57
5.	Eucalyptus LE at 2.0 %	11.50	11.25	17.75	21.50	45.00	45.25	5.00	5.02	2.06	0.13	2.12	0.14
6.	Eucalyptus LE at 4.0 %	9.75	9.25	13.75	12.25	25.00	26.00	2.77	2.88	0.0	0.0	0.0	0.0
7.	PDA Media (Control)	17.75	18.50	46.50	47.50	82.50	75.50	9.58	8.38	5.46	0.36	5.57	0.37
	CD at 5 %		1.89	2.86	3.14	2.98	3.64	3.52	-	0.35	-	0.27	-
	SE(m)		0.63	0.96	1.06	1.00	1.23	1.19	-	0.11	-	0.09	-

**Fig. 1.** Effect of different botanical leaf extracts on mycelial growth and dry matter weight of *P. sapidus* and *P. flabellatus* mushroom species.**Table 2.** Effect of botanical leaf powders on spawn growth (mm) of *P. sapidus* and *P. flabellatus* species of oyster mushroom

S. No.	Treatments	Mycelial growth (mm)								15 th day		15 th day growth rate (mm/day)	
		3 rd day		6 th day		9 th day		12 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>	<i>P. sapidus</i>	<i>P. flabellatus</i>	<i>P. sapidus</i>	<i>P. flabellatus</i>				
1.	Neem LP at 2.0 %	12.00	12.00	18.75	23.50	28.75	32.50	47.25	51.75	76.00	75.50	5.06	5.03
2.	Neem LP at 4.0 %	9.50	11.75	18.25	23.50	28.25	31.75	45.75	43.50	63.00	60.00	4.20	4.00
3.	Lantana LP at 2.0 %	12.50	12.25	18.75	26.00	31.25	32.50	47.50	58.75	77.75	76.50	5.18	5.10
4.	Lantana LP at 4.0 %	13.50	14.50	23.00	26.25	43.50	43.25	50.25	75.25	88.75	89.75	5.91	5.98
5.	Eucalyptus LP at 2.0 %	8.50	7.00	15.75	16.25	24.00	23.75	35.50	34.25	52.50	56.25	3.50	3.75
6.	Eucalyptus LP at 4.0 %	4.00	6.75	14.50	13.50	19.25	18.25	34.75	27.50	50.75	47.75	3.38	3.18
7.	Control	9.25	7.25	16.25	19.75	26.25	25.50	37.75	37.25	54.00	56.50	3.60	3.76
	CD at 5 %	3.66	3.13	3.40	4.44	3.27	3.38	4.54	4.20	4.69	3.28	-	-
	SE(m)	1.23	1.05	1.14	1.50	1.10	1.14	1.53	1.42	1.58	1.10	-	-

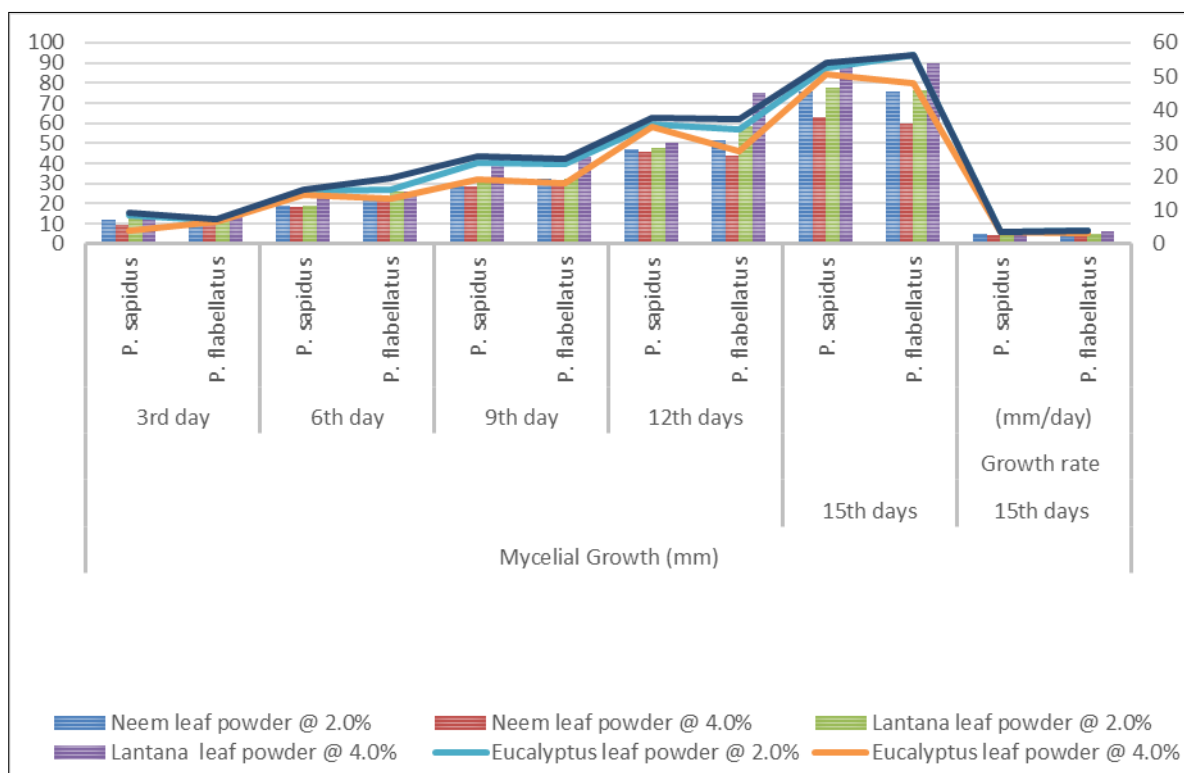


Fig. 2. Effect of different botanical leaf powders on spawn growth (mm) of *P. sapidus* and *P. flabellatus* mushroom species.

the mycelial growth of *P. sapidus* and *P. flabellatus*. Among the treatments, lantana LP at 4.0 % showed the highest mycelial growth for both *P. sapidus* (88.75 mm) and *P. flabellatus* (89.75 mm) by the 15th day, with the fastest daily growth rates of 5.91 mm/day and 5.98 mm/day respectively. This was followed by lantana LP at 2.0 % and neem LP at 2.0 %, both of which supported substantial growth compared to the control. However, eucalyptus LP, particularly at 4.0 %, had the slowest growth rates (3.38 mm/day and 3.18 mm/day) and the lowest mycelial growth for both species, with *P. sapidus* and *P. flabellatus* only reaching 50.75 mm and 47.75 mm respectively. The moderate growth of control treatment indicates that some botanicals, especially lantana at greater concentrations, may have an inhibitory influence on spawn development, whereas eucalyptus may have a stimulatory effect.

Effect of leaf powders on spawn run, cropping period and yield of *P. sapidus* and *P. flabellatus*

Growth and yield metrics of *P. sapidus* were considerably impacted by the addition of botanical leaf powders (Table 3, Fig. 3 & 4). The best overall performance was obtained by combining wheat straw with a 4.0 % concentration of lantana LP. This combination

produced the largest yield (861.25 g/kg dry substrate), the most fruiting bodies (65.75) and the highest BE (86.12 %) among all treatments. It also had the quickest times for both the initial harvest (21.25 days) and the entire spawn run (14.25 days), suggesting a faster growth cycle. On the other hand, the control treatment took the longest to spawn and harvest (21.00 and 28.00 days) and it performed the worst in terms of yield (555.00 g/kg), BE (55.50 %) and number of fruiting bodies (38.50). Among all treatments, eucalyptus LP, especially at 4.0 %, had the lowest yield (511.75 g/kg) and BE (51.17 %) due to its growth-inhibiting actions. These findings imply that lantana LP is a promising supplement for improving *P. sapidus* cultivation, particularly when used at a 4.0 % dosage.

The development and yield of *P. flabellatus* are significantly impacted by the various botanical LP, according to the data shown in Table 4. Although it had a relatively short spawn run (13.5 days) and an early first harvest (20.5 days), the wheat straw supplemented with lantana LP at 4.0 % performed the best among the treatments, achieving the maximum yield (878.25 g/kg dry substrate) and BE (87.82 %). Lantana LP at 2.0 %, which again demonstrated excellent output (821.25 g/kg) and BE (82.12 %), came in close second.

Table 3. Effect of botanical leaf powders on spawn run, cropping period and yield of *P. sapidus* species of oyster mushroom

S. No.	Treatments	DFSR	DFFH	DFCP	NOFB	Stipe length (cm)	Width (cm)	Yield (g/kg dry substrate)	Average weight (gm/FB)	Biological efficiency (%)
1.	Wheat straw + Neem LP at 2.0 %	15.00	22.00	78.50	54.00	10.17	8.50	731.25	13.54	73.12
2.	Wheat straw + Neem LP at 4.0 %	16.00	23.00	75.25	49.00	10.57	8.10	673.75	13.75	67.37
3.	Wheat straw + Lantana LP at 2.0 %	14.75	21.75	80.50	63.50	12.70	10.02	812.50	12.79	81.25
4.	Wheat straw + Lantana LP at 4.0 %	14.25	21.25	73.50	65.75	12.95	10.02	861.25	13.09	86.12
5.	Wheat straw + Eucalyptus LP at 2.0 %	17.75	24.75	83.75	46.75	10.77	8.77	581.25	12.43	58.12
6.	Wheat straw + Eucalyptus LP at 4.0 %	19.25	26.25	82.25	45.50	11.50	9.55	511.75	11.24	51.17
7.	Control	21.00	28.00	60.25	38.50	12.25	9.27	555.00	14.41	55.50
	CD at 5 %	1.33	1.33	5.34	5.15	1.48	1.22	9.78	-	-
	SE(m)	0.45	0.45	1.80	1.74	0.50	0.41	3.30	-	-

DFSR = Days for spawn run, DFFH = Days for first harvesting, DFCP = Days for cropping period, NOFB = Number of fruiting bodies

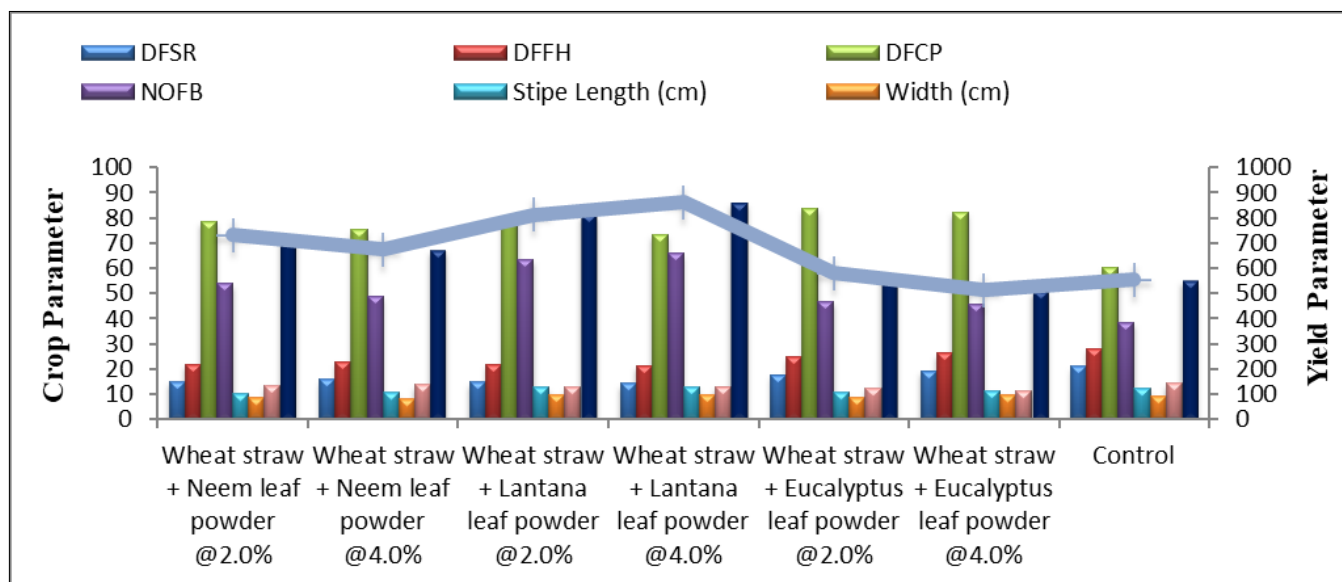


Fig. 3. Effect of different botanical powders on spawn run, cropping period and yield of *P. spidus* oyster mushroom.

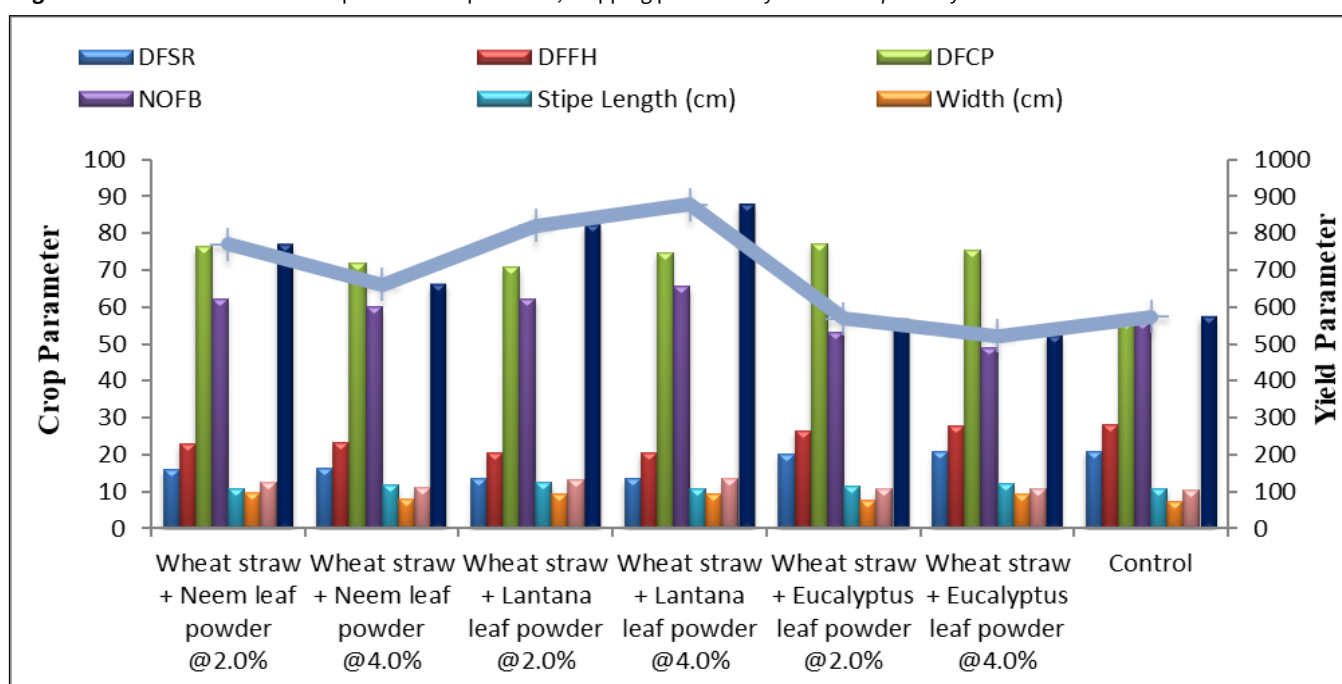


Fig. 4. Effect of different botanical powders on spawn run, cropping period and yield of *P. flabellatus* oyster mushroom.

Table 4. Effect of different botanical powders on spawn run, cropping period and yield of *P. flabellatus* species of oyster mushroom

S. No.	Treatments	DFSR	DFFH	DFCP	NOFB	Stipe length (cm)	Width (cm)	Yield (g/kg dry substrate)	Average weight (gm/FB)	Biological efficiency (%)
1.	Wheat straw + Neem LP at 2.0 %	16.00	23.00	76.25	62.25	10.85	9.62	769.25	12.35	76.92
2.	Wheat straw + Neem LP at 4.0 %	16.25	23.25	71.75	60.00	11.80	7.95	661.25	11.02	66.12
3.	Wheat straw + Lantana LP at 2.0 %	13.50	20.50	70.75	62.25	12.45	9.40	821.25	13.19	82.12
4.	Wheat straw + Lantana LP at 4.0 %	13.50	20.50	74.75	65.50	10.85	9.45	878.25	13.40	87.82
5.	Wheat straw + Eucalyptus LP at 2.0 %	20.00	26.50	77.25	53.25	11.52	7.72	569.25	10.69	56.92
6.	Wheat straw + Eucalyptus LP at 4.0 %	20.75	27.75	75.50	49.00	12.20	9.22	521.25	10.63	52.12
7.	Control	21.00	28.00	56.00	56.00	10.82	7.45	573.75	10.24	57.37
	CD at 5 %	1.27	1.39	4.71	5.47	0.74	0.68	10.54	-	-
	SE(m)	0.43	0.46	1.59	1.84	0.25	0.23	3.56	-	-

DFSR = Days for spawn run, DFFH = Days for first harvesting, DFCP = Days for cropping period, NOFB = Number of fruiting bodies

Eucalyptus LP at 4.0 % demonstrated the lowest yield (521.25 g/kg) and BE (52.12 %), while the control and eucalyptus treatments, especially at 4.0 %, reported lower yields and efficiencies. Moderate performance was demonstrated by neem treatments, with better outcomes at the lower concentration (2.0 %). Overall, under the investigated conditions, lantana LP was most successful in increasing *P. flabellatus* productivity at 4.0 %.

Discussion

Similarly mycelial growth was noted in lantana LE (51.25 %) and neem LE (47.75 %) at concentrations of 5 % and 10 % (15). The botanical that was shown to be less successful than the others at preventing the growth of *P. ostreatus* mycelium was neem (4.4 %). Another study reported that the mycelium growth of *P. ostreatus* was 6.7 % in extracts of *Pongamia pinnata* (karanja), 8.9 % in extracts of *Clerodendron indicum* (clerodendron) and 11.1 % in extracts of eucalyptus (16). Also, similar observations were recorded in *P. florida*, maximum radial growth (90.00 mm) in *Trigonella foenum-graecum* extract media, followed by *Piper nigrum* extract media (89.30 mm) and minimum growth in *Cuminum cyminum* extract media (78.00 mm). Regarding the growth rate (mm/day) was observed in *P. florida*, the maximum mycelial growth rate (10.00 mm/day) was observed in *Trigonella foenum-graecum* extract medium. Dry matter growth was observed in *P. florida* on *Piper nigrum* broth media, followed by *Elettaria cardamomum* medium in (5.13 mg/50 mL) (17). Also reported that in *P. sapidus*, maximum growth (88.75 mm) and growth rate 9.86 mm/day in lantana LE at 4 % followed by lantana LE at 2 % (87.25 mm) growth rate of 9.69 mm/day. Minimum growth (15.75 mm) and minimum growth rate 1.75 mm/day were observed after 9 days in eucalyptus LE at 4.0 % (18). The study was done on the mycelial growth of *P. djamor* against 3 different botanicals (*onion*, *garlic* and *coriander*) with 2 different concentrations (2.5 % and 3.0 %) in the laboratory. Plant extract more or less increased as well as decreased the radial growth of mycelium of *P. djamor*. Maximum radial growth was observed in coriander leaf extract at 3 % (90.00 mm), followed by coriander leaf extract at 2.5 % (88.00 mm) and 82.00 in the control (without any plant extract). Minimum radial growth was observed in garlic leaf extract at 3 % (32.00 mm), followed by garlic leaf extract at 2.5 % (52.25 mm) (19).

Neem, lantana and datura extracts were shown to be compatible with *P. ostreatus* in a compatibility test of various plant extracts at 5 % concentration, whereas onion and turmeric were much worse and completely incompatible (20). The effectiveness of all botanical powders on *Pleurotus* species spawn development. *P. djamor* showed a minimum spawn growth of 48.33 mm at the turmeric leaf, whereas the control showed a maximum spawn growth of 90.00 mm. *P. sajor-caju* showed the lowest spawn growth (77.33 mm) in onion leaf 6 % and the highest spawn growth (89.66 mm) in control. The outcomes were nearly identical (21).

Earlier studies reported that medicinal plant powder in different concentrations viz. *Citrus lemon*, *Eucalyptus camaldulensis*, *Cymbopogon marginatus* and *A. indica* add leaf powder to substrates made of cotton. *P. florida* and *P. ostreatus* were spawned in each compost bag containing a particular medicinal plant product at a particular concentration. When eucalyptus plant material was added to 2 % w/w compost, the output of mushrooms increased (22). This rise persisted up to 4 %. When 5 % eucalyptus plant product was added, the yield of mushrooms (1282.50 g) began to

decline. Thus, the antibacterial activity of the eucalyptus plant product was responsible for the greatest output of 1286.0 g at 4 % (23). Another scientist reported 8 botanicals, including neem and lantana, also showed the highest mean increase in yield (36.89 %) in polybags that received lantana in an *in-vivo* test (15). Additionally, it was reported that *P. ostreatus* was cultivated using an extract from 8 plants. The treated substrate showed increase in BE of 35.20 %. Similar patterns were also observed with the botanicals *in vivo*, where the substrate treated with *A. indica* produced the highest output of mushrooms (95.10 % BE). *P. pinnata* 92.20 % BE and *Clerodendron indicum* treated substrate 89.00 % came next, in that order (16).

Conclusion

Thus, the study revealed that the performance of botanicals on mycelial growth and production of *P. sapidus* and *P. flabellatus* can be achieved best in lantana LE and powder at 4 % maximal mycelial growth, spawn run and highest BE obtained. Eucalyptus leaves had the least amount of mycelial development, significantly inhibiting mycelium growth. These botanicals can be further explored for the commercial production of oyster mushrooms after proper validation under field trials.

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Authors' contributions

SK wrote, collected data and designed the research paper. GS wrote under the supervision, analysed, reviewed and edited the research article. NS formatted and checked the plagiarism. RT and C done sequence alignment and PC helped in the statistical analysis. GDB contributed to sequence alignment, while KS assisted with statistical analysis. AN reviewed the article, and VKP helped by checking for spelling errors. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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