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Akash Singh
Research Scholar, Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India

SN Rahul
Assistant Professor, Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India

SK Singh
Associate Professor, Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India

Subhash Chandra
Assistant Professor, Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India

Shivam Singh
Assistant Professor, Department of Plant Protection, Career Point University, Alaniya, Kota, Rajasthan, India

Corresponding Author:
Akash Singh
Research Scholar, Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India

Effect of various caffeine and yeast extract concentrations on the mycelial growth of *Pleurotus ostreatus*

Akash Singh, SN Rahul, SK Singh, Subhash Chandra and Shivam Singh

Abstract
In tropical nations, *Pleurotus ostreatus* is regarded as a suitable alternative source of protein-rich diet. The ability of Pleurotus to concurrently produce fruiting bodies and reduce or degrade harmful chemicals found in the substrate is equally impressive. This research's goal was to examine how cultivating *Pleurotus ostreatus* was affected by various caffeine and yeast concentrations. *P. ostreatus* strain AVT-PP-22-102, also known as *P. ostreatus*, was created on a PDA using various concentrations of caffeine and yeast. Based on optimal mycelial growth, caffeine and yeast extract play a vital role in the development of *Pleurotus ostreatus*. The maximum radial mycelium growth (mm) was recorded maximum 8.15 mm, 13.15 mm, 19.25mm and 25.42 mm) in T_8 at 24, 48, 72 and 96 hours followed by T_7 (7.72 mm, 12.47 mm, 18.42 mm and 22.5 mm), T_1 (6.45 mm, 9.7 mm, 15.7 mm and 18.5 mm), T_2 (5.45 mm, 8.45 mm, 14.35 mm and 18.15 mm), T_6 (4.55 mm, 9.55 mm and 14.85 mm and 17.12 mm), T_5 (3.47 mm, 8.47 mm, 10.47 mm and 12.35 mm) and T_3 (2.25 mm, 5.25 mm, 10.25 mm and 10.75 mm). T_4 have the lowest radial growth recorded among all the treatment which gave 0.95 mm, 3.7 mm, 5.5 mm and 6.5 mm at 24, 48, 72 and 96 hours respectively.

Keywords: Cultivation, *Pleurotus ostreatus*, Caffeine, Yeast, Mushrooms, Radial growth, Strain and Mycelium

Introduction
Mushrooms have recently become an essential component of the typical human diet. The popularity of several mushroom species has dramatically increased. More than 2000 different species of mushrooms are edible. A handful of them are severely dangerous by nature and should not be consumed. Some of them are grown commercially. There are 40 different species of mushrooms in the genus *Pleurotus* and they are all commonly referred to as "Oyster mushrooms". One of the most accessible edible mushrooms is the oyster mushroom. Flank, a German, was the first to produce the oyster mushroom in 1917. In tropical nations, *Pleurotus ostretus* is regarded as a beneficial substitute for sources of protein-rich food (Chang and Miles, 2004) [5]. *Pleurotus* also shows good ability in producing a fruiting body and simultaneously reducing or degrading toxic substances present in the substrate (Mata, 1994; Chang and Miles, 2004) [8, 5]. Bermudez *et al.*, (2001) [4], Baars *et al.* (1994) [2] studied the production of *Pleurotus* with caffeine, obtaining good results. Barbosa (1996) [3] and Maziero (1990) [9] used coffee husk to produce *Pleurotus*, but had no success. It was reported that mycelia were initially strong and vigorous, but after some days the growth was interrupted. Evidently, the coffee husk had a higher quantity of toxic substances than the coffee pulp did.

Material and Methods
Pleurotus ostreatus strain AVT-PP-22-102 (hereafter referred to as *P. ostreatus*). The strain was maintained on potato dextrose agar (PDA) and incubated at 25 °C in constant dark.

Radial growth
All of the strains were grown in a medium that contained coffee husk extract. This extract was made by heating coffee husks (40gL^{-1}) in distilled water for one hour, straining them, and then bringing the volume to one litter. Its pH was raised to 7, and the medium was then blended with 2% agar before being autoclaved at 121°C for 15 minutes. Petri dishes (9 cm in diameter) were each given 15 mL of the medium. According to Soccol's (1998) description, the fungal cultures were used to inoculate the plate.

Mycelia's growth was quantified in accordance with Soccol's (1998) methodology.

Effect of caffeine and yeast

The experiments were conducted in PDA plates with addition of different concentrations of caffeine and yeast using the selected strain. The concentrations of caffeine and yeast tested were: 3%, 5%, 8%, 10% respectively.

Cultivation of *Pleurotus ostreatus*

For experiments using solid media (PDA), plates were inoculated with 0.5 x 0.5 cm square plugs of agar taken from the periphery of the colony containing 6-day-old mycelium from a homologous culture of *P. ostreatus* (Fig. 3.1). Inoculated plates were incubated at 25 °C for 7 43 days. Transfer from the mother culture (stored at 5 °C) was done immediately before experimentation.

Table 1: Evaluation of different concentration of caffeine and yeast extract on the growth of *Pleurotus ostreatus*

Treatments	Composition of the substrate (Caffeine and Yeast extract)
T_1	Caffeine (3%)
T_2	Caffeine (5%)
T_3	Caffeine (8%)
T_4	Caffeine (10%)
T_5	Yeast extract (3%)
T_6	Yeast extract (5%)
T_7	Yeast extract (8%)
T_8	Yeast extract (10%)

Statistical analysis

The Complete Randomized Design (CRD) was used, and the results were statistically analysed. For comparison with different treatments, the crucial difference (CD) was computed using the analysis of variance (ANOVA) method and a 1% level of significance.

Results and Discussion

It is evident from the table that is clearly indicated that different concentrations of caffeine and yeast extract play significant roles in the growth of *Pleurotus ostreatus*. The maximum radial mycelium growth (mm) was recorded (8.15 mm, 13.15 mm, 19.25 mm and 25.42 mm) in T_8 at 24, 48, 72 and 96 hours followed by T_7 (7.72 mm, 12.47 mm, 18.42 mm and 22.5 mm), T_1 (6.45 mm, 9.7 mm, 15.7 mm and 18.5 mm), T_2 (5.45 mm, 8.45 mm, 14.35 mm and 18.15 mm), T_6 (4.55 mm, 9.55 mm and 14.85 mm and 17.12 mm), T_5 (3.47 mm, 8.47 mm, 10.47 mm and 12.35 mm) and T_3 (2.25 mm, 5.25 mm, 10.25 mm and 10.75 mm). T_4 have the lowest radial growth recorded among all the treatment which gave 0.95 mm, 3.7 mm, 5.5 mm and 6.5 mm at 24, 48, 72 and 96 hours respectively. Caffeine degradation was seen while *P. ostreatus* on SCG was being grown, and the colonized substrate and fruiting bodies both accumulated metabolic byproducts of caffeine degradation. Given the presence of six intermediary metabolites (Paraxanthine, theophylline, theobromine, 7-methylxanthine, 1-methylxanthine, and 3-methylxanthine) in the colonized substrate, caffeine degradation is most likely to occur during the vegetative phase. Additionally, the colonized substrate included xanthine, the final byproduct of caffeine breakdown, which accumulated in the fruiting bodies.

It is clear that breakdown stages may take place at various speeds based on the buildup of chemicals linked to caffeine metabolism in both the substrate and fruiting bodies. It was not feasible to identify how much of the measured compounds were released into the substrate and how much was contained in the mycelium itself since the substrate is deeply colonized by the mushroom hyphae. Other filamentous fungus from the genera *Penicillium*, *Aspergillus*, *Rhizopus*, and *Fusarium* cultivated in a variety of substrates have reported varying rates for caffeine breakdown (Kurt *et al.* 2010, Galzada, *et al.*, 1987, Martinez, *et al.*,1990) [7, 6, 1]. Despite having different rates of caffeine breakdown, *Rhizopus arrhizus* (87%) and *Aspergillus* sp. (89%) as well as the basidiomycete *Phanerochaete chrysosporium* (71%) all saw significant reductions in caffeine when grown on coffee husks as substrate (Brand *et al.* 2000) [11]. Even though most of these fungi are not closely related to *P. ostreatus*, research like this are an important place to start when figuring out how basidiomycetes and other fungi break down caffeine. It has been discovered that a separate strain of *P. ostreatus* and a different species of Pleurotus can both absorb caffeine without degrading it; however, again, no comparison research is available for the current investigation.

Table 2: Effect of different concentrations of caffeine and yeast extract on the mycelium radial growth of *Pleurotus ostreatus*

Treatment	Radial growth (mm)			
	24 Hours	48 Hours	72 Hours	96 Hours
T_1 Caffeine (3%)	6.45	9.7	15.7	18.5
T_2 Caffeine (5%)	5.45	8.45	14.35	18.15
T_3 Caffeine (8%)	2.25	5.25	10.25	10.75
T_4 Caffeine (10%)	0.95	3.7	5.5	6.5
T_5 Yeast extract (3%)	3.475	8.475	10.475	12.35
T_6 Yeast extract (5%)	4.55	9.55	14.85	17.125
T_7 Yeast extract (8%)	7.725	12.475	18.425	22.5
T_8 Yeast extract (10%)	8.15	13.15	19.25	25.425
T_9 (Caffeine and Yeast extract Free)	1.07	4.50	6.78	8.30
C.D. at 1%	1.009	1.065	1.244	1.739
SE(m)	0.341	0.36	0.42	0.587
SE(d)	0.482	0.509	0.594	0.831
C.V.	13.982	8.133	6.178	7.158

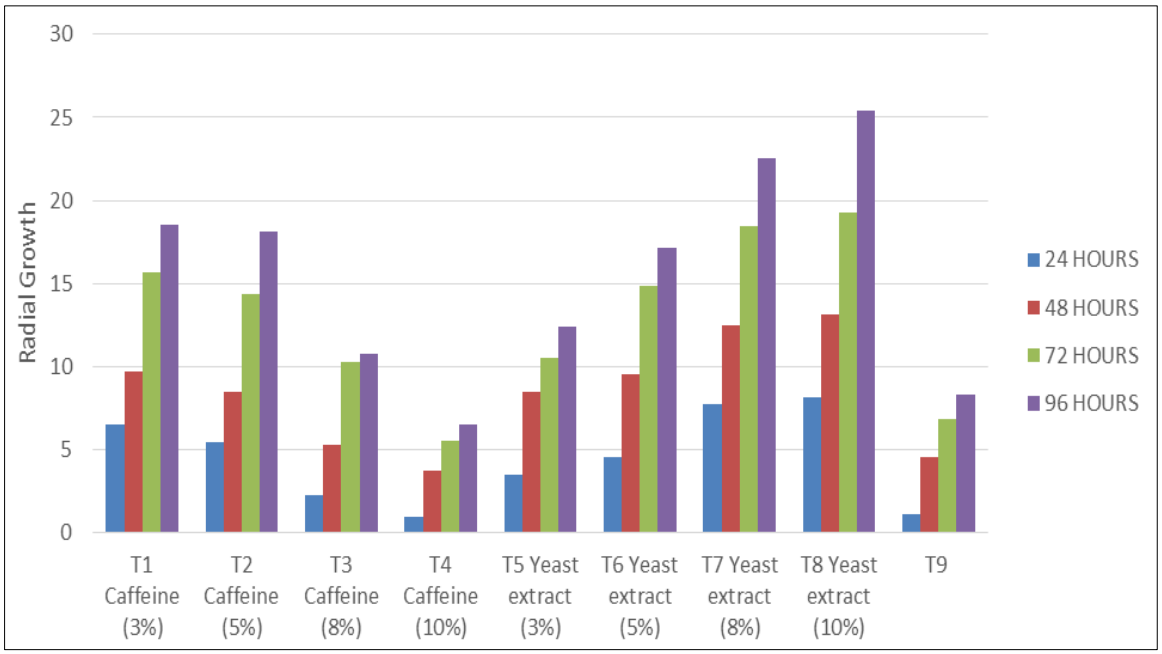

Fig 1: Effect of different concentrations of caffeine and yeast extract on the mycelium radial growth of *Pleurotus ostreatus*

Conclusion

In conclusion, this work has shed light on *Pleurotus ostreatus* cultivation and highlighted the importance of caffeine and yeast concentrations in affecting its mycelial growth. The strain AVT-PP-22-102 responded to various treatment scenarios with diverse degrees of radial mycelium development. The highest development shown in T8 Yeast extract (10%), followed by T_7 Yeast extract (8%), and T_1 Caffeine (3%), showed the best mycelial growth was attained under specified doses of caffeine and yeast extract. In contrast, T4 showed the least amount of radial expansion among all the analysed time frames. These results highlight the complex interaction between substrate elements and *Pleurotus ostreatus* growth dynamics.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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