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Mycelial growth performance of *Lepista* species on various culture media

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ABSTRACT

The genus *Lepista* encompasses edible and medicinal mushrooms of significant economic and pharmacological value, necessitating efficient cultivation protocols. A critical first step in their domestication is the optimization of *In vitro* mycelial growth, which is highly influenced by the nutrient composition of the culture medium. This study evaluated the effect of four different media Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Corn Meal Agar (CMA), and Carrot Dextrose Agar (CDA) on the mycelial growth of *Lepista* spp. under controlled conditions. The results demonstrated a highly significant effect of culture medium on radial mycelial growth. PDA proved superior, yielding the highest mean colony diameter (77.20 mm), accompanied by dense, uniform mycelial expansion. CMA supported robust growth (65.40 mm), while CDA facilitated moderate development (53.80 mm). In contrast, MEA was markedly inferior, resulting in severely restricted growth (20.60 mm), indicating its inadequacy for the metabolic requirements of *Lepista* spp.

KEYWORDS

Lepista, Culture Media, Mycelial Growth, Potato Dextrose Agar, Spawn, Mushroom Cultivation

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The genus *Lepista*, belonging to the family Tricholomataceae, comprises a group of saprotrophic basidiomycete fungi renowned for their culinary and medicinal value. Commonly known as "blewits," species within this genus, such as *Lepista nuda* (wood blewit) and *Lepista sordida*, are prized edible mushrooms sought after for their distinctive flavor and aroma (Pinto *et al.*, 2020). Beyond their gastronomic appeal, *Lepista* species have been a focus of pharmacological research due to their rich bioactive compound profile, demonstrating antioxidant, antimicrobial, anticancer, and immunomodulatory properties (Alves *et al*., 2013 and Tel-Cayan *et al*., 2021).

The increasing demand for these valuable fungi, coupled with the challenges and ecological impacts of wild harvesting, has spurred significant interest in developing reliable methods for their controlled cultivation (Pardo-Giménez *et al*., 2020). A critical first step in the domestication and cultivation of any mushroom species is the optimization of *in vitro* mycelial growth. This stage is fundamental for generating high-quality spawn, which directly influences the efficiency of substrate colonization, fructification, and ultimately, yield (Song *et al*., 2017).

The growth and development of mushroom mycelium are profoundly influenced by nutritional factors, including the source of carbon, nitrogen, and minerals, all of which are provided by the growth medium (Mleczek *et al*., 2021). While Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) are commonly used as standard media in mycological studies, their suitability is not universal across all fungal taxa. The nutritional requirements for optimal mycelial growth are highly species-specific (Ozturk *et al*., 2021). What constitutes an ideal medium for one genus may be suboptimal for another. Therefore, screening a range of culture media is an essential prerequisite for developing an efficient protocol for mycelial biomass production.

Although studies have investigated the cultivation parameters of various medicinal and edible mushrooms, targeted research on the *in vitro* requirements of *Lepista* spp. remains relatively limited. A systematic evaluation of different nutrient sources is necessary to identify the most conducive substrate for its vegetative growth. This study, therefore, aims to evaluate the effect of different culture media namely Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Corn Meal Agar (CMA), and Carrot Dextrose Agar (CDA) on the mycelial growth rate of *Lepista* spp. The findings will provide crucial baseline data for future cultivation efforts and contribute to the sustainable utilization of this valuable fungal resource.

Methods and Materials

The present research, entitled was conducted under the All India Coordinated Research Project (AICRP) on Mushrooms at the Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology (MPUAT), Udaipur. The study was carried out during the rainy season of 2024-25 under the natural climatic conditions of Udaipur. The environmental parameters in the cropping room were maintained within a temperature range of 20-25°C and a relative humidity range of 80–95%. This chapter delineates the comprehensive methodology and procedures adopted for the cultivation of *Lepista* mushrooms, along with the criteria established for evaluating the various treatments throughout the investigation.

A pure culture of *Lepista* spp. was procured from the culture repository of the All India Coordinated Mushroom Improvement Project unit, located within the Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur, for the purpose of this research. The procured culture was multiplied and maintained on Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) media. For long-term preservation, the pure culture was maintained in test tubes containing slants of 2% PDA or MEA. The freshly inoculated slants were incubated at 20 ± 1°C until satisfactory mycelial growth was observed.

The following apparatus and equipment were utilized during the course of the experiments: laminar airflow chamber, test tubes, Petri dishes, conical flasks, 500 ml glass bottles (for master spawn preparation), maximum-minimum thermometer, autoclave, hot air oven, refrigerator, incubator, spirit lamp, inoculation needle, pH meter, hot plate, plastic drums, buckets, tubs, and a weighing balance. The media were prepared according to the standard method. For potato dextrose agar (PDA), carrot dextrose agar (CDA), and corn meal agar (CMA), 200 g of washed, peeled, and sliced potatoes (for PDA), carrots (for CDA), or half-ground corn (for CMA) were added to a 1 L beaker containing 500 ml of distilled water. For malt extract agar (MEA), 20 g of malt extract was used instead of potato or carrot. The mixture was gently boiled for 30 minutes or until the solid ingredients could be easily penetrated with a glass rod. The resulting broth was filtered through muslin cloth, and the liquid was collected. Then, 20 g of dextrose and 20 g of agar-agar were added to the filtrate. The volume was adjusted to 1000 ml by adding distilled water. The medium was dispensed into five conical flasks (200 ml per flask) and sterilized by autoclaving. After sterilization, Petri plates were aseptically poured with 20.0 ml of the sterilized medium.

Results and Discussion

The choice of culture medium exhibited a highly significant ($p<0.05$) influence on the radial mycelial growth of *Lepista* spp. after a defined incubation period. The results, detailing mean colony diameters, they are presented in table 2 and graphically illustrated in fig 1.

Table 1. Constituents of different culture media used for the growth of *Lepista* spp.

Medium Name	Constituents	Quantity (per 1000 ml)
Malt Extract Agar (MEA)	Malt Extract	20 g
	Dextrose	20 g
	Agar-Agar	20 g
	Distilled Water	1000 ml
Potato Dextrose Agar (PDA)	Potato Infusion (from 200 g peeled potato)	-
	Dextrose	20 g
	Agar-Agar	20 g
	Distilled Water	1000 ml
Corn Meal Agar (CMA)	Corn Meal	20 g
	Dextrose	20 g
	Agar-Agar	20 g
	Distilled Water	1000 ml
Carrot Dextrose Agar (CDA)	Carrot Infusion (from 200 g carrot)	-
	Dextrose	20 g
	Agar-Agar	20 g
	Distilled Water	1000 ml

Potato Dextrose Agar (PDA) proved to be the most superior medium for promoting mycelial growth of *Lepista* spp., yielding a significantly higher mean colony diameter of 77.20 mm. The mycelium on PDA was typically dense, fluffy, and exhibited a uniform radial expansion, indicating excellent nutrient assimilation. While, Corn Meal Agar (CMA) was the second most effective medium, supporting a robust mean growth of 65.40 mm. Although the growth was significantly less than that on PDA, it was at par with itself as a distinct statistical group. The mycelial morphology on CMA often differed, sometimes appearing slightly less fluffy but more rhizomorphic than on PDA. However, Carrot Dextrose Agar (CDA) facilitated moderate mycelial development with a mean colony diameter of 53.80 mm.

This growth was significantly lower than that on both PDA and CMA but was at par with itself, forming a separate statistical grouping. The growth on CDA was stable and consistent, suggesting it provides a viable, though not optimal, nutrient base. Whereas, Malt Extract Agar (MEA) was distinctly the least favourable medium for the growth of *Lepista* spp., resulting in markedly restricted mycelial expansion with a mean colony diameter of only 20.60 mm. This growth was significantly inferior to all other treatments and was at par only with itself, forming the lowest statistical group.

The poor performance shows that the nutrient composition of MEA is inadequate or imbalanced for the metabolic requirements of this genus. The statistical analysis underscores the reliability of these observations. Since the difference in mean values between the top-performing medium (PDA). The results of the present study are in confirmation with the findings of (EI-Fallal *et al*., 2017) who also reported potato dextrose agar medium (PDA) was most effective medium for the growth of mycelium of *Lepista* spp. Similar results have also been reported by (De *et al*., 2022) that potato dextrose agar (PDA) was the ideal growing medium for mycelium development of fungal strains, *Lepista sordida.* The results of the present study align with the findings of (Zhong *et al.,* 2013), who also identified potato dextrose agar (PDA) as the most effective medium for mycelial growth of *Lepista sordida*.

Similarly (EI-Fallal *et al.,* 2017) evaluated multiple media and observed that PDA and malt extract agar supported the best mycelial growth, with the highest growth rate (8.5 mm/day) occurring on PDA. Consistent with these reports, (De *et al.*, 2022) also confirmed that potato dextrose agar is the optimal medium for mycelial development in *Lepista sordida*. These collective findings underscore the suitability and superiority of PDA for cultivating *Lepista* species.

Table 2. Effect of different media on mycelial growth of *Lepista* spp.

Name of the Medium	Colony Diameter (mm)
Malt extract agar	20.60
Potato dextrose agar	77.20
Corn meal agar	65.40
Carrot dextrose agar	53.80
S.Em.±	1.41
CD at 5%	4.93

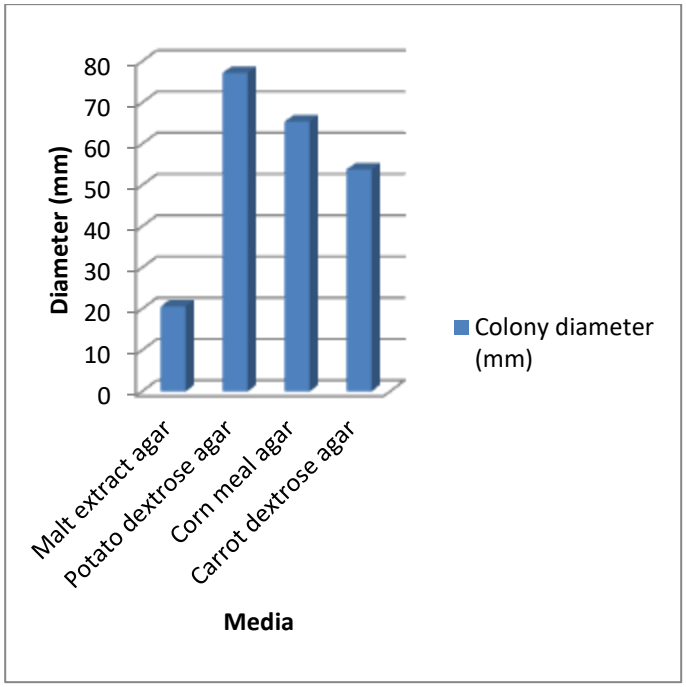

Fig 1. Effect of different media on mycelial growth of *Lepista* spp.

Conclusion

Based on the findings of this study, it is conclusively established that Potato Dextrose Agar (PDA) is the optimal medium for the cultivation of *Lepista* spp., yielding superior mycelial growth characterized by a dense, fluffy, and uniform colony with a significantly higher mean diameter of 77.20 mm. Corn Meal Agar (CMA) serves as a suitable secondary option, promoting robust but rhizomorphic growth, while Carrot Dextrose Agar (CDA) provides a moderate yet stable alternative. In contrast, Malt Extract Agar (MEA) is decidedly unsuitable due to its inadequate nutritional composition, resulting in severely restricted growth. Therefore, for efficient mycelial propagation of *Lepista* spp., the use of PDA is strongly recommended.

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