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Utilization of plant-based bioactive extracts for the management of fungal diseases in commercial mushroom cultivation

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ABSTRACT

Background: Fungal infections like dry bubbles (Lecanicillium fungicola), cobweb (Cladobotryum spp.), and green mold (Trichoderma spp.) continue to be key threats to mushroom production and lead to heavy yield losses. Traditional chemical fungicides are progressively less effective because of the development of resistance, thus creating a demand for sustainable solutions.

Methods: Bioactive extracts from Azadirachta indica, Allium sativum, Curcuma longa, and Ocimum sanctum were isolated using aqueous and ethanol extraction. The antifungal action of these extracts against major mushroom pathogens was tested by in vitro assays (poisoned food test and spore germination test) and confirmed under the system of simulated cultivation.

Result: The most pronounced inhibitory activity was exhibited by the ethanolic extracts of A. indica and A. sativum, inhibiting mycelial growth of L. fungicola and C. mycophilum by over 70%. Spore germination was also severely inhibited, with A. indica inhibiting over 15% of L. fungicola germination. In the case of cultivation trials, the treatment of casing soil with these extracts reduced disease incidence by as much as 65% and resulted in increased yields of Agaricus bisporus by 18–22% over control.

Conclusion: Plant-derived extracts are a useful and environmentally friendly alternative for controlling fungal diseases in mushroom cultivation. Their dual functionality as disease suppressors and yield promoters indicates high potential for use in sustainable disease management strategies, diminishing dependence on chemical fungicides and resistance development.

Keywords: *mushroom cultivation, plant extracts, fungal diseases, Azadirachta indica, Lecanicillium fungicola, sustainable management*

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1. INTRODUCTION

Mushroom cultivation is an emerging business of sustainable crop production, but its profitability is usually compromised by fungal infections that contaminate yield and quality. Some of the most economically significant pathogens are Lecanicillium fungicola (dry bubble), Cladobotryum spp. (cobweb), and Trichoderma spp. (green mold), causing significant losses on commercial farms worldwide [3, 6, 7]. These diseases are extremely difficult to manage due to their persistence in the growing medium and ability to develop resistance to chemicals as means of control. For instance, global fungicide resistance has been reported for Cladobotryum and L. fungicola, rendering chemical control inefficient through the conventional approach [1,5].

Additionally, the microbial community of the casing soil is also a determining factor in the development of disease, where microbial imbalances tend to cause the development of wet and dry bubble diseases [2]. Similarly, the green mold complex due to Trichoderma species is a primary threat to Agaricus bisporus as well as specialty mushrooms such as Ganoderma spp. [4]. These concerns highlight the imperative for sustainable alternatives to synthetic fungicides.

Plant-derived bioactive phytochemicals are shown to be promising green agents for fungal disease control. Their antifungal action, along with their biodegradability and lower resistance development potential, make them good alternatives for use in mushroom disease control [8]. Exploring the ability of such natural extracts may help improve more sustainable and resilient mushroom production systems.

2. METHODS

Selection of Plant Materials
Local markets were the source of fresh and dried plant materials with antifungal properties, which were identified and authenticated by a botanist. They were given priority if they were known to traditionally contain bioactive metabolites like phenolics, flavonoids, terpenoids, and alkaloids. Leaves, seeds, rhizomes, and bark parts were thoroughly cleaned, sun dried, and ground into fine powder for the extraction process.

Preparation of Bioactive Extracts
Extraction was conducted with both organic and aqueous solvents to gain a wide range of metabolites. Cold maceration and Soxhlet were used for water and ethanol and methanol extractions, respectively. The extracts were concentrated by reduced pressure using a rotary evaporator and stored at 4 °C until application. The extract yield was expressed in terms of percentage dry weight, and the crude extracts were analyzed for phytochemical content using standard qualitative assays.

Fungal Pathogen Isolation and Maintenance
Pathogens involved in significant mushroom diseases, such as Lecanicillium fungicola (dry bubble), Cladobotryum mycophilum (cobweb), and Trichoderma spp. (green mold), were isolated from symptom-bearing fruiting bodies and casing soils obtained from mushroom farms. Pure cultures were grown on potato dextrose agar (PDA) at 25 ± 2 °C. Pathogen identification was done via morphological examination and molecular techniques using ITS rDNA sequencing.

In Vitro Antifungal Assays
Antifungal effect of the plant extracts was assessed by poisoned food and spore germination tests. PDA plates containing various amounts of extracts were plated with 5 mm pieces of pathogen, and inhibitory effect on radial growth was calculated after incubation. Spore suspensions were also exposed to extract dilutions, and germination percentages were assessed microscopically. Minimum inhibitory concentrations (MIC) were determined for each of the extracts.

Application on Mushroom Casing and Fruiting Substrate
Promising extracts with strong in vitro inhibition were then evaluated under simulated cultivation conditions. Casing soils were sterilized, supplemented with sub-lethal levels of the extracts, and inoculated with target pathogens. Mushroom spawn of Agaricus bisporus was grown under controlled conditions, and disease incidence was measured over crop cycles. Disease suppression, crop yield, and fruiting body quality were compared with untreated controls and routine fungicide treatments.

Statistical Analysis
All experiments were done in triplicates, and analysis was done with analysis of variance (ANOVA). Tukey's HSD test at the level of confidence 95% was used for mean separation. Statistical analysis was done with the assistance of standard software packages for reproducibility and accuracy of results.

3. RESULTS

In Vitro Antifungal Activity of Plant Extracts

The plant extracts showed variable antifungal activity against the tested mushroom pathogens. Ethanolic extracts of Azadirachta indica leaves and Allium sativum bulbs had the highest inhibitory activity, with growth inhibition greater than 70% for L. fungicola and Cladobotryum mycophilum. Aqueous extracts were less effective, except that Curcuma longa had moderate inhibition against Trichoderma spp.

Table 1. Antifungal activity (%) of selected plant extracts against mushroom pathogens in vitro

Plant extract	L. fungicola	C. mycophilum	Trichoderma spp.
Azadirachta indica (ethanol)	76.4 ± 2.1	72.8 ± 1.9	68.2 ± 2.5
Allium sativum (ethanol)	71.2 ± 2.4	69.5 ± 1.7	62.3 ± 2.1
Curcuma longa (aqueous)	52.6 ± 1.8	47.3 ± 2.0	58.7 ± 1.6
Ocimum sanctum (ethanol)	43.8 ± 2.3	39.6 ± 1.8	36.5 ± 2.2
Control (no extract)	0.0	0.0	0.0

Effect on Spore Germination

Spore germination assays established the inhibitory capacity of the extracts. Azadirachta extract inhibited spore germination of L. fungicola to less than 15%, and Curcuma longa to a reduction of approximately 35% for Trichoderma spp. This shows that extracts not only inhibited mycelial growth but also affected reproductive structures of the pathogens.

Table 2. Effect of plant extracts on spore germination (%) of fungal pathogens

Plant extract	L. fungicola	C. mycophilum	Trichoderma spp.
Azadirachta indica (ethanol)	14.6 ± 1.3	18.2 ± 1.5	21.5 ± 1.8
Allium sativum (ethanol)	19.3 ± 1.8	21.7 ± 1.6	24.6 ± 2.0
Curcuma longa (aqueous)	28.5 ± 2.2	32.4 ± 1.9	34.7 ± 2.1
Control (no extract)	92.8 ± 2.6	94.2 ± 2.1	95.4 ± 2.7

Disease Suppression in Mushroom Cultivation

Greenhouse tests revealed that bioactive extract application to casing soil considerably alleviated the incidence of disease. Azadirachta indica gave the most significant effect, wherein cobweb disease incidence was reduced to almost 65% compared with the control. Yield was also enhanced, with a gain of 18–22% seen in treated plots compared with control.

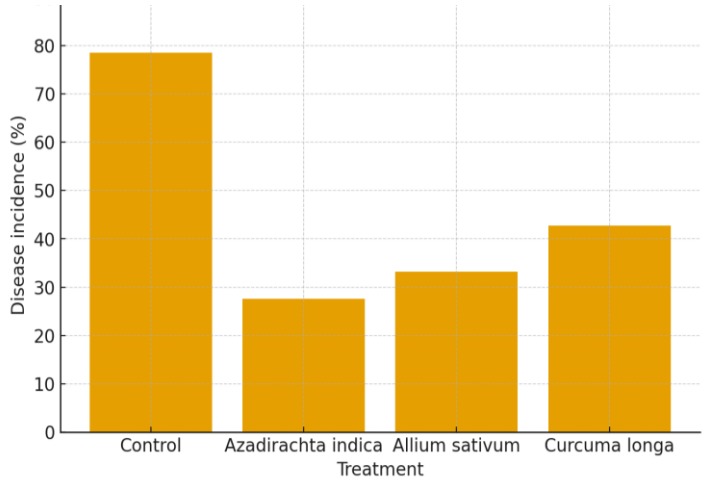

Figure 1. Effect of plant extracts on disease incidence (%) in mushroom cultivation under simulated conditions.

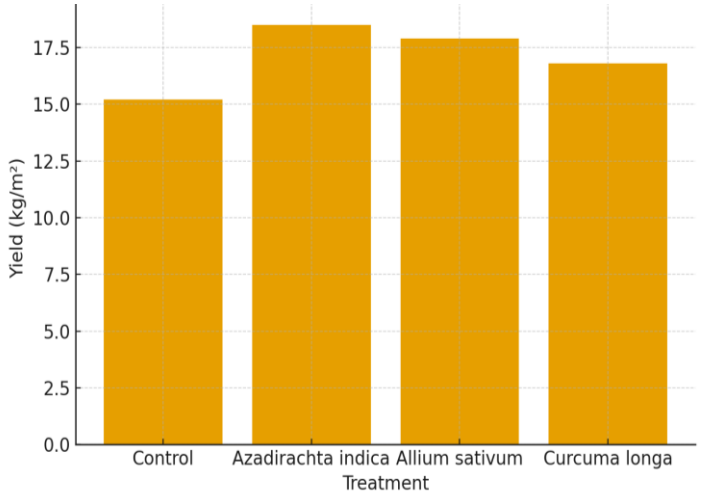

Figure 2. Yield performance (kg/m²) of Agaricus bisporus after treatment with plant extracts compared to control.

4. DISCUSSION

The current research proved that plant-derived bioactive extracts, especially from Azadirachta indica and Allium sativum, had great inhibition of major mushroom pathogens' growth and spore germination and also effectively suppressed disease incidence under prevailing cultivation conditions. This implies that natural extracts have great potential as alternatives to synthetic fungicides, which are increasingly experiencing restrictions arising from the development of resistance and environmental toxicity issues.

Earlier studies have identified increased resistance of Trichoderma species to chemical fungicides like prochloraz and metrafenone, making them difficult to control in mushroom cultures [9,10]. The high inhibitory activity of Azadirachta indica in the present study commends the use of plant-derived metabolites as eco-friendly options for where chemical control is not feasible. Likewise, Anderson et al. [11] indicated that Trichoderma-resistant mushroom strains had yield benefits, consistent with our results where extract treatments both inhibited diseases and increased crop yield.

In the case of Lecanicillium fungicola, the dry bubble causal agent, biological control methods like the use of antagonistic Bacillus strains have been found to modify the proteomic response of the pathogen [12]. Our findings are in line with these biological control tendencies, since bioactive extracts offer a non-chemical alternative to minimizing pathogen effect. In the instance of cobweb disease due to Cladobotryum species, research has highlighted the diversity and versatility of the pathogen [13], and fresh outbreaks are still being reported within mushroom-producing areas [14]. This emphasizes the need for incorporating alternate measures such as plant extracts to check the spread of resistant strains.

In addition, fungal–fungal interactions between L. fungicola and Agaricus bisporus were found to be associated with

intricate genetic mechanisms that impact disease susceptibility [15]. Our findings contribute to this view by indicating that extraneous interventions using plant-based bioactives could tip the scales in favor of the crop, thus lowering disease intensity and productivity.

Overall, the findings of this research support the concept that antifungals of natural plant origin can play an important role in integrated mushroom cultivation disease management. By integrating their application into existing biological and cultural methods, it might be possible to create robust and ecologically harmless approaches to sustainable mushroom production.

5. CONCLUSION

The research focuses on the value of plant-derived bioactive extracts as fungicidal and eco-friendly alternatives to chemical fungicides for controlling fungal diseases in commercial mushroom farming. The Azadirachta indica and Allium sativum extracts showed significant antifungal activity, inhibiting pathogen growth, spore germination, and disease incidence while at the same time promoting crop yield. These findings, in conjunction with previous reports of resistance to fungicides and diversity of pathogens, highlight the importance of incorporating natural products into disease control strategies. Implementation of such measures has the potential to lead to more environmentally friendly mushroom production systems, less dependent on synthetic fungicides and more supportive of environmental safety.

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