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EVALUATION OF *IN VITRO* ANTIFUNGAL EFFECTS OF *ASPARAGUS RECEMOSUS L.* AND *TERMINALIA BELLERICA ROXB.* ON POST-HARVEST FUNGAL PATHOGENS AFFECTING APPLE FRUITS

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ABSTRACT

The primary objective of this study was to evaluate the antifungal activity of natural medicinal herbs against post-harvest fungal pathogens. Ethanolic extracts of *Asparagus racemosus* L. and *Terminalia bellerica* Roxb. were tested at varying concentrations (500, 1000, 1500, 2000, 2500, and 3000 µg/ml) against dominant fungal pathogens isolated from naturally infected apple fruits, viz. *Penicillium expansum*, *Aspergillus flavus*, and *Mucor piriformis*. Plant materials were collected from various locations across Maharashtra and taxonomically identified. Antifungal susceptibility of ethanolic leaf extracts was assessed using the dilution method, with known antifungal agents serving as positive controls and water extracts as negative controls. The ethanolic extracts exhibited significantly higher antifungal activity compared to the controls. *Asparagus racemosus* L. demonstrated up to 99% inhibition of mycelial growth at 3000 µg/ml against *Penicillium expansum* and *Aspergillus flavus*, while *Terminalia bellerica* Roxb. showed complete (100%) inhibition of *Mucor piriformis* at the same concentration. These findings suggest that the leaf extracts possess potent antifungal properties and could serve as effective, natural alternatives for managing post-harvest diseases in fruits.

KEYWORDS: *Asparagus racemosus* L., *Terminalia bellerica* Roxb., Plant extracts, Post-harvest pathogens, Disease management.

INTRODUCTION

Apple (*Pyrus malus* L.), a pomaceous fruit belonging to the family Rosaceae, is the most important temperate fruit crop of the North Western Himalayan region. It holds significant agricultural and nutritional value and is predominantly cultivated in Jammu & Kashmir, Himachal Pradesh, and Uttar Pradesh, contributing to nearly 90% of the country's apple production. Post-harvest, apples are frequently affected by fungal pathogens such as *Penicillium expansum*, *Aspergillus flavus*, and *Mucor piriformis* during storage. Although synthetic fungicides are commonly used to manage blue mould and other post-harvest diseases, their indiscriminate application has raised concerns regarding environmental safety and public health. Hence, there is a growing interest in eco-friendly and sustainable alternatives. The use of plant-based solvent extracts presents a promising approach to disease management, offering potential as natural fungicides. Apple fruits are known to be affected by a range of dominant fungal pathogens, including *Penicillium expansum*, *Aspergillus flavus*, *Alternaria alternata*, *Aspergillus fumigatus*, *Botrytis cinerea*, *Venturia inaequalis*, *Erwinia amylovora*, *Botryosphaeria obtusa*, *Leptodontium elatius*, *Rhizopus arrhizus*, *Mycosphaerella pomi*, *Mucor piriformis*, and *Monilinia fructigena*. Among these, *Penicillium expansum*, *Aspergillus flavus*, and *Mucor piriformis* are particularly serious pathogens. Their management through ethanolic extracts of medicinal plants remains underexplored. Several plants are known for their antifungal and antibacterial properties, making them promising biocontrol agents.[3,4,9,10,12]

Eco-friendly herbal extracts have shown potential as alternatives to synthetic fungicides, being biodegradable and safe for human health.[6,8,11] Despite this, the application of such extracts in managing post-harvest pathogens of apples remains limited. Therefore, the present study aims to evaluate the effectiveness of ethanolic extracts from *Asparagus racemosus* L. and *Terminalia bellerica* Roxb. in inhibiting post-harvest fungal pathogens in apple fruits.

MATERIALS AND METHODS

Collection of Diseased Fruits

A survey was conducted at the APMC Fruit Market, Vashi, Navi Mumbai, from September to December 2024 to document common post-harvest disease symptoms in apple fruits. The predominant symptoms observed included the appearance of bluish, black, and brown mould on the surface of the fruits. Randomly selected symptomatic apple fruits were collected and

transported to the Research Laboratory, Department of Botany, K. V. Pendharkar College, Dombivli (E)-421203, Maharashtra, India, for further investigation. The fruits were washed with sterile distilled water, surface-sterilized using 0.1% mercuric hypochlorite solution, and subsequently cultured on Potato Dextrose Agar (PDA) medium. The cultures were incubated under aseptic conditions at 27 ± 2°C for 8 days to facilitate the isolation, identification, and single spore propagation of the pathogens.

Isolation and Identification of Test Pathogens

After incubation, fungal colonies were examined for their morphological and cultural characteristics. Based on standard literature, the fungal isolates were identified as *Penicillium expansum*, *Aspergillus flavus*, and *Mucor piriformis*. Single spore isolations were performed, and pure cultures were maintained on PDA at 25°C for 8 days. Spores were harvested by flooding the agar surface with sterile distilled water, and the suspensions were stored at 4°C for subsequent experiments.

Collection and Preparation of Plant Extracts

Fresh leaves of *Asparagus racemosus* L. and *Terminalia bellerica* Roxb. were collected from various locations across Maharashtra. The leaves were washed thoroughly under running tap water and oven-dried overnight at 40°C. A total of 100 grams of each dried plant material were ground into a fine powder using an electric blender and stored in labelled, airtight glass bottles.

A modified extraction method was used.[5] Fifty grams of each powdered sample were macerated with 100 ml of 95% ethanol and stirred continuously for 30 minutes. The mixtures were filtered sequentially through four layers of muslin cloth followed by Whatman No. 1 filter paper. The filtrates were evaporated at 60°C for 1 hour in an evaporating dish. The concentrated, spongy residues were dried at 37°C for 48 hours and stored in sterilized 5 ml screw-capped glass vials at 4°C for further use.

Preparation of Plant Extract Dilutions

The dried ethanolic extracts of *Asparagus racemosus* and *Terminalia bellerica* were brought to room temperature before use. Serial dilutions were prepared by dissolving 0.5 g, 1.0 g, 1.5 g, 2.0 g, 2.5 g, and 3.0 g of each extract in distilled water to obtain final concentrations of 500, 1000, 1500, 2000, 2500, and 3000 µg/ml, respectively.

In Vitro Antifungal Screening

PDA was prepared and autoclaved in 250 ml conical flasks for 20 minutes at 15 lbs pressure. After cooling to approximately 45°C, 5 ml of each plant extract concentration was added to 20 ml of molten PDA. The mixture was poured into sterile 9 cm Petri dishes (three replicates per treatment) under aseptic conditions. One ml of spore suspension from each test pathogen (*Penicillium expansum*, *Aspergillus flavus*, and *Mucor piriformis*) was inoculated at the center of each plate. Control plates contained PDA without plant extract. All plates were incubated at 25°C for 8 days.

The radial growth of fungal colonies was measured in millimetres, and antifungal activity was assessed by calculating the percentage inhibition of mycelial growth using the following formula.[15]

$$P = \frac{(gC - gT)}{gC} \times 100$$

Where:

C = radial growth in the control plate (mm)

T = radial growth in the treatment plate (mm)

RESULTS AND DISCUSSION

Table 1: Mycelial Growth Inhibition (in percentage) by the ethanolic extracts of the test plants at different concentrations.

	Asparagus recemosus L.			*Terminalia bellerica Roxb.*		
Concentration (µg/ml)	***Penicillium expansum***	***Aspergillus flavus***	***Mucor piriformis***	***Penicillium expansum***	***Aspergillus flavus***	***Mucor piriformis***
500	47.80	51.55	54.55	52.50	49.30	54.75
1000	55.55	54.55	62.00	63.20	54.50	62.32
1500	61.95	62.00	73.25	78.55	63.80	76.00
2000	80.50	73.95	81.10	89.55	75.30	86.50
2500	86.52	88.50	89.95	94.10	88.55	95.00
3000	98.85	99.25	100	100	100	100

The post-harvest fungal pathogens viz. *Penicillium expansum*, *Aspergillus flavus*, and *Mucor piriformis* were identified based on their cultural and morphological characteristics and were subsequently tested for susceptibility to plant extracts. Potato Dextrose Agar (PDA) media supplemented with varying concentrations (0 µg/ml as control, 500, 1000, 1500, 2000, 2500, and 3000 µg/ml) of *Asparagus racemosus* L. extract demonstrated

significant antifungal activity (Table 1). *Penicillium expansum* exhibited a progressive reduction in colony growth, with inhibition rates of 47.80%, 55.55%, 61.95%, 80.50%, 86.52%, and 98.85% at the respective concentrations. Similarly, *Aspergillus flavus* showed inhibition rates of 51.55%, 54.55%, 62.00%, 73.95%, 88.50%, and 99.25%, while *Mucor piriformis* displayed 54.55%, 62.00%, 73.25%, 81.10%, 89.95%, and 100% inhibition, respectively. No inhibition was observed in the control treatments. Notably, the highest concentration (3000 µg/ml) exhibited strong inhibitory effects on all three fungi.

A comparable trend was observed with *Terminalia bellerica* Roxb. extract, which also demonstrated potent antifungal efficacy. At 3000 µg/ml, it achieved nearly 100% mycelial growth inhibition in all three fungal species. The data presented in Table 1 confirm that both plant extracts were most effective at the highest concentration tested.

These findings align with previous studies showing that various plant extracts can effectively inhibit fungal pathogens such as *Fusarium*, *Alternaria*, and *Helminthosporium*.[14] Furthermore, similar antifungal activity has been reported for extracts of garlic[7], neem[13], *Withania somnifera*[2], mustard, and horseradish[1] against fungi like *Penicillium digitatum*, supporting the current results.

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