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IDENTIFICATION AND IN VITRO MANAGEMENT OF APPLE BLUE MOLD DISEASE IN BALOCHISTAN

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Abstract

Apple blue mold is a serious disease that affects apple production worldwide. It is caused by the fungus Penicillum expansum. Additionally, the study explored the impact of essential oils on the linear colony growth of Penicillium expensum under controlled in-vitro conditions. Essential oils, such as cinnamon, clove, black pepper, ginger, garlic and mustard displayed varying inhibitory effects on Penicillium expensum growth. Clove and Cinnamon oil exhibited strong inhibitory effects. These findings enhance our understanding of interactions between agents and the fungus, paving the way for further fungal growth control research. The study contributes practical insights for managing Penicillium expensum growth, potentially advancing strategies in the field of fungal growth control and management. The results indicate that the greatest increase in colony growth (mm) of Penicillium expansum (25, 23.27 and 20.37) occurred when mustard oil was applied at concentrations of 5%, 10% and 15%. Following this, colony growth of Penicillium expansum (23.93, 21.64 and 17.25) was recorded under black pepper oil at the concentrations of 5%, 10%, and 15%, respectively. In contrast, the colony growth of Penicillium expansum was observed under ginger oil (23.31, 20.41 and 15.83) and respectively the moderate growth of Penicillium expansum (22.25, 18.12 and 14.20) was recorded in garlic oil, in contrast, the minimum colony growth of Penicillium expansum was observed under cinnamon oil (21.87, 15.62 and 11.33) and the lowest colony growth of Penicillium expansum was observed under clove oil (19.08, 14.05 and 10).

Keywords: *Apple, Blue mold, Essential oils, Fungus, Penicillium expensum*

INTRODUCTION

Apple mainly grown in temperate countries, the apple (Malus domestica Borkh) is one of the most significant and fleeting fruits in the world. It is a member of the Rosaceae family and Pomoideae subfamily (1). Though there are over 7500 apple varieties identified, only few have significant economic value. Some cultivars are widely cultivated around the world, including Red Delicious, Golden Delicious, Granny Smith, Rome Beauty, and McIntosh (2). Apples can be made into sauce, slices, juice, or used in pastries, cakes, pies, and tarts. The juice can be made into vinegar, distilled into brandy, fermented into wine or cider, or consumed straight (3). Apple pomace contains antioxidant properties and is an excellent source of dietary fiber and polyphenols (4). Apple cider vinegar has the potential to treat a wide range of illnesses, regulate nervous system function, and provide essential nutrients and energy. It works well against gout, uric acid, urticarial infections, and kidney-related illnesses. It also fortifies the heart's muscle and gums (5). After bananas, which produced 115 million tons, apples came in second place with 86 million tons produced (6). Many experiments have been carried out in recent years to enhance the quality of apples while they are being stored utilizing different methods (7). However, current worries about global warming have led to a decrease in apple yields, mainly affecting the size, color, shape, and timing of flowering (8). Transgenic techniques that improve fruit quality, resistance to bacterial or fungal diseases, and rootstocks with better rooting or dwarfing capacities have all contributed to the growth of the apple (9).

There are several biotic and abiotic factors contributing to the low apple yield, the disease being the

most significant. More often than not, diseases can cause a 30–50 percent loss in apple fruit production, making them a more significant impact in fruit production reduction than weeds and insects. The production of important horticultural and agricultural crops is greatly impacted by a number of fungus that cause illness. In this context, fungal infections are a key factor leading to an average yield loss of over 13% over the whole agriculture and horticulture industry (10). *Penicillium expansum* is the culprit behind blue mold rot, a major post-harvest disease that affects apples globally and poses a danger to the fresh and processed fruit sectors. In wealthier nations, this illness can result in direct losses of 5 to 20%, whereas in underdeveloped nations, the number is closer to 50% (11). *P. expansum* enters the fruit by cuts in the pericarp, causing infection in the orchard or during handling after harvest, which leads to storage rots (12). Blue mold is caused by the pathogen, and it begins as light brown, squishy lesions with blue-green conidia (13).

Fungicides are frequently used to control post-harvest diseases, such as blue mold rot (14). Various other strategies to combat postharvest decay have therefore been explored. Despite the fact that pathogenic fungi are increasing due to the progressively wetter and warmer weather, postharvest fungicide applications are completely prohibited in the Nordic countries, and access to chemicals for pre-harvest application is also becoming more restricted (15,16). Essential oils, which are rich in bioactive chemicals with antibacterial and antioxidant capabilities, are fragrant, volatile, oily liquids that are extracted from plants and spices using hydrodistillation or supercritical fluid extraction (17). Due to its strong antibacterial, antifungal, and antioxidant properties, clove essential oil (CEO) is one of the most well-known EOs and is utilized for anticancer, fungicidal, antiviral, antioxidant, and cosmetic purposes (18).

MATERIALS AND METHODS

INFECTED SAMPLE COLLECTION

The infected samples of Apple fruits with blue mold were collected at Quetta city. The Apple fruits were promptly transported to the Department of Plant Pathology at Balochistan Agriculture College, Quetta. The collected fruits were immediately utilized as the experimental material for *in-vitro* propagation and transformation studies.

ISOLATION AND IDENTIFICATION OF FUNGUS

Isolation of *Penicillium spp*, from infected samples of Apple we picked contaminated portions for further culturing. Prepared PDA media along with other required material i.e., glassware forceps, loop, scalpel and distill water was autoclaved 121°C for 20 minutes. After autoclaving the petri plates were incubated in dry-oven at 100 °C for 10 minutes once the required materials and media are autoclaved, the laminar flow cabinet cleaned with ethanol-soaked cotton. Then materials kept under UV for 15 minutes. Then transfered single colony inoculation through sterile loop in laminar flow cabinet and plates were incubated in at 27°C for 6-8 days observation. Then incubated petri were identified microscopically and observed the slides with a drop of lacto phenol kept single spores for structure identification (19, 20).

ESSENTIAL OILS

In-vitro experiments were conducted to assess the effect of essential oils, Essential oils (EOs) known for their antifungal properties against various species of *Penicillium* were carefully selected for this study. The findings demonstrated that all the tested essential oils significantly inhibited the radial mycelial growth of the target pathogen, *Penicillium expensum*. Six specific essential oils were employed: Clove (*Syzgium aromaticum*), Ginger (*Zingiber officinale Roscoe*), Cinnamon (*Cinnamomum verum*), Garlic (*Allium sativum*), Black Pepper (*Piper nigrum*) and Mustard (*Brassica nigra*). These essential oils were emulsified using 5% Tween 20 (v/v) to prepare different concentration of 5%, 10% and 15% which were subsequently used for the experiments (Table I).

Table I. 5%, 10% and 15% (v/v) essential oil preparation

%age	Essential oil	Ethanol	5% Tween 20 (Fixed)		Grand Total
			Tween 5%	DH_2O	
5%	0.25 ml	4.75 ml	0.25 ml	4.75 ml	10 ml
10%	0.50 ml	4.50 ml	0.25 ml	4.75 ml	10 ml
15%	0.75 ml	4.25 ml	0.25 ml	4.75 ml	10 ml

For this technique, each essential oil was dissolved in 5% (v/v) Tween-20, and the appropriate quantity was added to individual 9 cm Petri plates, each containing 20 ml of PDA-agar at a temperature of 25 °C. A 0.5 mm disc of *Penicillium expansum* mycelium was positioned on the treated PDA medium, and the plate was subsequently incubated at 24 °C. The extent of radial mycelial growth was assessed at two-day intervals over the course of eight days. The inhibitory percentage (IP) was calculated using the following formula:

$$IP = \frac{dc - dt}{dc} x\ 100$$

Here, 'dc' represented the mycelium diameter in a control Petri dish, and 'dt' represented the mycelium diameter in the Petri dish treated with the essential oil, as measured after each two-day interval (21).

RESULTS

ISOLATION AND IDENTIFICATION

The colony morphology of *Penicillium* was varied depended on the species, however, some general features were observed. Typically, *Penicillium* colonies were fast-growing, flat and velvety or cottony in texture (Fig. 1).

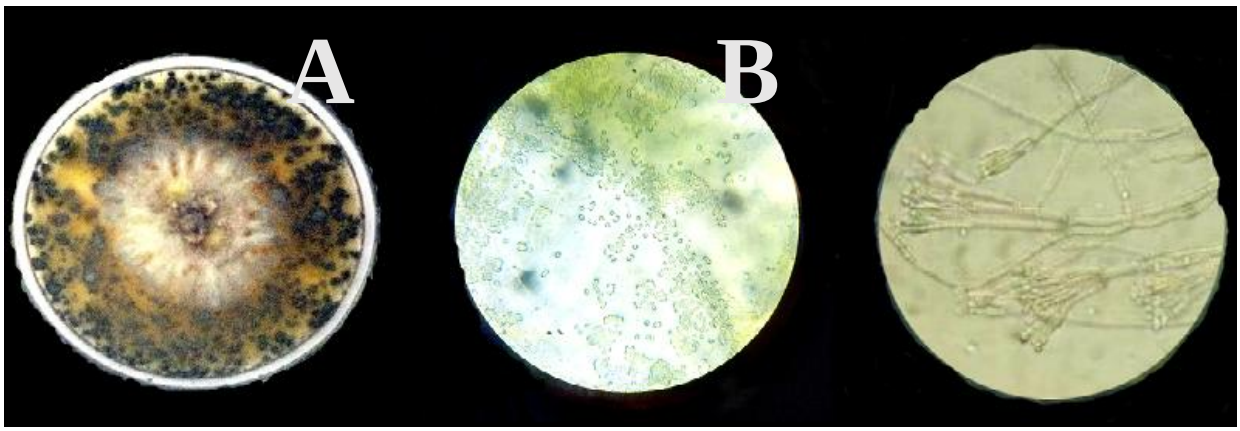

Fig. 1. Morphology Microscopy; A. Colony, B. Penicillium expansum morphology, C. spores

The colonies were color ranges from white to blue-green, olive-grey, or yellowish, depended on the pigmentation of the conidia and the mycelium. The conidiophores were long, branched, and bear flask-shaped. The isolated fungal species were subsequently sub-cultured to ensure the purification of the definitive pathogenic agent under microscopy and morphological characteristics causing apple fruits blue mold was confirmed as *Penicillium expansum.*

IN-VITRO ANTIFUNGAL EFFECTS OF ESSENTIAL OILS ON MYCELIAL RADIAL GROWTH

The experimental design encompassed a range of treatments, each conducted in vitro. Six distinct essential oils clove, cinnimum, garlic, ginger, black pepper and mustard were employed, and each oil was subjected into three distinct concentrations (5%, 10%, and 15%). The methodology established by Ozden and Bayindirli in 2002.

COMPARATIVE ANALYSIS OF LINEAR COLONY GROWTH OF *PENICILLIUM EXPANSUM* UNDER THE INFLUENCE OF VARIOUS ESSENTIAL OILS

The Fig. 2 presents the findings related to the linear colony growth of *Penicillium expansum* when exposed to various essential oils. The results indicate that the greatest increase in colony growth (mm) of *Penicillium expansum* (25, 23.27 and 20.37) occurred when mustard oil was applied at concentrations of 5%, 10% and 15%. Following this, colony growth of *Penicillium expansum* (23.93, 21.64 and 17.25) was recorded under black pepper oil at the concentrations of 5%, 10%, and 15%, respectively. In contrast, the colony growth of *Penicillium expansum* was observed under ginger oil (23.31, 20.41 and 15.83) and respectively the moderate growth of *Penicillium expansum* (22.25, 18.12 and 14.20) was recorded in garlic oil, in contrast, the minimum colony growth of *Penicillium expansum* was observed under cinnamon oil (21.87, 15.62 and 11.33) and the lowest colony growth of *Penicillium expansum* was observed under clove oil (19.08, 14.05 and 10). Under the control conditions, *Penicillium expansum* exhibited a colony growth of 27.79.

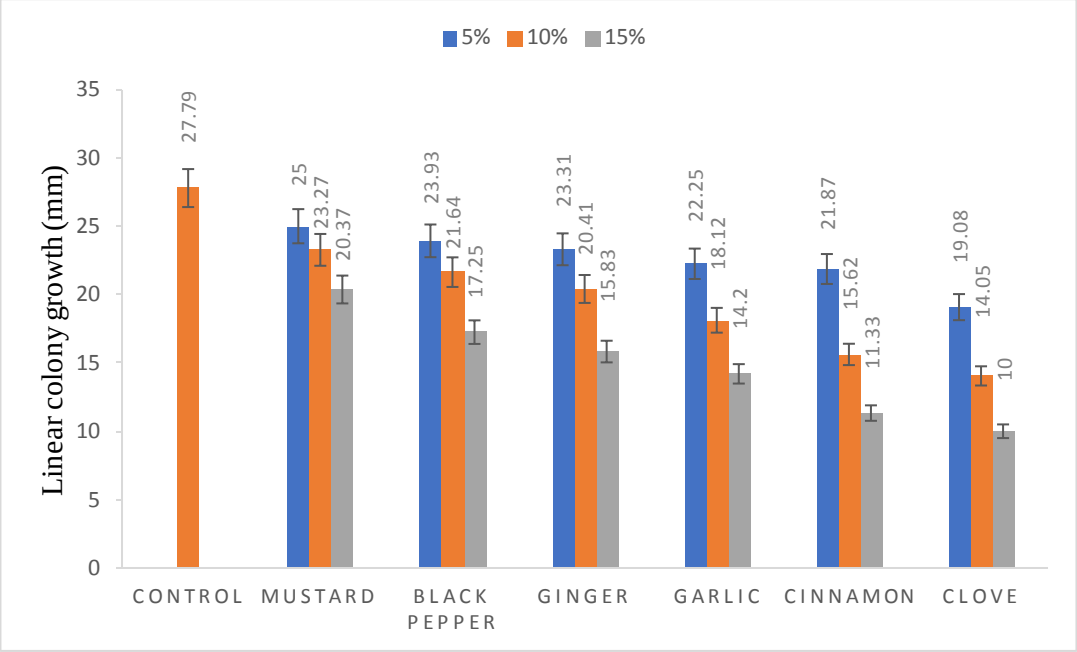

Fig. 2. Linear colony growth (mm) of Penicillium expansum at various essential oil concentrations of Mustard, Cinnimum, Garlic, Ginger, Black pepper and Mustard with a control group

DISCUSSION

The similar morphological characteristics of the colonies, such as color, shape, and texture, and microscopic features of the conidiophores and conidia, such as structure, shape, size, and ornamentation, were examined on lactophenol cotton blue (LPCB) mounts under a light microscope were observed the same (19). On these bases our study expresses and confirmed the pathogen as *Penicillium expansum.* The similar studies showed the according results (20). The conidiophores are long, branched, and bear flask-shaped phialides that produce chains of conidia. The conidia are usually spherical, smooth-walled, and green in color in their studies (22-24).

CEO was the most effective in reducing the percentage of germinated spores. The minimum inhibitory concentration (MIC) against blue mold was observed at 0.5, 0.5-, and 1-mL L−1 for clove, Essential oil. Clove EO was not able to provide a complete inhibition against blue mold (MGI between 70–90%) But having effectiveness in reducing *P. expansum* mycelial growth (25). But our study showed minimum growth at 5% concentration, was 19.08 mm, and while at 15% indicated total colony growth of 10 mm.

The garlic essential oil results showed showed minimum growth at 5% concentration, was 22.25 mm, and while at 15% indicated total colony growth of 14.2 mm. while another similar study garlic essential oil had a significant inhibitory effect on the growth of *P. expansum in-vitro*, with a minimum inhibitory concentration (MIC) of 0.5 ml. This is consistent with previous studies that reported similar or lower MIC values of garlic essential oil against *P. expansum* (26, 27). The cinnamon essential oil result showed minimum

growth at 5% concentration, was 21.87 mm, and while at 15% indicated total colony growth of 11.33mm. Another scientist used the same oil and tested the antifungal activity of cinnamon essential oil against *Penicillium exaporum* was tested *in-vitro* and *in-vivo*. And exhibited that it had a strong inhibitory effect on the growth and sporulation of *Penicillium exaporum in-vitro*, with minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of 0.5% and 1%, respectively (28). The Ginger essential oil results showed minimum growth at 5% concentration, was 23.31 mm, and while at 15% indicated total colony growth of 15.83 mm. This essential oil inhibited the mycelial growth of the fungus in vitro, with a minimum inhibitory concentration (MIC) of 0.5 ml. The essential oil also reduced the incidence and severity of blue mold on apple fruits inoculated with *P. expansum* spores, with a maximum inhibition rate of 83.3% at a concentration of 2mL (25). Several studies have investigated the antifungal activity of BPEO against different species of *Penicillium*. The results showed that BPEO exhibited different degrees of inhibition depending on the concentration, fungal strain, and assay method. BPEO was found to affect the fungal cell membrane, spore germination, mycelial growth, and enzyme activity. BPEO also reduced the production of patulin by *P. expansum*. Furthermore, MEO effectively controlled blue mold of apple in vivo, with a disease incidence of 6.7% and a disease severity of 1.3% at 2.0 μL/mL after 7 days of storage at 25 °C, compared to 100% and 43.3%, respectively, in the control treatment. (29, 30).

CONCLUSION

Considering the mean values, clove oil exhibited the highest efficacy, ranking 1st, followed by cinnamon oil in 2nd place, garlic oil in 3rd place, ginger oil in 4th place, black pepper at 5th place and mustard on 6th place in terms of inhibiting the colony growth of *Penicillium expansum* under *in-vitro* conditions. A statistical analysis of the collected data reveals a notable disparity in the linear colony growth of *Penicillium expansum* when exposed to different essential oils at varying concentrations.

Conflict of Interest:

Authors have no conflict of interest.

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