Characterization and management of post-harvest fungal diseases in banana (*Musa paradisiaca*)

Abstract

Banana (*Musa* spp.) is a vital crop globally, contributing significantly to food security and income generation, particularly in tropical regions like India. However, post-harvest fungal diseases pose a considerable threat to banana production, affecting both fruit quality and marketability. The current study investigated the isolation, characterization, and management of post-harvest fungal diseases in banana (*Musa paradisiaca*). Infected banana fruits were collected from the Dharashiv fruit market (Maharashtra, India), and pathogenic fungi were isolated and identified. *Fusarium napiforme*, *Talaromycesatroroseus*, *Cladosporium cladosporioides*, *Fusarium sp.*, and *Fusarium equiseti* were the primary pathogens identified. DNA extraction and sequencing were employed for accurate identification, and sequences were submitted to GenBank.The antifungal activity of essential oils and plant extracts was evaluated using the Poisoned Food Technique. Essential oils from *Syzygium aromaticum*, *Mentha piperita*, and *Punica granatum* showed significant inhibition ($p<0.05$) of fungal growth, with clove and peppermint oils achieving 100% inhibition at higher concentrations. Plant extracts of *Ocimum sanctum*, *Eucalyptus globulus*, *Mentha piperita*, *Zingiber officinale*, *Curcuma longa*, *Azadirachtaindica*, *Piper betel*, and *Cymbopogon citratus* were also tested, revealing notable efficacy, particularly with neem and peppermint extracts.The study that the efficacy of essential oils was more compared to aqueous plant extracts. The results suggested sustainable strategies for managing post-harvest fungal diseases in bananas and explained the importance of conducting field trials to validate laboratory results.

Keywords: Banana, ITS, Post -harvest, Plant extracts,Essential oils

Introduction

Banana (*Musa* spp.) is a perennial plant renowned for its edible fruits, playing a crucial role in food security and income generation for communities worldwide. Bananas are extensively cultivated in subtropical and tropical regions, with annual production

Comment [AM1]: Add losses caused by postharvest diseases on banana

Comment [AM2]: Write common name of all plants

estimated at over 102 million tonnes of fresh fruit worldwide with India alone producing 26.5 million tonnes of banana(Maseko *et al.*, 2024; Sathiya *et al.*, 2022). According to Canton (2021), global banana production is expected to rise to 140 million tonnes over the next decade, with India projected to remain the leading producer, reaching an output of 35 million tonnes by 2032. However, according to the OECD/FAO (2023), there has been a decline in banana production and export, from 20.5 million tonnes in 2021 to 19.6 million tonnes in 2022. One of the important reasons for the decline might be because of the diseases primarily fungal diseases that not only attack the banana plant during its growth stages but also pose a substantial threat to the fruit during the post-harvest period.

The most severe out of all the fungi is *Fusarium oxysporum* f. sp. cubense (Foc) tropical race 4, that has devastated banana fields globally, causing the well-knownPanama disease(Viljoen *et al.*, 2020). In June 2015, *Fusarium oxysporum* f. sp. cubense Tropical Race 4 (Foc TR4) was detected in Bihar, India's largest banana-producing province (Thangavelu *et al.*, 2019). Previously, Fusarium wilt caused by Foc race 1 affected Cavendish bananas in Theni, southern India (Thangavelu & Mustaffa, 2010). Beyond the field, fungal infections continue to threaten bananas during post-harvest handling and storage. Common post-harvest fungal pathogens include*Burkholderia, Pseudomonas, Elaphocordyceps, Penicillium,* and*Talaromyces*(Godana *et al.*, 2023). These infections can significantly reduce fruit quality, marketability, and shelf life, leading to substantial economic losses.Numerousstudieshave reported post-harvest fungal diseases affecting bananas across various regions of India(Chakrabarti *et al.*, 2003; Jones, 2000; Sarkar *et al*., 2009; Sarkar *et al*., 2013; Snehalatharani *et al*., 2021).

Application of essential oils and plant extracts from the plants is another effective way to control post-harvest diseases. Essential oils and plant extracts are known to be safe and would therefore be acceptable among the human population. The oils are known to be biologically active in their vapour phase. In the vapour phase they might act as fumigants and thereby manage post-harvest pathogens. Since the essential oils are more complex with different metabolites, the chances of pathogen resistance to the oils is low (Snehalatharani *et al.*, 2021). Studies have already been conducted on the efficacy of

Comment [AM3]: Describe briefly about the effectiveness of Essential Oil and Plant extracts,

essential oils from *Cinnamomum zeylanicum, Azadiractha indica,* and *Mentha arvensis*thatexhibited 100% efficacy against postharvest banana pathogens(Singh & Tripathi, 2015). Other alternatives like cinnamon, thyme, and almond oils have been used to protect bananas (Abd-Alla *et al.*, 2014).

The current study focuses on the isolation, characterization, and management of post-harvest fungal diseases in bananaby the use of alternative and natural strategies like plant extracts and essential oils.

Material and method

Collection and isolation of the pathogenic fungi from the fruit samples

Infected banana fruits were collected from three different locations within the Dharashiv fruit market. The fruit samples were randomly selected, sealed in sterile polyethylene bags, and promptly transported to the laboratory for fungal isolation. To obtain pure cultures, infected patches of the fruit samples were used. The isolation process was conducted immediately to minimize the presence of other saprophytic fungi on the fruit surface.

Comment [AM4]: Mention the locations. Also attach pictures showing symptoms of diseases.

The infected tissue was excised into small pieces of 2 mm^2 each that contained actively growing conidiawere cut from these fruits using a sterile scalpel. The tissues were placed to sterile potato dextrose agar plates (PDA) and incubated at 28 °C for seven days(Nicosia *et al.*, 2016). Petri dishes were observed daily, and the distinct colonies of fungi were picked. The isolated fungi were purified using a single spore technique (Leyronas *et al.*, 2012), and the pure colonies of fungal isolates were maintained on PDA slants.

Identification of fungal isolates

DNA extraction followed the method described by Saitoh *et al.* (2006). The Internal Transcribed Spacer (ITS) region of ribosomal DNA was amplified using ITS4 and ITS5 primer pairs. The PCR reaction mixture consisted of 2.5 µl of 10X PCR buffer, 1 µl of 200 mM dNTPs, 0.2 µl of Taq polymerase (1U/µl), 1 µl each of 10 pM/µl ITS5 and ITS4 primers, and 2.5 µl of 10 ng/µl template DNA. Sequencing of the amplified

product was performed using an Applied Biosystems Sanger sequencer (ABI 3100 Avant Prism). Consensus sequences were obtained from forward and reverse complementary sequences, and sequence identification was conducted using nBLAST alignment. Phylogenetic analysis for identifying the isolates was performed using MEGA 11 (Tamura *et al*., 2021). The identified pathogenic fungal sequences were submitted to GenBank.

Comment [AM5]: Attach picture data of DNA bands and its sequencing

***In-vitro* antifungal activity using poisoned food technique**

Fresh plant materials including *Ocimum sanctum, Eucalyptus globulus, Mentha piperita, Zingiber officinale, Curcuma longa, Azadirachta indica, Piper betel,* and *Cymbopogon citratus* were collected. The leaves were washed with sterilized distilled water, shade-dried, and ground into a fine powder. To prepare the plant extracts, 1000 grams of the powdered leaves were dissolved in 1000 milliliters of distilled water. The mixture was thoroughly stirred and then filtered through a double-layered muslin cloth to create a stock solution(Sahi *et al.*, 2012). The stock solution was then dried using a rotary evaporator and prepared in two concentrations (10,000 ppm and 20,000 ppm) for testing against the isolated post-harvest fungi. These plant extracts, known for their effectiveness against fungal pathogens, were evaluated *in vitro* using the Poisoned Food Technique(Nene & Thapliyal, 1993) and Potato Dextrose Agar (PDA) as the base medium.

Comment [AM6]: Only discuss the method of Plant Extract preparation where is E.O preparation method, describe it also

Three replicates were maintained for both the test pathogens and the control (without plant extract addition). The petri plates were inverted and incubated at 28 ± 2°C. Observations on radial mycelial growth and percent inhibition of the test fungi were recorded at 24-hour intervals until the test pathogen on the untreated control plate completely covered the medium(Jagtap *et al.*, 2012).

Comment [AM7]: Attach pictures of your experiment

The percentage of inhibition for the test pathogen was determined (Vincent, 1947).

$$\text{Percent inhibition} = \left(\frac{C - T}{C}\right) x\ 100$$

Were,

C = Growth of the test fungus in untreated control plates

T = Growth of the test fungus in treated plates

Essential oils of *Ocimumtenuiflorum, Eucalyptus citriodora hook, Azadirachta indica, Pongamia pinnata, Cymbopogon citratus, Syzygium aromaticum, Mentha piperita L.,* and *Punica granatum L.*were procured from local market and tested for the antifungal activity in similar way as performed for plant extracts. Percent inhibition were also calculated for the effect of essential oils.

Statistics

The mean, standard error of mean and analysis of variance with Tukey's HSD post hoc analysis was done using IBM SPSS ver 24.

Results and discussion

Post- harvest diseases of banana fruits i.e. crown rot, anthracnose, cigar end rot, fungal scald, stem end rot, main stalk rot, and botryodiplodia finger rot are some of the major diseases on different banana cultivars in India (Patil *et al.*, 2020). These diseases cause significant losses during storage, transportation, and marketing, affectingdifferent banana cultivars. Globally, postharvest diseases account for 10-30% of total crop yield losses, and in some perishable crops, especially in developing countries, they can destroy over 30% of the yield(Agrios, 2005).

Isolation and identification of post- harvest fungi in banana

Table 1 outlined various postharvest diseases affecting *Musa paradisiaca* (banana) and their respective causative agents along with their accession numbers. The isolation of pathogens from post-harvested *Musa paradisiaca* fruits revealed several significant diseases. *Fusarium napiforme* was identified as the pathogen responsible for Fusarium wilt, also known as Panama disease, which severely affected the banana plants. *Talaromycesatroroseus* was found to cause fruitlet core rot, compromising the quality and marketability of the fruit. *Cladosporium cladosporioides* led to crown rot, a common

Comment [AM8]: Also attach some pictures during your research work (as identification of pathogen)

post-harvest disease resulting in significant losses during storage and transport. Another Fusarium species, *Fusarium* sp., was also identified as a cause of Fusarium wilt or Panama disease, similar to*Fusariumnapiforme*. Additionally, *Fusarium equiseti* was found to contribute to Fusarium wilt or Panama disease, further highlighting the widespread impact of this disease on banana production. Similar results were obtained by Sarkar *et al.* (2009) who also reported the incidence of *Cladosporium cladosporioides*and *Fusarium sp.* from post- harvest fruits of banana from the Warangal region.Aradhana Pal *et al.* (2017)also reporteda total of 12 fungiisolated from diseased banana fruit samples viz. *Aspergillus flavus, Aspergillus niger, Alternaria spp., Botryodiplodiatheobromae, Colletotrichum musae, Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum, Fusarium solani, Mucor circinelloides, Penicillium spp., and Rhizopus stolonifer*from Uttar Pradesh state. The species of fungi infecting the fruit may vary depending on the cultivar of the crop, the host range of the fungi and geographical location as well as the environmental factors affecting the crop(Drenth & Guest, 2016).

Table 1 Isolated pathogens from the *Musa paradisiaca* post-harvested fruits

Sr No.	Name of Pathogen Isolated	Accession number	Disease name
1	*Fusarium napiforme*	OL711962	Fusarium wilt or Panama disease
2	*Talaromycesatroroseus*	OL711963	Fruitlet core rot
3	*Cladosporium cladosporioides*	OL711965	Crown rot
4	*Fusarium sp.*	OL711966	Fusarium wilt or Panama disease
5	*Fusarium equiseti*	OL711967	Fusarium wilt or Panama disease

Bio efficacy of essential oils and plant extracts on the fungus

The bioefficacy of different plant extracts and essential oils at different concentrations was tested against the post- harvest pathogenic fungus of banana fruit. The essential oils and aqueous plant extracts significantly exhibited the percent inhibition of the selected post- harvest pathogens of banana fruits at $p<0.05$ level of significance. With the increase in the concentration of essential oils and plant extracts, the percent

inhibitonalso increased. At 1500 ppm concentration, essential oils exhibited varying degrees of bio-efficacy against post-harvest fungal pathogensTable ***2***. *Syzygium aromaticum* and *Mentha piperita* L. oils achieved 100% inhibition of *Fusarium equiseti*. *Punica granatum* L. oil was most effective against *Fusarium napiforme* (46.83%) and *Talaromycesatroroseus* (73%). *Pongamia pinnata* oil showed high efficacy against *Talaromycesatroroseus* (67%) and *Cladosporium cladosporioides* (52.31%).

At 2000 ppm concentration, clove and peppermint oils maintained 100% inhibition of *Fusarium equiseti*(Table 3). Pomegranate oil significantly inhibited *Cladosporium cladosporioides* (59.65%), while *Pongamia pinnata* oil effectively inhibited *Fusarium napiforme* (33.28%) and *Cladosporium cladosporioides* (50.47%).In the current study, the essential oils of *Ocimumtenuiflorum*, Mentha piperita and *Syzygiumaromaticum*were effective in inhibiting the growth of the fungi.

In a similar bioassay, *Ocimumbasilicum* oil demonstrated fungistatic effects on the mycelial growth of *Fusariummoniliforme, Botrydiplodiatheobromae,* and *Colletotrichum sp*. at a low concentration of 1.5 ml/L (0.15% v/v)(Dube *et al.*, 1989).Researchers in Sri Lanka identified *Lasiodiplodiatheobromae, Fusarium proliferatum,* and *Colletotrichum musae* as crown rot pathogens in bananas, which could act alone or in combination, with greater severity when combined. *Cymbopogon nardus*and*Ocimumbasilicum* oils effectively inhibited *Colletotrichum musae* and *Fusarium proliferatum*at 0.2–0.6% concentrations, with even lower doses effective in liquid bioassays. These oils showed synergistic effects in *in-vivo* tests(Anthony *et al.,* 2004). They reportedultrastructural changes, including plasmalemma disruption, mitochondrial deformation, and alterations in hyphal wall thickness, were observed after treatment, indicating the mode of action of *Cymbopogon nardus*essentialoil.Similar results were obtained by Singh and Tripathi (2015) with essential oils from *Cinnamomum zeylanicum, Azadiractha indica,* and *Mentha arvensis* exhibit 100% efficacy against postharvest banana pathogens. *Cinnamomum zeylanicum*oilwas fungistatic at 100 ppm and fungicidal at 200 ppm.

At 10000 ppm concentration, aqueous plant extracts demonstrated significant efficacy against post-harvest fungal pathogens (Table 4). *Azadirachta indica* extract showed the highest inhibition of *Fusarium napiforme* (63.34%) and

Talaromycesatroroseus (68.79%). *Mentha piperita* L. and *Punica granatum* L. extracts notably inhibited Fusarium sp. (77.86% and 60.15% respectively). At 20000 ppm concentration, neem extract continued to show high efficacy against *Fusarium napiforme* (56.67%) and *Cladosporium cladosporioides* (75.47%) (Table 5). Peppermint extract effectively inhibited *Fusarium napiforme* (67.78%) and *Talaromycesatroroseus* (69.9%), while pomegranate extract showed notable inhibition of *Cladosporium cladosporioides* (79.93%).

Similar results were obtained by Pant *et al.* (2020)using three botanicals (*Azadirachta indica, Justicia adhatoda,* and*Eucalyptus globules*) tested against *Fusariumoxysporum.* With Eucalyptus was the most effective (36.67% inhibition at 10%), followed by *Justicia adhatoda* (9.10%) and Neem (5.32%). Another study demonstrated the antifungal efficacy of *Ocimumgratissimum*and *Moringa oleifera* leaf extracts against Eleven different fungi, including *Penicillium digitatum, Saccharomyces cerevisiae, Aspergillus niger, and Fusarium oxysporum*among others. The leaf extracts of *O. gratissimum and M. oleifera* demonstrated inhibitory effects on *Aspergillus* and *Fusarium* species, suggesting their potential as safe and effective alternatives to chemical fungicides for controlling post-harvest fruit deterioration(Kinge & Besong, 2021).

Biological control of post- harvest diseases of bananas with natural compounds, antioxidants, inorganic salts, fungicides, or even biosurfactants have already been studied. The combination of such compounds along with essential oils and plant extracts could also enable the improvement of the biological control. Once the inhibition activity has been confirmed under controlled conditions, it is crucial to conduct tests under natural infestation conditions and through real export scenarios to evaluate the true effectiveness of the biocontrol strategy. The current experiments were performed under rigorous conditions, using artificial inoculations using the high inoculum levels of the pathogenic species to study the effectiveness against them. These severe conditions are rarely encountered in practical situations, suggesting that the protection level may be even higher under natural infestation.

Conclusion

Based on our findings, essential oils proved more effective than plant extracts in controlling the pathogenic fungi isolated from the specified niche. Given the significant degree of infection by these fungi, combining essential oils with other compounds such as metal nanoparticles or biosurfactants could further enhance their efficacy in combating post-harvest diseases. While our experiments were confined to laboratory tests, it is imperative to conduct field trials for real-world validation. These results offer valuable insights for implementing sustainable strategies to control post-harvest fungi.

Comment [AM9]: Specify the concentration of E.O which was more effective against the diseases, as future recommendation.

Table 2 Bio-Efficacy of the essential oils against post-harvested fungal pathogens at 1500 ppm oil concentrations

Percent inhibition	*Ocimumtenuiflorum*	*Eucalyptus citriodora hook*	*Azadirachta indica*	*Pongamia pinnata*	*Cymbopogon citratus*	*Syzygium aromaticum*	*Mentha piperita L.*	*Punica granatum L.*
Fusarium napiforme	24.25±0.1	11.71±0.12	19.74±0.11	21.74±0.1	16.23±0.11	41.81±0.08	26.76±0.1	46.83±0.07
Talaromycesatroroseus	22±0.15	15±0.17	6±0.18	67±0.06	18±0.16	57±0.08	64±0.07	73±0.05
Cladosporium cladosporioides	35.13±0.04	1.53±0.06	5.64±0.06	52.31±0.03	49.74±0.03	42.05±0.04	55.9±0.03	30±0.04
Fusarium sp.	29.27±0.06	26.45±0.01	28.33±0.04	26.92±0.02	23.63±0.06	22.85±0.09	24.57±0.04	31.77±0.07
Fusarium equiseti	13±0.27	26±0.23	20±0.25	6±0.3	22±0.24	100±0a	100±0a	18±0.26

The readings are in Percentage inhibition. Any value followed by ± denotes standard error of the mean. The mean values with similar alphabets are similar means at $p<0.05$ level of significance.

Table 3 Bio-Efficacy of the essential oils against post-harvested fungal pathogens at 2000 ppm oil concentrations

Percent inhibition	*Ocimumtenuiflorum*	*Eucalyptus citriodora hook*	*Azadirachta indica*	*Pongamia pinnata*	*Cymbopogon citratus*	*Syzygium aromaticum*	*Mentha piperita L.*	*Punica granatum L.*
Fusarium napiforme	30.77 ± 0.09	27.26 ± 0.1	20.74 ± 0.11	33.28 ± 0.09	35.29 ± 0.09	50.34 ± 0.07	47.33 ± 0.07	28.27 ± 0.1
Talaromycesatroroseus	11 ± 0.4cd	9 ± 0.41bc	9 ± 0.41bd	34 ± 0.3	-1 ± 0.46	64 ± 0.16	42 ± 0.26	52 ± 0.22

Cladosporium cladosporioides	44.87 ± 0.03	35.69 ± 0.04	59.65 ± 0.02	50.47 ± 0.03	2.27 ± 0.87	31.29 ± 0.04	40.48 ± 0.03	10.12 ± 0.05
Fusarium sp.	15.35 ± 0.31	41.73 ± 0.21bc	40.55 ± 0.22c	31.89 ± 0.25	36.61 ± 0.23a	37.8 ± 0.23a	42.52 ± 0.21b	48.03 ± 0.19
Fusarium equiseti	11.89 ±0.6	24.32 ±0.51b	18.67 ±0.55	7.37 ±0.63	23.19 ±0.52b	100 ±0a	100 ±0a	1.73 ±0.66

The readings are in Percentage inhibition. Any value followed by ± denotes standard error of the mean. The mean values with similar alphabets are similar means at $p<0.05$ level of significance.

Table 4 Bio-Efficacy of the aqueous plant extracts against post-harvested fungal pathogens at 10000 ppm concentrations

Percent inhibition	*Ocimumtenuiflorum*	*Eucalyptus citriodora hook*	*Azadirachta indica*	*Pongamia pinnata*	*Cymbopogon citratus*	*Syzygium aromaticum*	*Mentha piperita L.*	*Punica granatum L.*
Fusarium napiforme	43.34 ± 0.15	35.57 ± 0.17a	63.34 ± 0.1	50.01 ± 0.13	44.45 ± 0.15	54.45 ± 0.12	65.56 ± 0.09	35.57 ± 0.17a
Talaromycesatroroseus	72.13 ± 0.59ghi	56.52 ± 0.92cd	68.79 ± 0.66begi	65.44 ± 0.73aef	60.98 ± 0.83df	54.29 ± 0.97c	66.56 ± 0.71ab	73.24 ± 0.57gh
Cladosporium cladosporioides	55.41 ± 0.94begi	44.26 ± 1.18	64.33 ± 0.76h	57.64 ± 0.9adef	58.75 ± 0.87cfhi	28.65 ± 1.51	56.52 ± 0.92abc	52.06 ± 1.02dg

Fusarium sp.	73.43 ± 0.04	54.61 ± 0.07	65.68 ± 0.05	84.5 ± 0.02	57.93 ± 0.06	67.9 ± 0.05	77.86 ± 0.03	60.15 ± 0.06
Fusarium equiseti	13.65 ± 0.13	14.76 ± 0.13	11.44 ± 0.13	16.97 ± 0.13ade	16.97 ± 0.13cef	16.97 ± 0.13bdf	16.97 ± 0.13abc	12.55 ± 0.13

The readings are in Percentage inhibition. Any value followed by ± denotes standard error of the mean. The mean values with similar alphabets are similar means at $p<0.05$ level of significance.

Table 5 Bio-Efficacy of the aqueous plant extracts against post-harvested fungal pathogens at 20000 ppm concentrations

Percent inhibition	*Ocimumtenuiflorum*	*Eucalyptus citriodora hook*	*Azadirachta indica*	*Pongamia pinnata*	*Cymbopogon citratus*	*Syzygium aromaticum*	*Mentha piperita L.*	*Punica granatum L.*
Fusarium napiforme	35.57 ± 0.17	31.12 ± 0.18	56.67 ± 0.11	44.45 ± 0.15	34.46 ± 0.17	45.56 ± 0.14	67.78 ± 0.08	32.23 ± 0.18
Talaromycesatroroseus	72.13 ± 0.59di	62.1 ± 0.8efg	65.44 ± 0.73bfhkm	68.79 ± 0.66ahij	66.56 ± 0.71cgjlm	53.18 ± 0.99	69.9 ± 0.64abcd	63.21 ± 0.78cekl
Cladosporium cladosporioides	72.13 ± 0.59cfg	57.64 ± 0.9	75.47 ± 0.52bef	68.79 ± 0.66cd	67.67 ± 0.68d	73.24 ± 0.57eg	77.7 ± 0.47ab	79.93 ± 0.43a
Fusarium sp.	66.92 ± 0.14a	50.38 ± 0.21	61.4 ± 0.16	77.94 ± 0.09	54.79 ± 0.19	66.92 ± 0.14a	72.43 ± 0.12	55.89 ± 0.19

Fusarium equiseti	15.87 ± 0.13cef	15.87 ± 0.13adeg	13.65 ± 0.13	15.87 ± 0.13bdf	13.65 ± 0.13g	9.23 ± 0.14	15.87 ± 0.13abc	11.44 ± 0.13

The readings are in Percentage inhibition. Any value followed by ± denotes standard error of the mean. The mean values with similar alphabets are similar means at $p<0.05$ level of significance.

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