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. *Fusariumnapiforme, Talaromycesatroroseus, Cladosporiumcladosporioides, Fusarium sp.,* and*Fusariumequiseti* 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 *Syzygiumaromaticum, Menthapiperita,* 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, Menthapiperita, Zingiberofficinale, Curcuma longa, Azadirachtaindica, Piper betel,* and*Cymbopogoncitratus*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 estimated at over 102 million tonnes of fresh fruit worldwide with India alone producing 26.5 million tonnes of banana(1, 2). According to Canton (3), 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 (4), 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[32,33,34].

The most severe out of all the fungi is *Fusariumoxysporum* f. sp. cubense (Foc) tropical race 4, that has devastated banana fields globally, causing the well-knownPanama disease(5). In June 2015, *Fusariumoxysporum* f. sp. cubense Tropical Race 4 (Foc TR4) was detected in Bihar, India's largest banana-producing province (6). Previously, Fusarium wilt caused by Foc race 1 affected Cavendish bananas in Theni, southern India (7). 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(8)*. 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(9-13).

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 (13). Studies have already been conducted on the efficacy of essential oils from

Cinnamomumzeylanicum, *Azadiracthaindica*, and*Menthaarvensis*thatexhibited 100% efficacy against post-harvest banana pathogens(14). Other alternatives like cinnamon, thyme, and almond oils have been used to protect bananas (15).

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

100 Infected banana fruits were collected from three different locations within the Dharashiv fruit market(18°11’12.3”N, 76°02’30.4”E).All the 100fruit samples were selected, sealed in sterile polyethylene bags, and promptly transported to the laboratory for fungal isolation. To obtain pure cultures, infected patches of the peel offruit samples were used. The isolation process was conducted immediately to minimize the presence of other saprophytic fungi on the fruit surface.

The infected tissue was excised into small pieces of each that contained actively growing conidiawere cut from these fruits using a flame sterilised scalpel. The tissues were placed to sterile potato dextrose agar plates (PDA) and incubated at 28 °C for seven days(16). Petri dishes were observed daily, and the distinct colonies of fungi were picked. The isolated fungi were purified using a single spore technique (17), and the pure colonies of fungal isolates were maintained on PDA slants.

Identification of fungal isolates

DNA extraction followed the method described by Saitoh, Togashi (18). The Internal Transcribed Spacer (ITS) region of ribosomal DNA was amplified using ITS4(5'-TCCTCCGCTTATTGATATGC -3')and ITS5(5'- GGAAGTAAAAGTCGTAACAAGG -3')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,

United States). 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 (19). The identified pathogenic fungal sequences were submitted to GenBank.The phylogenetic analysis of ITS region of the fungal samples are shown from figure 1 to 5.

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

Fresh plant materials including *Ocimum sanctum, Eucalyptus globulus, Menthapiperita, Zingiberofficinale, Curcuma longa, Azadirachtaindica, Piper betel,* and*Cymbopogoncitratus* were collected. The leaves were washed with sterilized distilled water, shade-driedfor 5 days, and ground into a fine powder using electric blender. To prepare the plant extracts, 1000 g of the powdered leaves were dissolved in 1000 mlof distilled water. The mixture was thoroughly stirredovernight and then filtered through a double-layered muslin cloth to create a stock solution(20). The stock solution was then dried using a rotary evaporator(Generic RE-201D 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(21) and Potato Dextrose Agar (PDA) as the base medium.

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°Cand 65% to 70% RH. 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(22).

The percentage of inhibition for the test pathogen was determined as described by (23).

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

Where,

C = Growth of the test fungus in untreated control plates

T = Growth of the test fungus in treated plates

Essential oils of *Ocimumtenuiflorum, Eucalyptusglobulus, Azadirachtaindica, Pongamiapinnata, Cymbopogoncitratus, Syzygiumaromaticum, Menthapiperita L.,* and *PunicagranatumL.*were procured from local market and tested for the antifungal activity in similar way as performed for plant extracts. The concentration of essential oils used was 1500 and 2000 ppm.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 (24). 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(25).

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. *Fusariumnapiforme* 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. *Cladosporiumcladosporioides* led to crown rot, a common

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, *Fusariumequiseti* 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, Girisham (11) who also reported the incidence of *Cladosporiumcladosporioides*and*Fusarium sp.* from post- harvest fruits of banana from the Warangal region.*Aradhana Pal, Singh (26)*also reporteda total of 12 fungisolated from diseased banana fruit samples*viz. Aspergillus flavus, Aspergillus niger, Alternaria spp., Botryodiplodiatheobromae, Colletotrichummusae, Fusariumequiseti, Fusariummoniliforme, Fusariumoxysporum, Fusariumsolani, Mucorcircinelloides, Penicillium spp., and Rhizopusstolonifer*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(27).

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

Sr No.	Name of Pathogen Isolated	Accession number	Disease name
1	*Fusariumnapiforme*	OL711962	Fusarium wilt or Panama disease
2	*Talaromycesatroroseus*	OL711963	Fruitlet core rot
3	*Cladosporiumcladosporioides*	OL711965	Crown rot
4	*Fusarium sp.*	OL711966	Fusarium wilt or Panama disease
5	*Fusariumequiseti*	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 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 inhibiton also increased. At 1500 ppm concentration, essential oils exhibited varying degrees of bio-efficacy against post-harvest fungal pathogens as depicted inTable 2. *Syzygiumaromaticum* and *Mentha piperita* L. oils achieved 100% inhibition of *Fusariumequiseti*. *Punica granatum* L. oil was most effective against *Fusariumnapiforme* (46.83%) and *Talaromycesatroroseus* (73%). *Pongamiapinnata* oil showed high efficacy against *Talaromycesatroroseus* (67%) and *Cladosporiumcladosporioides* (52.31%).

At 2000 ppm concentration, clove and peppermint oils maintained 100% inhibition of *Fusariumequiseti*(Table 3). Pomegranate oil significantly inhibited *Cladosporiumcladosporioides* (59.65%), while *Pongamiapinnata* oil effectively inhibited *Fusariumnapiforme* (33.28%) and *Cladosporiumcladosporioides* (50.47%).In the current study, the essential oils of *Ocimumtenuiflorum*, Menthapiperita 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)(28).Researchers in Sri Lanka identified *Lasiodiplodiatheobromae, Fusariumproliferatum,* and*Colletotrichummusae* as crown rot pathogens in bananas, which could act alone or in combination, with greater severity when combined. *Cymbopogonnardus*and*Ocimumbasilicum* oils effectively inhibited *Colletotrichummusae* and *Fusariumproliferatum*at 0.2–0.6% concentrations, with even lower doses effective in liquid bioassays. These oils proven synergistic effects in *in-vivo* tests(29). They reportedultrastructural changes, including plasmalemma disruption, mitochondrial deformation, and alterations in hyphal wall thickness, were observed after treatment, indicating the mode of action of *Cymbopogonnardus*essentialoil.Similar results were obtained by Singh and Tripathi (14) with essential oils from *Cinnamomumzeylanicum, Azadiracthaindica,* and*Menthaarvensis* exhibit 100% efficacy against postharvest banana pathogens. *Cinnamomumzeylanicum*oilwasfungistatic 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). *Azadirachtaindica* extract showed the highest inhibition of *Fusariumnapiforme* (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 *Fusariumnapiforme* (56.67%) and *Cladosporiumcladosporioides* (75.47%) (Table 5). Peppermint extract effectively inhibited *Fusariumnapiforme* (67.78%) and *Talaromycesatroroseus* (69.9%), while pomegranate extract showed notable inhibition of *Cladosporiumcladosporioides* (79.93%).

Similar results were obtained by Pant, Manandhar (30)using three botanicals (*Azadirachtaindica, Justiciaadhatoda,* and*Eucalyptus globules*) tested against *Fusariumoxysporum*. Eucalyptus was the most effective (36.67% inhibition at 10%), followed by *Justiciaadhatoda* (9.10%) and Neem (5.32%). Another study demonstrated the antifungal efficacy of *Ocimumgratissimum*and*Moringaoleifera* leaf extracts against eleven different fungi, including *Penicilliumdigitatum, Saccharomyces cerevisiae, Aspergillus niger, and Fusariumoxysporum*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(31).

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.

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

Percent inhibition	*Ocimumtenuiflorum*	*Eucalyptus citriodora hook*	*Azadirachtaindica*	*Pongamiapinnata*	*Cymbopogoncitratus*	*Syzygiumaromaticum*	*Menthapiperita L.*	*Punicagranatum L.*
Fusariumnapiforme	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
Cladosporiumcladosporioides	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
Fusariumequiseti	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 3Bio-Efficacy of the essential oils against post-harvested fungal pathogens at 2000 ppm oil concentrations

Percent inhibition	*Ocimumtenuiflorum*	*Eucalyptus citriodora hook*	*Azadirachtaindica*	*Pongamiapinnata*	*Cymbopogoncitratus*	*Syzygiumaromaticum*	*Menthapiperita L.*	*Punicagranatum L.*

Fusariumnapiforme	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
Cladosporiumcladosporioides	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
Fusariumequiseti	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*	*Azadirachtaindica*	*Pongamiapinnata*	*Cymbopogoncitratus*	*Syzygiumaromaticum*	*Menthapiperita L.*	*Punicagranatum L.*
Fusariumnapiforme	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
Cladosporiumcladosporioides	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
Fusariumequiseti	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 5Bio-Efficacy of the aqueous plant extracts against post-harvested fungal pathogens at 20000 ppm concentrations

Percent inhibition	*Ocimumtenuiflorum*	*Eucalyptus citriodora hook*	*Azadirachtaindica*	*Pongamiapinnata*	*Cymbopogoncitratus*	*Syzygiumaromaticum*	*Menthapiperita L.*	*Punicagranatum L.*
Fusariumnapiforme	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
Cladosporiumcladosporioides	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
Fusariumequiseti	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.

Figures

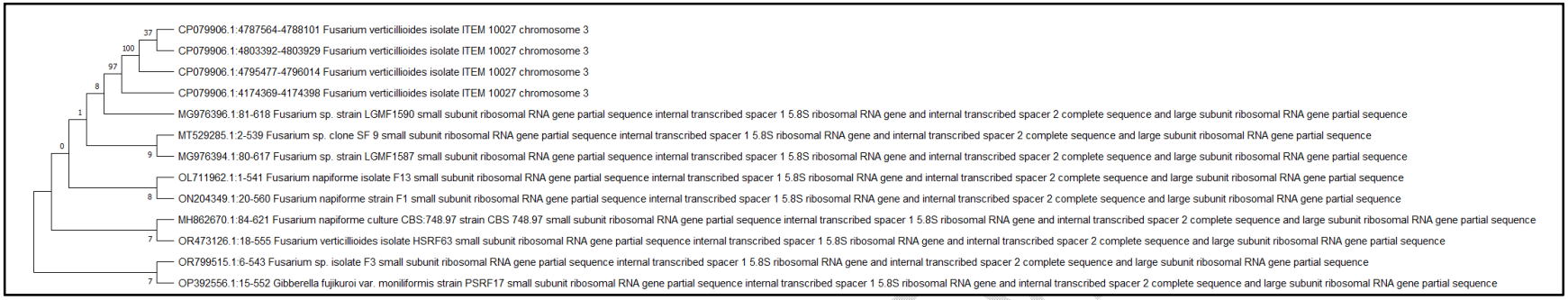

Figure 1Phylogenetic analysis of Fusariumnapiforme registered as accession OL711962

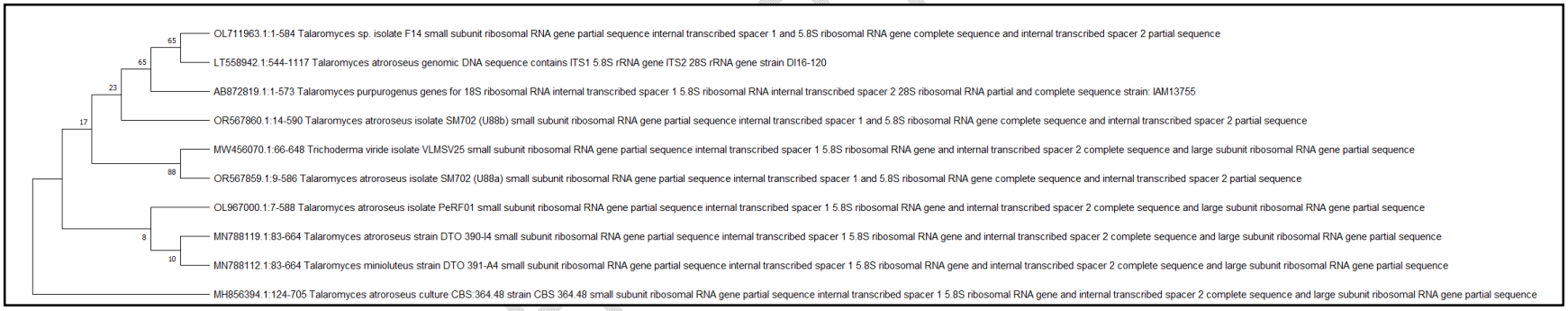

Figure 2Phylogenetic analysis ofTalaromycesatroroseus registered as accession OL711963

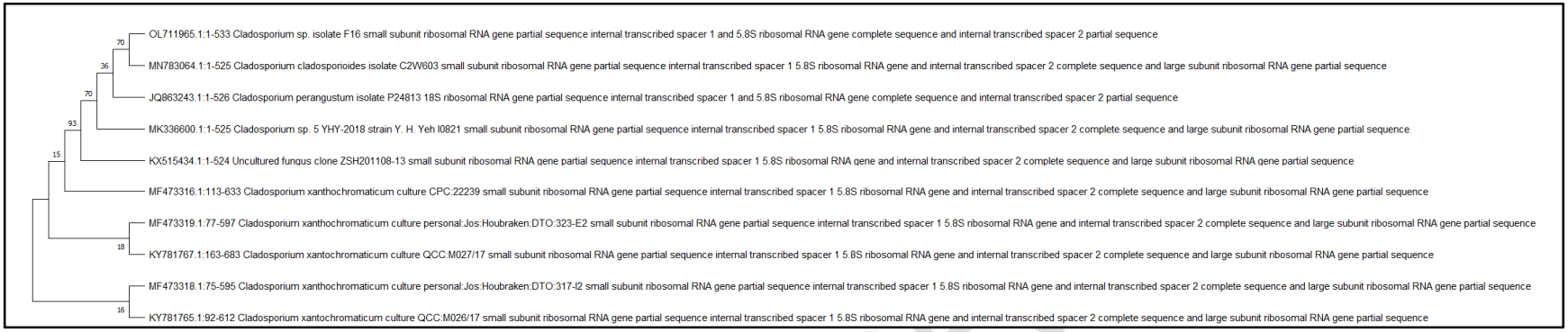

Figure 3Phylogenetic analysis of Cladosporiumcladosporioides registered as accession OL711965

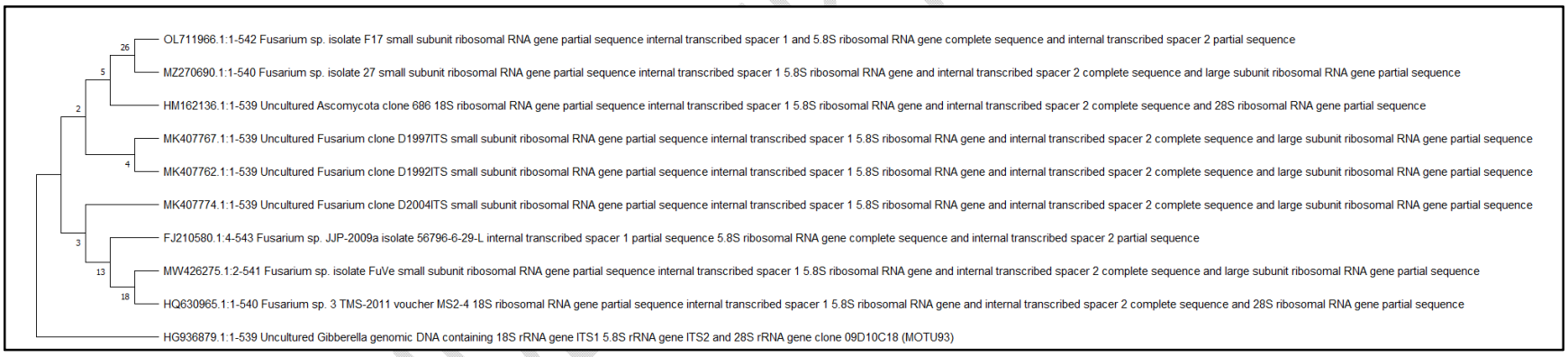

Figure 4Phylogenetic analysis of Fusarium sp. registered as OL711966

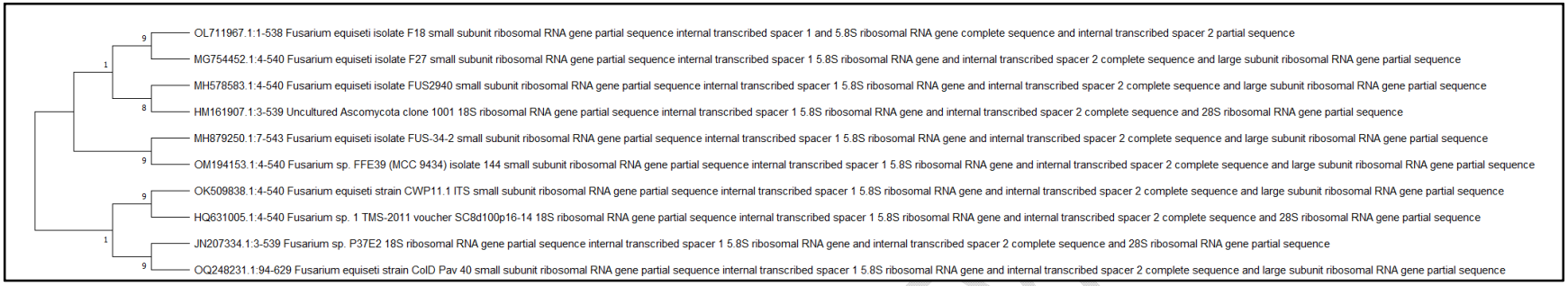

Figure 5Phylogenetic analysis of Fusariumequiseti registered as OL711967

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