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Identification of a fungus contaminating orchid tissue culture media from Darjeeling

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Plant tissue culture is an artificial plant propagation technique employed for large-scale plant multiplication.In addition to other limitations, the most vulnerable effect of tissue culture is the contamination of culture media by endophytes and other microorganisms.Contaminated orchid tissue culture media were collected from the Plant Tissue Culture Laboratory of the Darjeeling Government College.After subculturing in PDA, a pure fungal culture was obtained.The conidia of isolated fungus were catenating in densely branched chains of ellipsoid, ovoid, limoniform, aseptate, smooth, and conspicuous hila.Molecular characterization of the isolated fungus was performed by extracting amplification of specific genes such as partial 5.8s r RNA, ITS, and partial 18s r RNA using universal primers ITS1 and ITS4.The amplified product was then sequenced bidirectionally, and the 560-nucleotide sequence was submitted to GenBank after alignment and annotation.During BLAST analysis, the sequence displayed 99.82% similarity and 100% query coverage with *Cladosporium-tenuissimum*.Phylogenetic analysis revealed that this fungus shares a close relationship with the same pathogen isolated from the rich rhizospheric soil of India. This is the first report on the biological and molecular identification of *Cladosporium* sp. from orchid tissue culture media in India.

Keywords : *Cladosporium* sp., molecular identification,orchid tissue culture media

INTRODUCTION

Plant tissue culture, the cultivation of plant cells, tissues, organs, seeds, and other plant parts on a nutrient medium under sterile conditions, has played a pivotal role in advancing agricultural methods worldwide. Over the past few decades, it has been instrumental in rapidly multiplying various crops and in enhancing production systems (Zulkarnain *et al*. 2015).

However, plant tissue cultures are often compromised by microorganisms introduced either through the explant or by laboratory contamination. These contaminants, whether biological or chemical, visible or invisible, and harmful or seemingly harmless, invariably undermine the efficacy of tissue culture (Varghese and Joy, 2016).Bekalu *et al.* (2023) identified several factors that limit the success of tissue cultures.These include the source of plant material, which may introduce contaminants from both internal (endophytic microorganisms) and external (epiphytic microbes) origins, as well as the potential for microscopic organisms to enter culture media, culture bottles, less sterile equipment, workspaces, and cultural spaces. Human errors during the tissue culture process and increased contamination during wet months further exacerbate these issues. Microorganism contamination poses a serious challenge (Omamor *et al*. 2007). Microorganisms infiltrate living plant tissues, and the nutrient-rich medium used in tissue culture provides an ideal environment for microbial growth.These microorganisms compete with plant tissue cultures for essential nutrients, leading to irregular growth, tissue necrosis, decreased shoot proliferation, reduced root development, and ultimately higher culture mortality (Kane 2003; Odutayo *et al*. 2007; Cassells, 2012).

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A variety of microorganisms, including filamentous fungi, bacteria, yeast, viruses, and microarthropods that act as vectors, are recognized sources of contamination in plant tissue cultures (Leifert and Cassells, 2001).Notable bacterial contaminants include *Escherichia coli*, *Proteus* sp., *Pseudomonas fluorescens*, *Micrococcus* sp., *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus cereus*, *Bacillus subtilis*, *Corynebacterium* sp., and *Erwinia*sp.. Contamination sources include tissue culture plant materials and the laboratory environment, such as human skin (Varghese and Joy, 2016; Odutayo *et al*. 2007). However, fungi are the predominant source of contamination in tissue cultures, compared to bacteria, viruses, and other microorganisms (Niehues *et al*. 2020). Yeasts, such as *Candida* and *Rhodotorula*, are also common contaminants in plant tissue cultures and can proliferate on indexing media used to detect latent bacteria.

Fungal contaminants identified in plant tissue cultures include *Alternaria tenuis*, *Aspergillus fumigatus*, *Cladosporium* sp., *Aspergillus niger*, *Saccharomyces* sp., *Rhizopus nigricans*, *Fusarium oxysporum*, and *Fusarium culmorum* (Odutayo*et al*. 2007). *Chrysosporium* sp. and *Aspergillus* sp. have been identified as fungal contaminants during the early stages of micropropagation of tissue-cultured abaca, leading to cell death owing to poor plant material management, culture vessel contamination, and suboptimal laboratory conditions (Cobrado and Fernandez, 2016). Fungal isolates like *Aspergillus*, *Penicillium*, *Cladosporium*, *Fusarium*, and *Cunninghamella* sp. have been isolated from various tissue culture laboratories (Odutayo*et al*. 2007; Msogoya*et al*. 2012; Ankur *et al*. 2014; Kithaku*et al*. 2019).

The genus *Cladosporium* belongs to *Ascomycota*, *Pezizomycotina*, *Dothide-omycetes*, *Pleosporomycetidae*, *Capnodiales*, and *Cladosporiaceae* (Jayawardena *et al*.2020; Bensch *et al*. 2012) and is commonly found in molds in various environments worldwide. *Cladosporium* has been reported as a tissue culture contaminant in several laboratories, causing damage to plants during their early stages (Kumar *et al*.2019; Odutayo*et al*.2007; Kithaku*et al*. 2019). In addition to being a laboratory contaminant, *Cladosporium* is known for its pathogenic properties, causing diseases in the leaves, flowers, and roots (Geng *et al*. 2022; Nam *et al*. 2015; Heo *et al*. 2023; Cui *et al*. 2023; Liu *et al*. 2022; Krasnow *et al*. 2022; Ragukul and Makander, 2023). Some species within this genus are plant pathogens, whereas others are spoilage agents in food or industrial products. *Cladosporium* has also been associated with endophytic fungi and asthma (Heuchert *et al*. 2005). *Cladosporium cladosporioides* and *Cladosporium pseudocladosporioides* have been identified as strong antagonists of *Puccinia horiana*, the pathogen responsible for chrysanthemum white rust (Torres *et al*. 2017). The genus *Cladosporium* is primarily composed of three species based on morphology: *C.cladosporioides*, *C. sphaerospermum*, and *C. herbarum* (El-Dawyet al. 2021). Two new species, *Cladosporium clitoriae* Haldar and *Cladosporium murshidabadense* Haldar, have recently been reported in West Bengal, India (Haldar, 2019).

Morphological and molecular characterization are essential for the accurate identification of fungi, including *Cladosporium* (Dugan *et al*. 2008). Molecular techniques have been successfully employed to identify and diagnose species, such as *Fusarium* spp., *Aspergillus* spp., and *Rhizoctonia solani* (Salem *et al*. 2019; Abedalred*et al*. 2019). One such molecular approach used to diagnose *Cladosporium* species is PCR amplification of the internal transcribed spacer (ITS) region, a technique known as the universal barcode of fungi.This method is effective for identifying different *Cladosporium* species (Hensel *et al*. 2020; Han *et al*. 2020).

ITS sequences are commonly used to identify fungi and to construct phylogenetic trees (Salem *etal*. 2019; Ao *et al*. 2020). *Cladosporium* isolates were identified at the species level using ITS sequencing and morphologicalanalysis (Abedy*et al*. 2021). According to Mng'omba*et al*. (2007), surface sterilization only removes external microbial contamination, whereas endogenous contaminants can thrive in nutrient-rich media.Endophytic contaminants can be effectively managed if identified early and

systemic fungicides are correctly applied before explants are collected (Habiba *et al*. 2002).

In this study, contaminated orchid tissue culture medium was collected from the plant tissue culture laboratory of Darjeeling Government College, and contaminating fungi were identified using both morphological and molecular methods.

MATERIALS AND METHODS

Sample Collection and Fungus Isolation

Contaminated tissue culture containers were collected from the Tissue Culture Lab of Darjeeling Government College in July, September, and October of 2023.The number of infected bottles was calculated by counting the number of contaminated bottles. Several samples were collected separately and labeled appropriately. The bottles were brought to the Mycopathology Laboratory, Department of Botany, Darjeeling Government College.The contaminated bottles were then opened in a sterile environment. Using a heat-sterilized loop, a small sample from the contaminated area was aseptically transferred to Potato Dextrose Agar slants in triplicate. The tubes were incubated at 28°C for 5 days.

Identification of Isolated Fungal Pathogens

The isolated fungal pathogens were identified based on phenotypic characteristics, colony features, microscopic analyses, and phylogenetic characterization using rRNA gene sequences obtained through PCR amplification.

Studies on Phenotype

Phenotypic investigations involved analyzing the morphology of conidial spores, hyphae, and colonies of the fungus.The cellular morphology was examined under a microscope.

Colony Characteristics

The fungus was grown on sterile PDA plates (9 cm diameter) at 28°C for 3–4 days to allow the colonies to form mycelial mats.Mycelial agar discs (4 mm) were cut from the radially spreading zone of the developing hyphae using a sterile cork borer.Each disc was positioned at the center of a sterile PDA plate.The plates were incubated at 28°C for ten days to ensure sufficient sporulation.Numerous culture characteristics were routinely monitored and recorded, including the number, color, and texture of spores; pattern of radial development; and color and texture of the mycelia.

Microscopy

Mycelia from the cultured fungal strains that produced spores were placed on grease-free glass slides and stained with cotton blue in lactophenol. The slides were mounted with cover glass, sealed, and examined using an Olympus compound microscope from India at 10X, 40X, and 100X (with oil immersion). The specific morphological characteristics of the fungus, including hyphal septation and spore type and shape, were examined and recorded. Measurements were also taken of spore length and width, as well as mycelial breadth.

Studies on Ribosomal RNA Genes (rRNA Gene)

The isolated fungus was identified by phylogenetic analysis using gene sequences containing the internal transcribed spacer (ITS) region and the entire 5.8S rRNA gene.This region, which does not contribute to the final ribosomal RNA, is located between the 18S and 5.8S rRNA genes (also known as ITS1) and 5.8S and 28S rRNA genes (also known as ITS2).Using genomic DNA as a template, the target genes were amplified by PCR to obtain sequences.The universal forward and reverse primers employed were ITS1 and ITS4, which bind to the end of the 18S rRNA gene and the beginning of the 28S rRNA gene, respectively (White *et al*. 1990).

Isolation and PCR Amplification of Genomic DNA

Genomic DNA was isolated from fungal cultures using the CTAB method (Dellaporta et al. 1983) as modified by Sharma et al. (2003). A UV-VIS spectrophotometer (Model No.Lasany LI, 285) was used to calculate the A260/A280 ratio to

determine the purity of the DNA. PCR amplification of the ITS regions, including the 5.8S, 28S, and 18S rRNA genes, was conducted using universal primers ITS1 (5'-TCCGTAGGT-GAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). 25 ìl reaction volume was amplified by 20 ng of genomic DNA from each isolate, 3 U Taq DNA polymerase, 2.5 mM MgCl, , dNTPs (2.5 mM each), and 1 ìM of the forward and reverse primers in 10X Taq buffer B (Genei, Bangalore). After combining all materials, DNA polymerase was added.The PCR products were purified using a Quick PCR Purification Kit (Genei, Bangalore) according to the manufacturer's instructions.Purified amplicons were sequenced in both directions at Xcelris Genomics Ltd., Bangalore.

Phylogenetic analysis for species discrimination

Partial ribosomal RNA gene sequences of pathogenic fungal strains were compared to those of similar strains in the GenBank database using the BLAST search facility at the NCBI (http://www.ncbi.nlm.nih.gov/) (Altschul *et al*. 1997). Cladosporium sp. was identified as the fungal strain (Acc. No. OR528898) using rRNA gene sequences delivered to GenBank with appropriate annotations.Their sequences were differentiated from the others for sequence distinctiveness and phylogenetic relationships.Reference ribosomal RNA (rRNA) sequences of 108 Cladosporium species were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/).For phylogenetic analysis, the Cladosporium sp. rRNA sequences acquired in this study were combined with other accessible sequences.ClustalW was used to align the sequences (Thompson *et al*. 1994). Sequences with possible contamination and unconfirmed species assignments were not included in the analysis.A total of 85 Cladosporium rRNA sequences were used for the analysis, and similar reference sequences were excluded. Using the Markov Chain Monte Carlo simulations (MCMC) technique for 1,000,000 generations with four cumulative hot chains beginning from random trees and sampling one every 100 generations, Bayesian phylogenetic inference was performed using MrBayes 3.12 (Ronquist and Huelsenbeck, 2003). As a burn-in, the first 25% of the rebuilt trees were removed (Zhang et al. 2018).

Estimation of genetic distance

Percent genetic distance was computed using the Kimura-2-Parameter model (Kimura 1980) and MEGA X (Kumar *et al*. 2019). Based on BI analysis, the cladosporium rRNA sequences were grouped into MEGA X cryptic species. The threshold value in species differentiation was established by computing the mean of the greatest "within species" genetic distance and noticeably smaller "between species" genetic distance of several *Cladosporium* species.

RESULTS AND DISCUSSION

Contamination is a significant problem in tissue cultures.An increase in contamination was noted during July-September, the monsoon season.Contaminated tissue culture bottles observed in the lab of Darjeeling Government College are presented (Fig.1). The medium appeared cloudy, with a whitish film, fuzzy growth, or patches resembling mold on the surface of the medium or along the bottle walls.Gas bubbles are also observed.As the contamination progressed in plant tissue culture, there was a deterioration in the appearance and growth of the plants due to contaminants competing for nutrients, resulting in reduced plant growth.Portions of the plant culture were distorted, dried, and exhibited stunted growth.

From the survey results, it was evident that during July, the risk of contamination in tissue culture tended to be higher than that in other months.

Morphological Characterizations of the Fungal Isolates

The growth and sporulation of the fungal isolates were observed after growth on PDA, and the morphology was examined under a microscope.The colonies of the isolates were gray to olivaceous green with cottony mycelium (Fig. 2), while the reverse side of the colony was greenish-black with regular colony margins.The growth was circular, with radial advancements. Radial growth rings were evident on mycelial mats.Microscopic examination of the fungus revealed that its mycelia and conidia were shiny black to brown. Sporulation was observed from

Fig. 1: (A) Orchid seedlings growing in tissue culture media. (B) Contaminated media with some hyphal growth collected from Tissue Culture Laboratory, Dept. of Botany, Darjeeling Govt. College

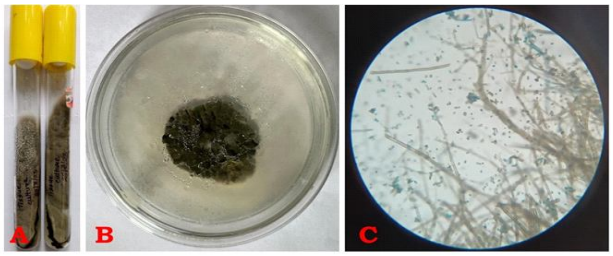

Fig. 2: Pure culture of the fungal contaminant isolated from tissue media after 7 days of incubation on PDA media (A= Slant and B= Plate culture). C- Microscopic photograph of fungus showing mycelia and conidia.

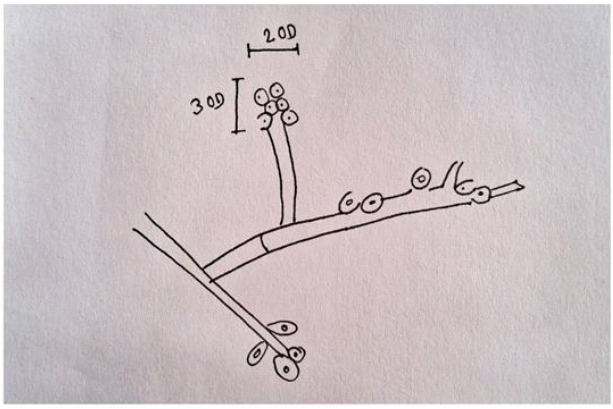

Fig.3: Camera lucida drawing of mycelia of isolated fungus

the 8th day onwards. Conidia were catenating in densely branched chains, ellipsoid, ovoid, limoniform, aseptate, 5.74±0.19 µm in length and 5.16±0.12 µm in breadth, smooth, and with conspicuous hila. They were easily detached from the conidia chain (Fig.3).

Molecular Identification of the fungus

Approximately 500 nt of DNA was amplified from the isolated fungal pathogens after successful PCR amplification using the extracted DNA.Alignment of the obtained rRNA sequences with those of the strains from the GenBank database was performed using the NCBI BLAST

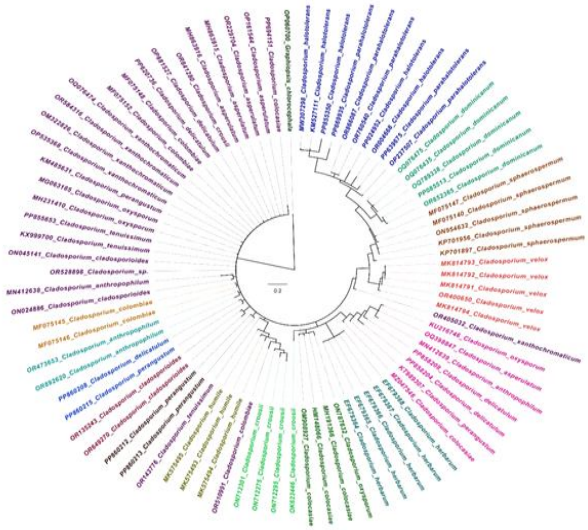

Fig. 4: Bayesian phylogenetic analysis of *Cladosporium* species generated using MrBayes 3.12, with Markov chain Monte Carlo simulations for 1,000,000 generations. GenBank accession numbers and species names are indicated at the end of each branch.

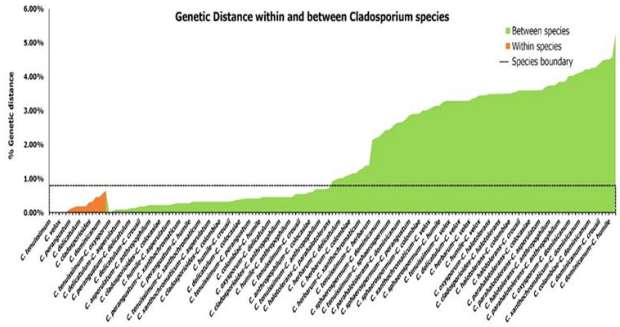

Fig.5: Percent genetic distance within and between different species of *Cladosporium*, with species boundary at 0.8% indicated by "—."

tool for each fungal isolate.Isolated TC-O2 (Acc. No. OR528898) showed 99.82% nucleotide (nt) identity with *Cladosporiumtenuissimum*.

Phylogeny of Cladosporium species

As presented in the study (Fig. 4), phylogenetic analysis of *Cladosporium* species provides a detailed picture of the evolutionary relationships among different species.The isolate showed the highest similarity (99.82%) with *Cladosporium tenuissimum* based on BLASTn analysis. However, rather than forming a distinct cluster with only *C. tenuissimum*, it was grouped with several related *Cladosporium* species.This indicates that these species share a close evolutionary relationship, making it challenging to delineate them based solely on the genetic data.

The phylogenetic tree includes several well-defined clusters such as *C. dominicanum*, *C. sphaerospermum*, *C. velox*, *C. herbarum*, and *C. perangustum*, indicating clear genetic differentiation from other species. *C. halotolerans* and *C. parahalotolerans* form a tight cluster together, reflecting their close genetic relationship and potentially similar ecological niches or physiological traits.

Genetic distance data (Fig. 5) supported these clusters.For example, within *C. tenuissimum*, the genetic distance was 0.00%, indicating no genetic variation within this species. The same applies to other species such as *C. oxysporum*, *C. herbarum*, and *C. velox*, all showing 0.00% genetic distance within species. However, interspecies genetic distances reveal why some species cluster together.For example, the genetic distance between *C. tenuissimum* and *C. oxysporum* is 0.00%, suggesting that these two species are genetically similar.Similarly, *C. halotolerans* and *C. parahalotolerans* had a genetic distance of 0.72%, indicating a close relationship.

The unnatural clustering of the isolate under study can be explained by the observation that the genetic distances between some species were less than the highest genetic distance within a species.For example, the genetic distance between *C. perangustum* and *C. asperulatum* was 0.05%, which is less than the distance within a single species.This suggests that traditional species boundaries might not fully capture the genetic diversity of *Cladosporium* spp.A proposed species boundary at 0.8% genetic distance helps clarify these relationships.Species with genetic distances greater than this threshold were considered separate, whereas those with smaller distances were grouped.This threshold helps explain why some species form distinct clusters, while others, such as the isolate under study, form mixed clusters with closely related species.The clustering patterns observed in the phylogenetic tree were supported by genetic distances, highlighting clear species boundaries and areas where genetic similarities blur traditional species definitions.

Increased humidity and moisture create favorable conditions for microbial growth. Several contaminated tissue culture bottles were collected from the laboratory and brought to the laboratory for pathogen isolation. Indoor air, tables/walls, and human skin are examples of sources of fungal contaminants, and among all airborne living contaminants, fungi make up two-thirds. *Penicillium*, *Cladosporium*, *Alternaria*, and *Aspergillus* are the most common indoor fungi, and furniture in tissue culture laboratories is a major source of fungal spores (Odutayo*et al*. 2007). Anything containing moisture in the tissue culture laboratory is a source of fungal contamination. A relative humidity of 60% is sufficient for fungal growth, and drain pans/drip pans under cooling coils (as in refrigerators) and steam from cooking are also considered sources of fungal growth.

Based on morphological characteristics, the isolate was preliminarily identified as *Cladosporium* sp. (Toresse*t al*. 2017). Such colony and conidial characteristics have been reported by several authors from different sources (Geng *et al*. 2022; Nam *et al*., 2015; Heo *et al*. 2023; Cui *et al*. 2023; Liu *et al*. 2022; Krasnow *et al*. 2022; Ragukul and Makander, 2023).

Cladosporium spp., a fungal tissue culture contaminant in southwestern Nigeria, was reported by Odutayo*et al*. (2007). Identification of the fungus was done using cultural characteristics and morphology and by comparison with standards. Liu *et al*. (2022) identified *Cladosporium* using ITS1 and ITS4 primers as the cause of fruit rot of grapes (*Vitis vinifera*) from China. Kumar *et al*. (2019) isolated *Cladosporium sphaerospermum* from tissue culture media of *Chlorophytum borivilianum*. *Cladosporium cladosporioides*, a causal pathogen of garden pea leaf blight, was identified by sequencing the ITS region using the ITS1 and ITS4 primers (Ragukul and Makandar, 2023).*Cladosporium* spp. and other fungi as major fungal contaminants of banana cultures in JKUAT laboratories have been reported by Kithaku*et al*. (2019). Identification was based on sequencing of the PCR-amplified product. *Cladosporium cladosporioides*, a pathogen of leaf spot disease of *Alalia cordata* in Korea, was reported based on ITS, Act, and TEF1 sequencing by Heo *et al*. (2023).

Owing to the desire to grow on a large scale in the past few decades, tissue culture techniques have been manipulated to improve plant growth, biological activities, transformation, and secondary metabolite production.For successful propagation,contamination free culture media is first foremost desirable. In present study identification of fungus from tissue culture media will help to take appropriate precaution measures to check fungal contamination.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

REFERENCES

Abedalred, E. M., Ismail, W. M. H., Abdulmoohsin, R. G., Al-Karhi, M. A. J. 2019. First molecular identification of *Fusarium fujikuroi* causing pollen rot of palm trees (*Phoenix dactylifera* L.) in Iraq and evaluation efficacy of some nanoparticles against it. *IOP Conf Ser Earth Environ Sci*. **388**:012007.https://doi.org/10.1088/1755-1315/388/1/012007

Abedy, A. N. A., Musawi, B. H. A., Isawi, H. I. N. A., Abdalmoohsin, R. G. 2021. Morphological and molecular identification of *Cladosporium sphaerospermum* isolates collected from tomato plant residues. *Braz J Biol*. **9**: 82. https://doi.org/10.1590/1519-6984.237428

Altschul, S. F. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*. **25**:3389-3402. https://doi.org/10.1093/nar/25.17.3389

Ankur, V., Meena, B., Harsh, N. 2014. Identification and bioassay of fungal contaminants observed during in vitro propagation of *Saracaasoca* (Roxb.) De Wilde. *Biotechnol Soc*. **7**:69-76.

Ao, T., Deb, C. R., Rao, S. R. 2019. Molecular strategies for identification and characterization of some wild edible mushrooms of Nagaland, India. *Mol. Biol. Rep*.**47**:621-630.https://doi.org/10.1007/s11033-019-05170-2.

Bensch, K., Braun, U., Groenewald, J. Z., Crous, P. W. 2012. The genus *Cladosporium*. *Stud Mycol*. **72**:1-401.https://doi.org/10.3114/sim0003.

Bekalu, Z. E., Panting, M., Bæksted, H. I., Brinch-Pedersen, H. 2023. Opportunities and challenges of in vitro tissue culture systems in the era of crop genome editing. *Int. J. Mol. Sci.***24**:11920. https://doi.org/10.3390/ijms241511920.

Cassells, A. C. 2012. Pathogen and biological contamination management in plant tissue culture: Phytopathogens, vitro pathogens, and vitro pests. In: *Plant Cell Culture Protocols*. p. 57-80.https://doi.org/10.1007/978-1-61779-818-4_6.

Cobrado, J., Fernandez, A. 2016. Common fungi contamination affecting tissue-cultured abaca (*Musa textiles Nee*) during initial stage of micropropagation. *Asian Res. J. Agric*.**1**:1-7. https://doi.org/10.9734/arja/2016/28353

Cui, Q., Lyu, H., He, L., Chi, M., Wang, W., Xie, C., et al. 2023. First report of *Cladosporium cladosporioides* causing leaf blight on *Sambucus chinensis* in China. *PlantDis*. **107**:950. https://doi.org/10.1094/pdis-05-22-1030-pdn.

Dellaporta, S. L, Wood, J., Hicks, J. B. 1983. A plant DNA minipreparation: Version II. *Plant Mol. Biol. Rep*.**1**:19-21.https://doi.org/10.1007/bf02712670.

Dugan, F. M., Braun, U., Groenewald, J. Z., Crous, P. W. 2008. Morphological plasticity in *Cladosporium sphaerospermum*. *Persoonia*. 21:9-16.https://doi.org/10.3767/003158508x334389.

El-Dawy,E., Gherbawy, Y. A., Hussein, M. A. 2021. Morphological, molecular characterization, plant pathogenicity and biocontrol of *Cladosporium* complex groups associated with faba beans. *Sci.Rep*. **11**: 14183.https://doi.org/10.1038/s41598-021-93123-w

Geng, Y., Wang, J. G., Zhang, J. L., Chen, G., Yu, J. H., Li, Y. T, et al. 2022. First report of *Cladosporium tenuissimum* causing leaf spot of *Arachis hypogaea* in China. *PlantDis*.**106**:2754.https://doi.org/10.1094/pdis-11-21-2461-pdn

Habiba, U., Reja, S., Saha, M. L., Khan, M. R. 2002.Endogenous bacterial contamination during in vitro culture of table banana: identification and prevention. *PlantTissueCult*. **12**:117-124.

Haldar, D. 2019. Two new *Cladosporium* species from Murshidabad District, West Bengal, India. *IntJ.Curr.Re.sRev*. **11**:10-13.https://doi.org/10.31782/ijcrr.2019.1013.

Han, X., Dai, Y., Hu, M., Xiao, R., Zhang, S., Li, J., Wang, Z., Hu, X., Gu, Z. Investigation of multiple pathogens in black-spotted *Dendrobium officinale* based on culture-independent and dependent methods. *J. Biobased Mater Bioenergy*.14:249-257.https://doi.org/10.1166/jbmb.2020.1959

Hensel, M., Meason-Smith, C., Plumlee, Q. D., Myers, A. N., Coleman, M. C., Lawhon, S., Rodrigues H. A, Rech, R. R. 2019. Retrospective analysis of aetiological agents associated with pulmonary mycosis secondary to enteric salmonellosis in six horses by panfungalpolymerase chain reaction. *J. Comp.Pathol*. **174**:1-7.https://doi.org/10.1016/j.jcpa.2019.10.002

Heo, Y., Kim, J., Hong, S. B., Jung, C. R., Jeon, Y. 2023. First report of *Cladosporium cladosporioides* causing leaf spot of *Aralia cordata* var. *continentalis* in Korea. *Plant Dis*.**107**:3282.https://doi.org/10.1094/pdis-02-23-0251-pdn

Heuchert, B., Braun, U., Schubert, K. 2005.Morphotaxonomic revision of fungicolous*Cladosporium* species (hyphomycetes). *Schlechtendalia***13**:1-78.

Jayawardena, R. S., Hyde, K. D, Chen, Y. J., Papp, V., Palla, B., Papp, D., et al. 2020. One stop shop IV: taxonomic update with molecular phylogeny for important phytopathogenic genera: 76–100 (2020). *FungalDivers*. **103**:87-218.https://doi.org/10.1007/s13225-020-00460-8

Kane, M.,2003. Bacterial and fungal indexing of tissue cultures. *J. Allergy Immunol.***94**:393-400.

Kithaku, E. M., Muigai, A. W., Neondo, J. O., Mweu, C. 2019. Screening of fungal contaminants in banana tissue cultures in Jkuat, Kenya. *Afr. J Microbiol Res*. **13**:675-688.https://doi.org/10.5897/AJMR2019.9182

Kimura, M. A. 1980. simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*.**16**:111-120.https://doi.org/10.1007/BF01731581

Krasnow. C., Raphael, G., Ziv, C. 2022. First report of fruit rot of sweet pepper caused by *Cladosporium cladosporioides* in Israel. *PlantDis*. **106**:2533.https://doi.org/10.1094/pdis-12-21-2755-pdn

Kumar, S., Yadav, A. K., Prabha, C. 2019. Microbial contamination in tissue culture of *Chlorophytum borivilianum*, a rare medicinal herb. *J. Plant Pathol.***101**:991-995.

Leifert, C., Cassells, A. C. 2001. Microbial hazards in plant tissue and cell cultures. I*n Vitro Cell Dev. Biol. Plant*.**37**:133-138.https://doi.org/10.1007/s11627-001-0025-y.

Liu. Z., Jiao, R., Chen, S., Ren, Y., Zhang, L., Zhang, D., et al. 2022. First report of fruit rot of grapes (*Vitis vinifera*) caused by *Cladosporium cladosporioides* in Xinjiang, China. *PlantDis*.**106**:315.https://doi.org/10.1094/pdis-01-21-0080-pdn

Mng'omba, S. A., du Toit, E. S., Akinnifesi, F. K., Venter, H. M. 2007. Effective preconditioning methods for in vitro propagation of *Uapacakirkiana*Müell Arg. tree species. *Afr. J.Biotechnol*. **6**: 1670-1676.

Msogoya, T., Kanyagha, H., Mutigitu, J., Kulebelwa, M., Mamiro, D. 2012. Identification and management of microbial contaminants of banana in vitro cultures. *J.Appl.Biosci*. **55**:3987-3994.

Nam, M. H., Park, M. S., Kim, H. S., Kim, T. I., Kim, H. G. 2015.*Cladosporium cladosporioides* and *C. tenuissimum* cause blossom blight in strawberry in Korea. *Mycobiology*. **43**:354-359. https://doi.org/10.5941/myco.2015.43.3.354.

Niehues, H., Jansen, P. A. M., Rodijk-Olthuis, D., Rikken, G., Smits, J. P. H., Schalkwijk, J., Zeeuwen, P. L. J. M., van den Bogaard, E. H. J. 2020. Know your enemy: Unexpected, pervasive and persistent viral and bacterial contamination of primary cell cultures. *Exp.Dermatol.***29**:672-676.https://doi.org/10.1111/exd.14126

Odutayo, O. I., Amusa, N. A., Okutade, O. O., Ogunsanwo, Y. R. 2007.Sources of microbial contamination in tissue culture laboratories in southwestern Nigeria. *Afr*. J.*Agric.Res*.**2**:67-72.

Omamor, I. B., Asemota, A. O., Eke, C. R., Eziashi, E. I. 2007.Fungal contaminants of the oil palm tissue culture in Nigerian Institute for Oil Palm Research (NIFOR). *Afr.J.Agric.Res*.**2**:534-537.

Ragukula, K., Makandar, R. 2023.*Cladosporium cladosporioides* causes leaf blight on garden pea in Telangana, India. *PlantDis*. **107**:2239.https://doi.org/10.1094/pdis-09-22-2175-pdn.

Ronquist, F., Huelsenbeck, J. P. 2003.MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics***19**:1572-1574.https://doi.org/10.1093/bioinformatics/btg180

Salem, M. Z. M., Behiry, S. I., El-Hefny, M. 2019. Inhibition of *Fusarium culmorum*, *Penicillium chrysogenum* and *Rhizoctonia solani* by n-hexane extracts of three plant species as a wood-treated oil fungicide. *J.Appl.Microbiol*. **126**:1683-1699.https://doi.org/10.1111/jam.14256

Sharma, R., Mahla, H. R., Mohapatra, T., Bhargava, S. C., Sharma, M. M. 2003.Isolating plant genomic DNA without liquid nitrogen. *PlantMol.BiolRep*. **21**:43-50.https://doi.org/10.1007/bf02773395

Thompson, J. D., Higgins, D. G., Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *NucleicAcidsRes*. **22**:4673-4680.https://doi.org/10.1093/nar/22.22.4673

Torres, D. E., Rojas-Martínez, R. I., Zavaleta-Mejía, E., Guevara-Fefer, P., Márquez-Guzmán, G. J., Pérez-Martínez, C. 2017.*Cladosporium cladosporioides* and *Cladosporium pseudocladosporioides* as potential new fungal antagonists of *Puccinia horiana* Henn., the causal agent of chrysanthemum white rust. *PLoSOne*.**12**. https://doi.org/10.1371/journal.pone.0170782

Varghese, N., Joy, P. P. 2016. Plant tissue culture contaminants identification and its response to fumigation. *Biotechnol.Adv*. **22**:32-36.

White, T. J., Bruns, T. D., Lee, S. B., Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols*(Eds. M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J.White) p. 315-322.https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Zhang, S., Yang, Y., Li, J., Qin, J., Zhang, W., Huang, W., Hu, H. 2018. Physiological diversity of orchids. *PlantDivers*. **40**:196-208.https://doi.org/10.1016/j.pld.2018.06.003

Zulkarnain, Z., Tapingkae, T., Taji, A. 2015. Applications of in vitro techniques in plant breeding. In: *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools*.p. 293-328.https://doi.org/10.1007/978-3-319-22521-0_10