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Research Article

Morpho-Molecular Identification of Plant Pathogen Infecting Bulb Onion (*Allium cepa* L.) Production Under Protected Cultivation

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ABSTRACT

This study was conducted to identify the fungal pathogen associated with bulb onion production under protected cultivation through combined morphological and molecular identification. Onion leaves showing yellowing, wilting, and purple-to-black lesions were collected from infected plants and subjected to fungal isolation, morphological examination, pathogenicity testing, and molecular analysis. Based on colony and spore characteristics, together with the results of pathogenicity test, the pathogen was identified as *Alternaria porri,* which is the causal agent of purple blotch disease, fulfilling Koch's postulates. However, molecular identification based on Internal Transcribed Spacer (ITS) ribosomal DNA sequencing revealed high similarity (98.58–98.79%) to *Nigrospora sphaerica*. This difference may be attributed to the faster growth rate and dominance of *N. sphaerica* in vitro, which likely overshadowed the slower-growing *A. porri* during DNA amplification and sequencing. Phylogenetic analysis additionally supported the clustering of isolates within the *N. sphaerica* clade, distinct from *A. porri*. The contrasting morphological and molecular findings show the occurrence of mixed fungal infections, where *A. porri* acts as the primary pathogen, and *N. sphaerica* functions as a secondary colonizer or opportunistic fungus. These results show the limitations of single-method identification and point out the need to integrate morphological, pathogenicity, and molecular approaches for accurate pathogen diagnosis in complex disease systems.

Keywords: *Alternaria porri, ITS rDNA, Mixed fungal infection, Nigrospora sphaerica, Purple blotch*

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Introduction

Onion (Allium *cepa* L.) is one of the most widely cultivated vegetable bulb crops due to its importance in daily food preparation and its nutritional and economic value. Although important, onion production is frequently constrained by several fungal diseases that reduce both yield and bulb quality, especially under environmental conditions favorable for disease development. Among these diseases, purple blotch caused by *Alternaria porri* is considered one of the major foliar diseases affecting onion production and has been reported to cause serious economic losses in many onion-growing areas (Schwartz & Mohan, 2008; Meena et al., 2017).

Identification of fungal pathogens has conventionally relied on morphological attributes such as colony appearance, growth pattern, and spore morphology. Although these methods remain useful, identification based solely on morphology can sometimes be difficult because several fungal species display similar cultural and microscopic characteristics. In addition, fungal morphology may vary with culture conditions and growth stage (Barnett & Hunter, 1999; Simmons, 2007). Plant diseases are also now recognized as more complex than formerly understood, as infected plant tissues may harbor not only the primary pathogen but also secondary or opportunistic fungi that colonize weakened tissues (Agrios, 2005).

With advances in molecular biology, DNA-based techniques have become important tools for fungal identification. Sequencing of the internal transcribed spacer (ITS) region is commonly used because it provides reliable and reproducible results for many fungal species (Schoch et al., 2012). However, molecular identification based on a single gene region and culture-derived isolates may still present limitations. Fast-growing fungi can dominate culture plates and interfere with DNA amplification, making it difficult to detect slower-growing pathogens in mixed infections (Dhingra & Sinclair, 1995).

Under protected cultivation systems such as screenhouses and greenhouse-like structures, environmental conditions, including high humidity, fluctuating temperatures, and limited air movement, may favor the development of fungal diseases and increase the possibility of mixed infections. Despite this, only limited information is available regarding the combined use of morphological, pathogenicity, and molecular approaches in identifying fungal pathogens associated with onion diseases under protected cultivation.

Hence, this study was conducted to identify the fungal pathogen associated with bulb onion production under protected cultivation using an integrated morpho-molecular approach. The study expressly aimed to compare results from morphological characterization, pathogenicity testing, and molecular analysis to clarify pathogen identity and better understand the potential occurrence of mixed fungal infections associated with purple blotch disease in onion.

Materials and Methods

Plant Sample Collection

Plant samples exhibiting symptoms of yellowing and wilting, with white and purple to black spot lesions on the leaves, were collected from the leaves of bulb onions (cv. Yellow Granex) planted at the ACIAR project site of the Department of Horticulture, Visayas State University. Sampling was conducted randomly across the entire field. The collected samples were enclosed in a brown paper bag and transported to the Plant Disease Diagnostic Laboratory (PDDL) for further analysis.

Fungal Isolation

Fungi were isolated from infected leaves of bulb onions. These tissues were cut, surface-sterilized in 70% ethanol for 1 minute, rinsed twice in sterile distilled water (SDW), air-dried on sterile filter papers, and placed onto potato dextrose agar (PDA) medium amended with chloramphenicol (Fig. 1). Cultures were incubated at room temperature for 7 days, and purification was performed on 2% water agar (WA) medium using the single-spore technique (Leslie & Summerell, 2006).

Figure 1. Fungal isolation of the samples collected at the ACIAR-GAP project site

Morphological Attributes Identification

The isolated fungi obtained from symptomatic onion leaf tissues were purified by single-spore isolation and sub-cultured on potato dextrose agar (PDA) to observe their morphological attributes, including macroconidia and microconidia. For microscopic observations, fungal cultures were prepared in lactophenol-cotton blue and examined under a microscope to assess conidiophore structure, septation, and other diagnostic traits. Microscopic photographs were captured using a digital camera.

Pathogenicity tests

Once purified, the isolate was grown on PDA culture medium to increase the inoculum. After seven days of growth, suspensions were prepared in sterile distilled water and adjusted to a concentration of 6×10^8 conidia mL^{-1}. Two-month-old seedlings were used, with 30 plants inoculated with the pathogen by spraying 2.5 mL of the suspension using an atomizer, while another 30 plants served as the control. The inoculated seedlings were placed inside a chamber in the Department of Pest and Management screenhouse and disinfected with 70% ethanol. Incubation was carried out at 28°C and 80% relative humidity, and symptoms were recorded daily for seven days. Once fungal symptoms became evident, 10 samples (0.5 cm^2 each) were taken from the infected tissues, disinfected with 2% sodium hypochlorite for 2 minutes, washed three times with sterile distilled water, and placed on PDA culture medium. The morphological aspects of the colonies and re-isolated fungi were compared with those of the first strains to verify Koch's postulates.

DNA Extraction and PCR Amplification

Representative pure fungal cultures obtained after single-spore isolation and grown on potato dextrose agar (PDA) for seven days were submitted to Kinnovette Analytical Laboratory Inc. (Quezon City, Philippines) for molecular identification. At Kinnovette, genomic DNA was extracted from actively growing fungal mycelia using a commercial DNA extraction kit following the manufacturer's protocol. Since molecular identification was performed from culture-derived isolates, fast-growing fungi associated with the infected tissues may have become dominant during in vitro culture prior to DNA extraction. The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using the universal fungal primers ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′).

DNA Sequencing and BLAST Analysis

The PCR-amplified ITS products were purified and sequenced by Kinnovette Analytical Laboratory Inc. using the same primer pair. The resulting chromatograms were examined and edited to produce clean consensus sequences using GeneStudio™ Professional software. The edited ITS sequences were compared with sequences in the NCBI GenBank database using BLASTn to identify the closest homologs. Sequence identification was based on percent identity, bit score, and E-value. Isolates showing ≥97% sequence identity were considered

conspecific or closely related to the reference species.

Phylogenetic analysis

The nucleotide sequences obtained from each isolate were compared with sequences in the NCBI GenBank database using BLASTn to confirm their identity. The consensus ITS sequences were then aligned with selected reference sequences retrieved from GenBank using ClustalW implemented in MEGA version 12.

Phylogenetic relationships among the isolates and reference sequences were inferred using the Maximum Likelihood (ML) method based on the Tamura–Nei substitution model (Tamura & Nei, 1993). The Tamura–Nei model was identified as the best-fit nucleotide substitution model according to the Bayesian Information Criterion (BIC) in MEGA.

The reliability of the inferred tree topology was assessed using 1,000 bootstrap replications. The final phylogenetic tree was generated and visualized using MEGA version 12, with branch lengths proportional to the number of nucleotide substitutions per site. Bootstrap values of 70% or higher were considered to indicate statistically well-supported clades.

Results and Discussion

Morphological identification of the fungal isolate

Field-collected onion plants exhibited typical symptoms of purple blotch disease, including white flecks, leaf yellowing and wilting, white to purple-black lesions, and chlorosis at the leaf tips (Fig. 2), consistent with observations reported by Miller and Lacy (1995), Suheri and Price (2001), and Black et al. (2012). From these symptomatic tissues, a single fungal isolate was obtained and subsequently purified by single-colony isolation on potato dextrose agar (PDA), assuring a pure culture for further morphological and molecular characterization.

Figure 2. Field symptoms observed in onion plants include white flecks, leaf yellowing, chlorosis, tip dieback, and elongated necrotic lesions on foliage

After seven days of incubation at room temperature, the fungal culture developed dense, cottony white mycelium that extended along the PDA slant (Fig. 3a), accompanied by slight yellowish discoloration of the underlying medium. As the colony matured, the central portion gradually turned grayish to slightly darkened, while the surface remained soft and fluffy. Based solely on cultural characteristics, was difficult to determine whether the isolate belonged to *Alternaria* or *Nigrospora*, as both genera can exhibit similar colony morphology at this stage. Microscopic examination of lactophenol cotton blue mounts, however, revealed septate, pigmented conidia borne singly or in short chains on septate conidiophores. The conidia were ellipsoid to clavate, with multiple transverse septa and occasional longitudinal septation (Fig. 3b). These structures are characteristic of *Alternaria* and are consistent with descriptions of species responsible for purple blotch of onion (Dar et al., 2020; Simmons, 2007; Woudenberg et al., 2014).

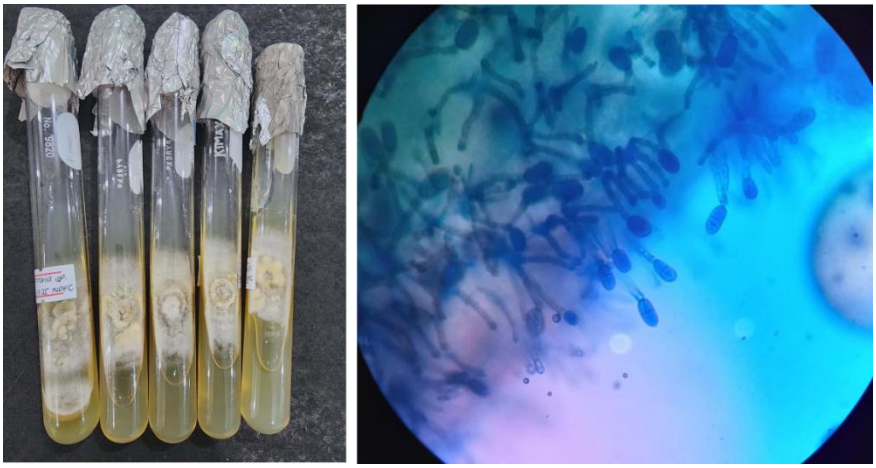

Figure 3. Cultural and microscopic morphology of the fungal isolate obtained from symptomatic onion leaves. (A) PDA slant cultures showing white-to-grayish, cottony colonies. (B) Microscopy of conidiophores and conidia exhibiting primary diagnostic traits of Alternaria porri, including obclavate conidia with several transverse septa

Pathogenicity Test

Such pathogenicity test confirmed that the fungal isolate obtained from infected leaves of bulb onion could induce disease symptoms similar to those observed in the field. Typical symptoms appeared 3 days after inoculation as described initial symptom, of small, water-soaked spots and leaf tip yellowing on the inoculated plants (Fig. 4). These lesions gradually enlarged and developed into elongated, black lesions with distinct concentric rings, extensive necrosis, and a surrounding chlorotic halo, which are characteristic of purple blotch disease.

Control plants sprayed with sterile distilled water remained healthy and symptomless throughout the observation period, confirming that the disease symptoms were specifically caused by the inoculated fungus.

Disease severity was assessed 7 days after inoculation, and the average severity rating was 4.5 on a 0–5 scale, indicating high virulence. The same fungal isolate was successfully re-isolated from artificially infected tissues and was found to be morphologically identical to the first culture, thereby satisfying Koch's postulates and confirming its pathogenicity.

Figure 4. Pathogenicity test showing symptom development on onion seedlings inoculated with the suspected Alternaria porri isolate.

Molecular identification based on ITS5 /ITS4 rDNA sequence data analysis

The internal transcribed spacer (ITS) region of the fungal isolate was successfully amplified using ITS5 and ITS4 primers, yielding an amplicon approximately 550–600 bp. The cleaned consensus sequence was subjected to BLASTn analysis relative to the NCBI GenBank database to determine species identity. BLAST results showed that the isolate exhibited 98.58–98.79% sequence similarity and 100% query coverage to multiple *Nigrospora sphaerica* reference sequences, all with E-values of 0.0, indicating highly significant matches. The highest-ranking hit corresponded to *N. sphaerica* isolate CTN13 (OK175859.1), which showed 98.79% identity to the query sequence. All of the top 100 BLAST hits were exclusively composed of *N. sphaerica* accessions, with no *Alternaria* species detected, thereby providing strong molecular evidence that the isolate belongs to *N. sphaerica*.

To additionally validate this identification, an expanded set of 27 closely related ITS reference sequences of *Nigrospora sphaerica* from GenBank was compiled for comparative assessment (Table 1). These included the highest BLAST matches—such as OK175859.1 (CTN13), OP596135.1 (CN136E2), MK333938.1 (PB-18), MF434826.1 (GDC1-2), PV938076.1 (GUCC25-0094), and KX256179.1 (Ns-12) as well as additional isolates representing broad ecological and geographic origins, such as MF400860.1 from *Adiantum philippense*, OR818025.1 from leaf-spot disease, MN215808.1 and KY019606.1 from fruit-host leaf blight, KJ767121.1 from calabash leaf spot, and several more including strains TS-53, NS-6, MANB1, HM2, and NG1. All reference sequences consistently clustered within the *N. sphaerica* lineage, demonstrating that the study isolate falls well within the known genetic diversity of the species.

The high percent identity values, complete query coverage, and exclusive BLAST alignment to *Nigrospora sphaerica* accessions provide robust and conclusive evidence that the fungal isolate obtained in this study is accurately identified as *Nigrospora sphaerica*.

Table 1. Reference Nigrospora sphaerica isolates from NCBI used for molecular identification based on ITS5/ITS4 rDNA sequence analysis

No.	Accession No.	Isolate / Strain Name	Source / Description	Percent Identity (%)
1	OK175859.1	*N. sphaerica* isolate CTN13	ITS reference sequence	98.79
2	OP596135.1	*N. sphaerica* strain CN136E2	ITS sequence from fungal isolate	98.79
3	MK333938.1	*N. sphaerica* strain PB-18	Environmental isolate	98.60
4	MF434826.1	*N. sphaerica* strain GDC1-2	Environmental isolate	98.60
5	JX179231.1	*Sordariomycetes sp.* DZY10 (Nigrospora lineage)	ITS sequence	98.58
6	PV938076.1	*N. sphaerica* isolate GUCC25-0094	ITS region	98.58
7	KX256179.1	*N. sphaerica* strain Ns-12	Leaf-blight associated isolate	98.58
8	OR640300.1	*N. sphaerica* isolate GUCC21-235	ITS sequence	98.58
9	OP596131.1	*N. sphaerica* strain CN136D2	ITS sequence	98.58
10	OR883761.1	*N. sphaerica* isolate UCDMyco_010_1.2	Environmental isolate	98.58
11	OW987626.1	*N. sphaerica* genomic DNA clone (ITS1–5.8S–ITS2)	Genomic clone	98.58

No.	Accession No.	Isolate / Strain Name	Source / Description	Percent Identity (%)
12	KY883352.1	*N. sphaerica* isolate Po7	Plant-associated isolate	98.58
13	OP596130.1	N. sphaerica strain CN136D1	ITS sequence	98.58
14	KX271308.1	*Fungal sp.* strain 26 (*Nigrospora* lineage)	Environmental isolate	98.58
15	MK807770.1	*Nigrospora sp.* isolate DS45	ITS isolate	98.58
16	KM510417.1	*N. sphaerica strain* BPBH1	Plant-associated isolate	98.58
17	KT966515.1	*N. sphaerica* isolate EUGW407	ITS sequence	98.58
18	MG832473.1	*N. sphaerica* strain TS-53	ITS sequence	98.58
19	PX351434.1	Fungal sp. isolate LLC1264	Environmental isolate	98.58
20	OR883765.1	*N. zimmermanii* isolate UCDMyco_012_1.2	Sister species	98.58
21	KJ863497.1	*Nigrospora sp.* TPS20	ITS sequence	98.58
22	KM510415.1	*N. sphaerica* strain MANB1	ITS sequence	98.58
23	KT224800.1	*Nigrospora sp.* 2 S22	ITS sequence	98.58
24	KX515800.1	Uncultured fungus clone ZFQ201207-35	Environmental clone	98.58
25	MF186864.1	*N. sphaerica* strain NS-6	ITS sequence	98.58
26	MN759078.1	*Nigrospora sp.* isolate NG1	ITS sequence	98.58
27	OM350445.1	*N. sphaerica* strain HM2	ITS sequence	98.58

Phylogenetic Relationship Based on Maximum Likelihood Analysis

The Maximum Likelihood phylogenetic tree constructed from the ITS (ITS5/ITS4) sequences revealed clear evolutionary relationships between the study isolates (A1 JC ITS5 and A2 JC ITS5) and reference sequences of *Nigrospora spp*. and *Alternaria porri* retrieved from GenBank, with bootstrap values demonstrating the level of support for each node. The analysis showed that the majority of reference sequences clustered within a large, well-supported *Nigrospora sphaerica* clade (Fig. 5), which included isolates originating from varied geographical regions (e.g., CTN13, BPBH1, MANB1, NS-6, TS-53, and 640J), along with several uncultured fungal clones and *Nigrospora sp.* sequences associated with the same lineage. This extensive grouping illustrates the high degree of ITS sequence conservation within *N. sphaerica*, resulting in the tight clustering observed. Consistent with this pattern, the study isolates A1 (Aciar isolates 1) and A2 (Aciar isolates 2) were positioned firmly within the *N. sphaerica* clade, exhibiting very short branch lengths relative to the authenticated *N. sphaerica* reference sequences, indicating high genetic similarity, shared ancestry, and strong confidence in their species-level identity.

In contrast, the reference sequence KR811362.1 corresponding to *A. porri* formed a distinctly separate and distant branch outside the *Nigrospora* lineage, reflecting substantial genetic divergence between the genera and confirming that the study isolates are not closely related to *A. porri*, which also helps explain earlier morphological inconsistencies.

Additional branching patterns indicated a small but distinct *N. zimmermanii* subclade, and several sequences labeled as Nigrospora sp. or fungal isolates aligned with the *Nigrospora* lineage; many of these grouped near *N. sphaerica* and may represent uncharacterized strains or potential cryptic species that justify further multi-locus investigation. While many internal nodes showed moderate bootstrap support, as is typical for ITS-only phylogenies, the major branches separating *Nigrospora spp.* from *A. porri* were strongly supported, therefore strengthening the clear genetic delimitation between these taxa. Notably, the sequence KR811362.1, an ITS sequence of *Alternaria*

porri (the pathogen initially suspected based on symptoms and morphology), clustered far from the *Nigrospora* sequences. This distant placement indicates no evolutionary relationship between the study isolate and *A. porri*, demonstrating that morphological observations alone could lead to misidentification, especially when fungal structures are similar (e.g., dark spores, pigmented conidia).

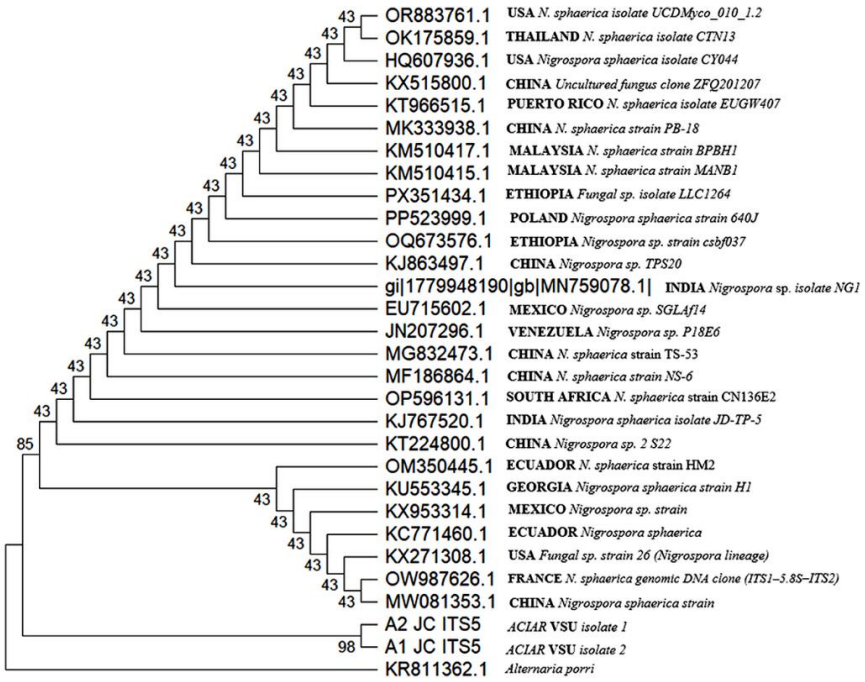

Figure 5. Phylogenetic relationships of the study isolates with reference Nigrospora species inferred using Maximum Likelihood analysis of ITS rDNA sequences

The phylogenetic analysis strongly confirms the conclusion that the fungal isolate recovered in this study belongs to *Nigrospora sphaerica*. The high bootstrap values, consistent clustering with authenticated *N. sphaerica* sequences, and clear separation from *Alternaria* and other *Nigrospora* species provide robust evidence confirming the molecular identification obtained through BLAST analysis.

The phylogenetic evidence provides strong confirmation that the isolates obtained in this study belong to the *Nigrospora sphaerica* lineage, fully consistent with BLAST results, and affirms the essential role of molecular tools in resolving species identity when morphology alone may lead to misidentification. In the symptomatic tissues examined, *Alternaria porri* was identified as the primary pathogen responsible for the major disease symptoms. However, the detection of additional fungal taxa, such as *Nigrospora,* indicates that mixed infections involving secondary or opportunistic fungi can occur and may influence symptom severity, disease progression, and management outcomes (Dutt et al., 2022).

Mixed fungal infections are increasingly recognized in plant disease epidemics, where more than one pathogenic species can simultaneously or sequentially inhabit the same host tissues. For example, mixed fungal infections involving multiple pathogenic species have been reported to contribute to increased disease severity and the development of complex disease in plants, suggesting possible synergistic or additive interactions among co-occurring pathogens (Dutt et al., 2022). Similarly, in pomegranate fruit diseases, *Alternaria* and *Colletotrichum* species were recovered from the same symptomatic tissues, demonstrating the need for molecular characterization to distinguish co-occurring pathogens (Manjunatha et al., 2022).

These observations emphasize that mixed pathogen complexes are common in plant pathology and that exclusive reliance on morphology can overlook secondary taxa that contribute to these complexes.

In onion purple blotch, variations between morphological, molecular, and virulence results can be explained by mixed infections. Although many pathogenicity studies have focused on non-Allium hosts, *Nigrospora spp*. have been repeatedly isolated from Allium crops, including bulb onions. Leaf surface and endophytic surveys of onion leaves have reported *Nigrospora sphaerica* among the fungi associated with onion leaves, together with common genera such as A*lternaria, Stemphylium, and Cladosporium,* suggesting that multiple fungi may occur in association with onion leaf tissues (Abdel-Hafez et al., 2015). *Nigrospora* has been recovered from both apparently healthy leaves and tissues exhibiting foliar blight, including purple blotch lesions, suggesting that this fungus may act as an endophyte, latent pathogen, or secondary invader depending on host stress and field conditions. Its persistent detection in onion foliage indicates its regular presence in Allium agro-ecosystems.

Plant diseases frequently involve "a complex of pathogens, in which secondary organisms invade host tissues already damaged by primary pathogens" (Agrios, 2005). In this study, pathogenicity testing successfully reproduced purple blotch symptoms on onion, and the re-isolated fungus consistently presented morphological aspects typical of *Alternaria*, supporting its role as the primary causal agent. This corresponds to established descriptions of onion purple blotch, in which *Alternaria* species produce characteristic necrotic lesions and diagnostic dematiaceous, septate conidia that are reliably recovered following pathogenicity assays (Simmons, 2007).

Although molecular analysis identified *Nigrospora*, this genus is commonly reported as a secondary colonizer, as species of *Nigrospora* are "frequently isolated as endophytes or saprobes and only occasionally reported as primary pathogens" (Wang et al., 2017). Secondary colonizers may control culture-based identification because "secondary colonizers or saprophytes may be isolated more frequently than the primary pathogen due to their rapid growth on artificial media" (Dhingra & Sinclair, 1995), and "the organism most frequently isolated from diseased tissue is not necessarily the primary causal agent of the disease" (Agrios, 2005).

Therefore, the successful reproduction of symptoms and recovery of *Alternaria* morphology following pathogenicity testing strongly indicate that *Alternaria porri* was the principal pathogen responsible for onion purple blotch in this study, while *Nigrospora sphaerica* likely functioned as a secondary invader. The molecular dominance of *Nigrospora* in sequencing results is likely attributable to its faster growth and higher abundance in culture, illustrating that culture-based and single-locus molecular approaches can sometimes overrepresent secondary or opportunistic fungi in mixed infections. This stresses the essential need to integrate morphological, pathogenicity, and molecular strategies to accurately identify causal agents. In particular, next-generation sequencing (NGS) or multilocus sequencing approaches provide higher resolution for detecting multiple co-occurring taxa within a single sample, helping researchers to distinguish primary pathogens from secondary colonizers and latent endophytes that may otherwise obscure disease etiology. Multilocus phylogenetic analyses verify species identity across multiple genomic regions, lowering the risk of misidentification that can arise from morphology or single-gene sequencing alone, while NGS-based community profiling can reveal the full spectrum of fungal diversity within symptomatic tissues, including rare or slow-growing species. Employing these advanced molecular tools is therefore essential in complex disease systems, such as onion purple blotch, where *Alternaria* is the primary pathogen but secondary fungi, such as *Nigrospora*, can be abundant and confound culture- or single-locus-based diagnoses. This integrative approach constitute a reliable characterization of mixed infections, informs efficient disease management strategies, and adds to a more complete understanding of pathogen interactions taking place within plant hosts.

Conclusions

This study confirmed the occurrence of mixed fungal infections in bulb onion under protected cultivation. Morphological characterization and pathogenic potential tests consistently identified *Alternaria porri* as the primary causal agent of purple blotch disease, as demonstrated by symptom reproduction and fulfillment of Koch's postulates. In contrast, molecular analysis based on ITS sequencing identified *Nigrospora sphaerica* as the dominant species in culture.

The discrepancy between these outcomes suggests that *N. sphaerica* functions as a secondary or opportunistic colonizer, while *A. porri* remains the principal pathogen responsible for disease development. These results show the disadvantages of relying on a single identification method and undeline the essentiality of integrating morphological, pathogenicity, and molecular approaches. Furthermore, the study shows the complexity of plant disease systems and the need of comprehensive diagnostic strategies, particularly in protected cultivation environments.

Recommendations

An integrated approach merging morphological, pathogenicity, and molecular methods is recommended for accurate pathogen identification. Early sampling and strict aseptic techniques should be used to minimize contamination to ensure reliable results. The use of advanced molecular tools, such as multilocus sequencing and next-generation sequencing, is encouraged to better resolve mixed infections. Additionally, improving environmental conditions in protected cultivation and adopting integrated disease management strategies, including the use of resistant varieties and proper cultural practices, are important for effecient disease control and improved onion productivity.

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References

Abdel-Hafez, S. I. I., Abo-Elyousr, K. A. M., & Abdel-Rahim, I. R. (2015). Leaf surface and endophytic fungi associated with onion leaves and their antagonistic activity against *Alternaria porri*. *Czech Mycology, 67*(1), 1–22.

Agrios, G. N. (2005). *Plant pathology* (5th ed.). Elsevier Academic Press.

Barnett, H. L., & Hunter, B. B. (1999). *Illustrated genera of imperfect fungi* (4th ed.). APS Press.

Black, L. L., Koike, S. T., O'Brien, R. G., & Hanson, P. (2012). *Compendium of onion and garlic diseases and pests* (2nd ed.). APS Press.

Dar, G. H., Beig, M. A., Bhat, J. A., & Bhat, N. A. (2020). Morphological and molecular characterization of *Alternaria porri* causing purple blotch disease in onion. *Journal of Plant Pathology*, *102*(2), 559-567.

Dhingra, O. D., & Sinclair, J. B. (1995). *Basic plant pathology methods* (2nd ed.). CRC Press.

Dutt, V., Sharma, S., & Singh, R. (2022). Mixed fungal infections and their role in plant disease development. *Plant Pathology Journal, 38*(2), 89–101.

Leslie, J. F., & Summerell, B. A. (2006). The Fusarium laboratory manual. *Blackwell Publishing*.

Manjunatha, N., Sharma, J., Pokhare, S. S., Sharma, K. K., Agarrwal, R., Hinge, V. R., Adiver, S. S., Gawande, S. P., Somkuwar, R. G., Jadhav, V. T., Ghadge, S. B., Bidwe, M. G., Bicchal, A., Ukale, A. S., & Marathe, R. A. (2022). Characterization of *Alternaria* and *Colletotrichum* species associated with pomegranate (*Punica granatum* L.) in Maharashtra State of India. *Journal of Fungi, 8*(10), 1040. https://doi.org/10.3390/jof8101040

Meena, M., Gupta, S. K., Swapnil, P., Zehra, A., Dubey, M. K., & Upadhyay, R. S. (2017).

Alternaria toxins: Potential virulence factors and genes related to pathogenesis. *Frontiers in Microbiology, 8*, 1451. https://doi.org/10.3389/fmicb.2017.01451

Miller, M. E., & Lacy, M. L. (1995). Purple blotch of onion. *Plant Disease, 79*(3), 242–248.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences, 109*(16), 6241–6246. https://doi.org/10.1073/pnas.1117018109

Schwartz, H. F., & Mohan, S. K. (2008). *Compendium of onion and garlic diseases and pests* (2nd ed.). American Phytopathological Society.

Simmons, E. G. (2007). *Alternaria: An identification manual.* CBS Fungal Biodiversity Centre.

Suheri, H., & Price, T. V. (2001). The epidemiology of purple leaf blotch on leeks in Victoria, Australia. *European Journal of Plant Pathology, 107*(5), 503–510.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution, 10*(3), 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Wang, M., Liu, F., Crous, P. W., & Cai, L. (2017). Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. *Persoonia, 39*, 118–142. https://doi.org/10.3767/persoonia.2017.39.06

Woudenberg, J. H. C., Seidl, M. F., Groenewald, J. Z., & Crous, P. W. (2014). *Alternaria* section *Alternaria*: Species, formae speciales or pathotypes? *Studies in Mycology, 75*, 1–21. https://doi.org/10.3114/sim0015