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Research Article

Molecular identification and phylogenetic analysis of *Alternaria perpunctulata GVKNSV7* causing leaf spot disease on *Alternanthera philoxeroides* (Mart.) Griseb : A first report in India

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Abstract
Leaf spot disease caused by *Alternaria perpunctulata* (E.G. Simmons) poses a significant threat to *Alternanthera philoxeroides* (Mart.) Griseb, commonly known as alligator weed and Ceylon Spinach, contributes to persistent weed proliferation in Indian paddy fields. This study focuses on the molecular identification and phylogenetic analysis of *the Alternaria perpunctulata strain GVKNSV7, which is* responsible for leaf spot disease on *A. philoxeroides*. Diseased plant leaves were collected from Kakumanu Mandal, Guntur, and local vegetable markets of Visakhapatnam, Andhra Pradesh, India, in December 2021, revealing symptomatic samples with pink to reddish spots on *A. philoxeroides* leaves. Employing a comprehensive approach that included morphological assessment, pathogenicity testing, and ITS region sequencing, the isolated fungus was conclusively identified as *A. perpunctulata* with a high sequence similarity of 98.54%. Molecular comparison with sequences in the NCBI database further validated this identification. The resulting genetic sequence, formally catalogued as "*Alternaria perpunctulata* GVKNSV7," with the GenBank accession number OQ073752, has been successfully submitted to the NCBI database. This study emphasized the utility of ITS sequencing and molecular analyses for accurately identifying and documenting emerging pathogens. Such documentation provides essential insights for developing future bioherbicides and effective weed management strategies. Furthermore, it highlights the potential use of *A. philoxeroides* as a leafy vegetable, contributing to food security and agricultural sustainability. Overall, this research contributes to understanding *Alternaria perpunctulata* and its implications for crop health and management practices.

Keywords: Bioherbicides, Czapek Dox agar (CZA), GenBank host-specific, Internal transcribed spacer (ITS), National Center for Biotechnology Information (NCBI)

INTRODUCTION

Paddy, scientifically known as *Oryza sativa* L., holds a crucial position as one of the world's primary food crops. With over half of the global population relying on rice for daily sustenance, it is India's second most prominent crop, following wheat. India, ranking second only to China, significantly contributes to global rice production (Savary *et al*., 2005). The data collected over two decades from different agro-ecological regions reveals the prevalence of weed species in various ecosystems, with reported yield losses ranging from 5% in commercial agriculture to 37–79% in agriculture (Dixit *et al*., 2009; Rao *et al*. 2020). Paddy fields, characterized by their unique flooded environment, provide an ideal stage for the interplay between rice plants and weeds (Olofsdotter *et al*., 1999; Chauhan *et al*., 2012). Biological control, a strategy aimed at managing undesirable species within ecosystems, involves intentionally introducing organisms. Over the past five decades, the focus of this approach, particularly in the context of controlling invasive plant species and weeds, has shifted towards bacteria and fungi (Li *et al*., 2003).

Fungi, in particular, have gained prominence in the biological control of weeds. Commercial products in North

America have predominantly utilized fungal formulations, yet only a few have demonstrated long-term success. Examples include BioMal, based on *Colletotrichum gloeosporioides* f.sp. *malvae*, targeting round leaf mallow (*Malva pusilla*) (Mortensen, 1988; PMRA, 2006), and *C. gloeosporioides* f.sp. *aeschynomene*, released for controlling northern jointvetch (*Aeschynomene virginica*) in 1982 as Collego (Daniel *et al*., 1973; Menaria, 2007), later reintroduced in 2006 as LockDown (EPA Registration Number 82681-1) (Bailey, 2014). Sarritor, a formulation of Sclerotinia minor, has also been introduced for dandelion (*Taraxacum officinale*), white clover (*Trifolium repens*), and broadleaf plantain (*Plantago major*) control in turf (PMRA, 2010). While these successes highlight the potential of fungal species in biological weed control, challenges such as environmental variability, host specificity, and weed adaptability must be addressed for sustained effectiveness. Ongoing research efforts, collaborative initiatives, and refinements in formulations are crucial to advancing biological weed control strategies and ensuring their responsible deployment for sustainable weed management (Harding and Raizada, 2015).

Weed infestations pose a formidable challenge to paddy cultivation in India. The alligator weed or Ceylon Spinach, belongs to the Amaranthaceae family, *Alternanthera philoxeroides (Mart.)* Griseb is emerging as a persistent and pervasive concern (Suthari *et al*. 2017), as a stoloniferous perennial herb, *A. philoxeroides'* propensity for emergent growth complicates efforts at weed management, necessitating a proactive approach. In light of the quest for sustainable and environmentally friendly weed control methods, this study pivots towards exploring the potential of endophytic fungi as bioherbicides. A widespread fungal genus encompasses saprobic, endophytic, and pathogenic species linked to diverse substrates. This omnipresent dematiaceous hyphomycete produces dark-colored multicellular conidia, also known as phaeodictyospores. Alternaria is found in association with various substrates, such as seeds, plants, agricultural products, humans, soil, and the atmosphere (Woudenberg 2015 *).* The primary objective of targeting endophytic fungi associated with *A. philoxeroides* was isolating and characterizing endophytic fungi extracted from *A. philoxeroides* that exhibit distinct host-specific pathogenicity to this weed.

MATERIALS AND METHODS

Isolation of Endophytic fungi

Samples of *A. philoxeroides* leaves exhibiting pink to reddish spots were meticulously collected from (16.1224° N, 80.4541° E) Kakumanu Mandal, Guntur, Andhra Pradesh, India, and (17.728405351857102, 83.32647259883225) local vegetable markets of Visakhapatnam, Andhra Pradesh, India during December 2021. Rigorous care was exercised to ensure the inclusion of infected portions in the collected samples. To eliminate extraneous contaminants, the isolated leaves underwent surface sterilization. This process involved immersing the leaves in a solution of 70% alcohol, followed by gentle agitation. Subsequently, the leaves were rinsed with sterile distilled water to remove residual alcohol.

Culturing and incubation

Sterilized leaves were carefully placed onto Petri dishes containing Potato Dextrose Agar (PDA) or Czapek-Dox Agar (CZA) medium. The Petri dishes were meticulously sealed with parafilm and subjected to an incubation period in darkness. Maintaining a controlled temperature range of 25-30°C, this environment facilitated optimal growth conditions for endophytic fungi (Reis *et al*., 2022). Sterilized leaves were carefully placed onto Petri dishes containing Potato Dextrose Agar (PDA) or Czapek-Dox Agar (CZA) medium. The Petri dishes were meticulously sealed with parafilm and subjected to an incubation period in darkness. Maintaining a controlled temperature range of 25-30°C, this environment facilitated optimal growth conditions for endophytic fungi.

Morphological characterization

Following the 7-15 days incubation interval, a meticulous examination of the Petri dishes ensued, focusing on any discernible growth of fungal colonies originating from the symptomatic leaf tissues (Gera *et al*., 2023 and Suwannarach *et al*., 2022). Colonies were subjected to scrutiny regarding aspects such as color, texture, and other morphological attributes. Utilizing microscopy range from 1–14µm, fungal structures, particularly conidia, were scrutinized to ascertain their dimensions, shapes, and septation patterns.

Pathogenicity testing

Validation of pathogenicity represented a pivotal stage in establishing a conclusive linkage between the isolated fungus and the observed leaf spot disease. This entailed the application of a conidial suspension, characterized by a concentration of 1x10^7 conidia/mL, onto the leaves of 25 mature and healthy plants. Concurrently, a set of control plants remained untreated. Over the subsequent monitoring period, vigilant observation was exercised for the potential emergence of leaf spot symptoms.

Re-isolation and Koch's Postulates

Upon the manifestation of leaf spot symptoms on the inoculated plants, samples were judiciously procured from the pink to red colour circular spots regions symptomatic regions. Employing culture on PDA or CZA medium, fungal isolates were obtained from these sam-

ples. A meticulous comparison of morphological attributes between the reisolated fungi and the initial isolate ensued. The causal association between the isolated fungus and the leaf spot disease was substantiated through the full-filing of Koch's postulates encompassing isolation, pathogenicity, re-isolation, and comprehensive morphological correlation (Long *et al*., 2009; Walker *et al*., 2006)

Molecular identification

To foster an enhanced and definitive identification process, molecular methodologies were enlisted. Amplification of the Internal transcribed spacer (ITS) region of the fungal DNA was executed through the deployment of specific primers, namely ITS 5F (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS 4R (TCCTCCGCTTATTGATATGC-3′) (Li *et al*., 2013). The resultant amplified DNA underwent sequencing, followed by a meticulous comparison against existing sequences within the NCBI database. The process of BLAST analysis contributed an additional layer of confirmation to the fungus' identity.

Phylogenetic tree construction

The phylogenetic tree was constructed by collecting DNA sequences of *A. perpunctulata GVKNSV7* and closely related fungal species from the NCBI GenBank database. These sequences were aligned to ensure accurate alignment, removing any gaps or ambiguities. The best-fit evolutionary model was selected using the MEGA software, and Maximum Likelihood (ML) analysis was performed to estimate the likely evolutionary tree topology. Bootstrap resampling was then conducted to assess the robustness of the inferred tree topology. The final phylogenetic tree, illustrating the evolutionary relationships between *A. perpunctulata GVKNSV7* and related fungal species, was visualized using the MEGA software (Hall, 2013).

RESULTS AND DISCUSSION

Isolation and identification of *Alternaria perpunctulata*

The isolated fungus was characterized by a spectrum of colony colors and textures on the culture medium. Microscopic analysis revealed langouste ellipsoidal subcylindrical conidia, typically measuring within the range of 80–100 × 10–14µm in diameter (Fig.1). Conidia exhibited sporadic septation, occasionally displaying 2–4 septa (Fig. 2). These morphological attributes harmonized with the established traits associated with the *Alternaria* genus.

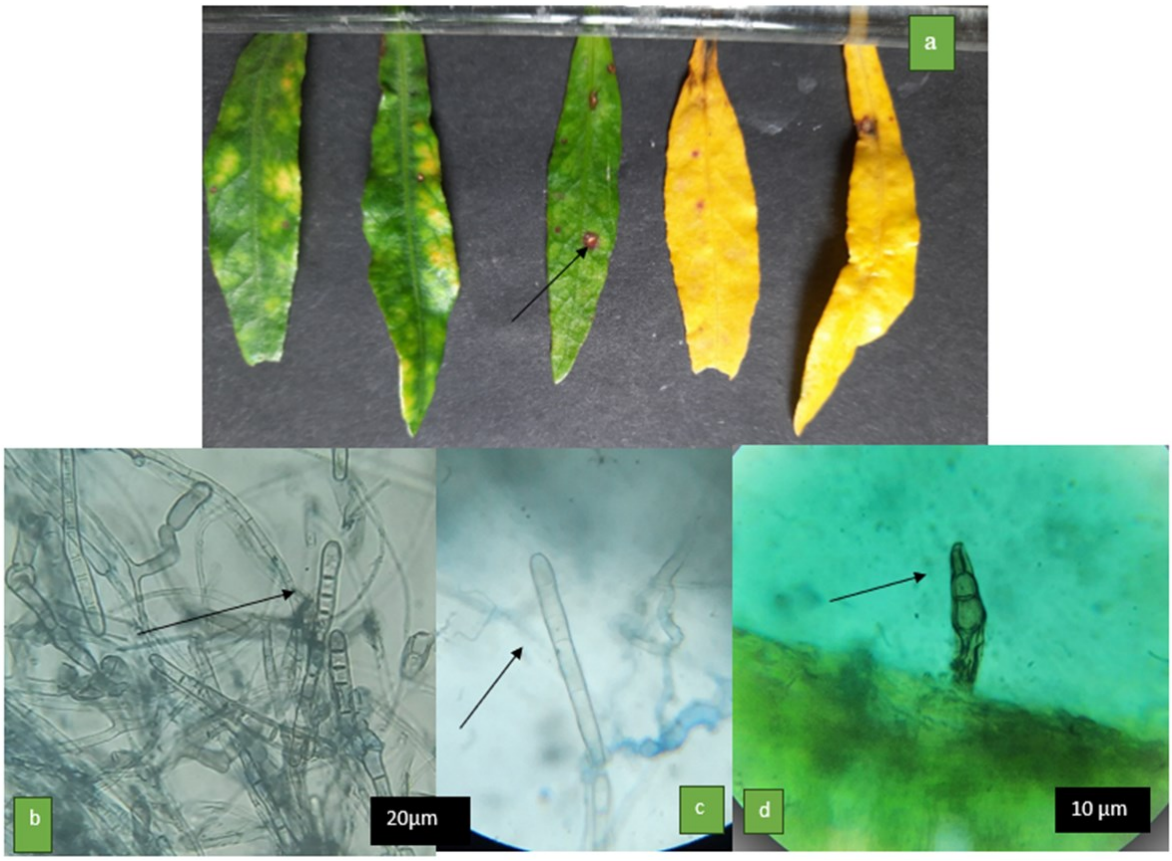

Fig. 1. a. *Alternanthera philoxeroides diseased leaves with pink spots.* ***b*** *&* ***c*** *showing microscopic images of Alternaria perpunctulata GVKNSV7 with septate langouste ellipsoidal subcylindrical conidia.* ***d****. conidia showing on the upper epidermis of the diseased leaf with two septa*

Pathogenicity confirmation using Koch's postulates

The substantiation of pathogenicity emerged as a pivotal stride in cementing the causal link between the isolated fungus and the observed leaf spot disease. Achievement of this goal involved the application of a conidial suspension, formulated to possess a concentration of 1x10^7 conidia/mL, onto healthy *A. philoxeroides* plants. Over the subsequent monitoring duration, the anticipated leaf spot symptoms materialized exclusively on the inoculated plants. In stark contrast, the untreated control plants remained resolutely devoid of such symptoms. A isolation phase bore fruit, with the identical fungus successfully re-retrieved from the symptomatic regions of the initially inoculated plants. Morphological attributes exhibited by the re-isolated fungus coherently aligned with those demonstrated by the initial isolate. This adherence to Koch's postulates conclusively fortified the substantiation of the isolated fungus' pathogenic nature.

Molecular Identification

To fortify the identification process, a strategic molecular approach was harnessed. Specifically, the fungal DNA's ITS region underwent amplification through primers with designated ITS 5F and ITS 4R nomenclature. Subsequent sequencing of the amplified DNA yielded a genetic sequence that underwent meticulous comparison against pre-existing sequences cataloged within the NCBI database. This incisive process of BLAST analysis yielded a compelling outcome, featuring a remarkable 98.54% similarity with *A. perpunctulata* CBS 115267(NR 151838.1), (KC584210), and JN383498. This robust molecular analysis effectively reinforced the identification of the isolated fungus as *A. perpunctulata*.

ITS region sequencing with this primers ITS5F+ITS4R

>ACATTCACAGATTTGAAGGGCGGGGCTGGGAACC
TCTCGGGGTTGCAGTCTTGCTGAATTTTCACCCGT
GTCTTTTGCGTACTTCTTGTTTCCGGGTGGGTTCG
CCCACCACCAGGACAAACCATGAACCTTTTGTAAT
TGCAATCAGCGTCAGTAACAACACAATCATTTACAA
CTTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAGTGTGAAT
TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCA
CATTGCGCCCTTTGGCATTCCAAAGGGCATGCCTG
TTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGT
GTTGGGCGTCTTTGTCTCTGGCTTTGCTGGAGACT
CGCCTTAAAGGAATTGGCAGCCGGCCTACTGGTTT
CGGAGCGCAGCACAAGTCGCACTCTCTTCCAGCC
ACGGTCTGGCATCCATGAAGCC<

Submission to NCBI Database and phylogeny

The conclusive identification profile, harmonizing both morphological attributes and the discerned molecular sequence, underwent formal submission to the esteemed NCBI database. The allocated designation for this isolate read "*A. perpunctulata* GVKNSV7," accompanied by the assignment of a GenBank accession number, specifically OQ073752 (Fig.3) and phylogenic tree was constructed based on the (ITS) molecular data by using MEGA software (Fig.4). This submission not only enshrined the isolate's identity but also ensured its accessibility for prospective research and scholarly discourse.

Historical taxonomic update

In a historical context, the fungus in question previously bore the designation *Nimbya perpunctulata*. The origin of its isolation and identification traces back to the work of E.G. Simmons in the United States (Simmons 2004). After a meticulous molecular characterization and phylogenetic analysis conducted by Lawrence *et al*. (2011), the fungus underwent a taxonomic metamorphosis, culminating in its reclassification as *A. perpunctulata*. The present study harmoniously aligns with and reaffirms this revised taxonomic classification, all while injecting valuable insights into the fungus' geographic distribution and consequential implications. Akhtar *et al*. (2012) reported *N. alternantherae* as a causal pathogen of leaf and stem necrosis of *A. philoxeroides* in Pakistan. It naturally causes severe infection on *A. philoxeroides,* producing leaf spots and resulting in severe defoliation and occasionally death. Barreto and

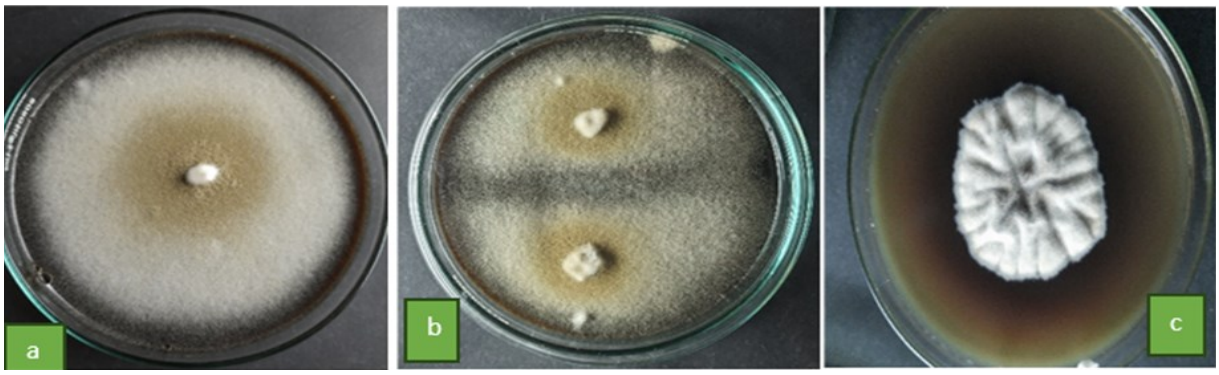

Fig. 2. (a & **b)**. *Isolated fungal pathogen growth on the CZA medium, and* ***c)****. growth on the PDA medium*

Alternaria perpunctulata **strain GVKNSV7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence**
GenBank:OQ073752.1
LOCUS OQ073752 479 bp DNA linear PLN 19-MAY-2023
DEFINITION :*Alternaria perpunctulata* strain GVKNSV7 internal transcribed spacer1, partial sequence; 5.8S ribosomal RNA gene, complete sequence;and internal transcribed spacer 2, partial sequence.
ACCESSI ON OQ073752
VERSION OQ073752.1
KEYWORDS .
SOURCE*Alternaria perpunctulata*
ORGANISM *Alternaria perpunctulata*
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;
Dothideomycetes; Pleosporomycetidae; Pleosporales; Pleosporineae;Pleosporaceae; Alternaria; Alternaria sect. Alternantherae.
REFERENCE1 (bases 1 to 479)
AUTHORS Gera Vinay Kumar,G.V.K. and Ratna Kumar,P.K.
TITLE Direct Submission
JOURNAL Submitted (19-DEC-2022) botany, andhra university, 4-15 s/o sivanageswararao, kondabalavaripalem, guntur, kakumanu/ANDHRA PRADESH 522124, India
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Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
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/strain="GVKNSV7"
/isolate="GVKNSV7"
/isolation_source="PLANT LEAVES"
/host="*Alternanthera philoxeroides*"
/db_xref="taxon:1187936"
/country="India"
/collection_date="20-Aug-2021"
/collected_by="GERA VINAY KUMAR"
/note="PCR_primers=fwd_name: ITS5F, rev_name: ITS4R"
misc_RNA<1..>479
/note="contains internal transcribed spacer 1, 5.8S ribosomal RNA, and internal transcribed spacer 2"
ORIGIN
1 acattcacagatttgaagggcggggctgggaacctctcggggttgcagtcttgctgaatt
61 ttcacccgtgtcttttgcgtacttcttgtttccgggtgggttcgcccaccaccaggacaa
121 accatgaacctttttgtaattgcaatcagcgtcagtaacaacacaatcatttacaacttttt
181 caacaacggatctcttggttctggcatcgatgaagaacgcagcgaaatgcgataagtagt
241 gtgaattgcagaattcagtgaatcatcgaatctttgaacgcacattgcgccctttggcat
301 tccaaagggcatgcctgttcgagcgtcatttgtaccctcaagctttgcttggtgttgggc
361 gtctttgtctctggctttgctggagactcgccttaaaggaattggcagccggcctactgg
421 tttcggagcgcagcacaagtcgcactctcttccagccacggtctggcatccatgaagcc

Fig. 3. *Isolated Alternaria perpunctulata GVKNSV7 fungal strain was submitted on the NCBI Database with A.no-OQ073752.*

Tôrres (1999) discussed *Nimbya alternantherae,* which has previously been reported to cause similar disease in *A. philoxeroides* in South America.

To the best of our understanding, based on existing literature, NCBI database, and Mycobank data, the identification of *Alternaria perpunctulata* GVKNSV7 in India represents the first report. Such exploration of endophytic fungi holds promise for devising innovative and eco-friendly strategies to manage *A. philoxeroides* infestations, thereby contributing to India's broader canvas of sustainable agricultural practices and other hand In India and Bangladesh, the plant's leaves are commonly consumed as a leafy vegetable. Meanwhile, in China, this versatile plant has found widespread use as a traditional remedy for treating various viral diseases such as measles, influenza, and haemorrhagic fever (Khatun *et al*., 2013; Zhang *et al*., 2018; Akbar *et al*., 2021; Nahar *et al*., 2022).

Conclusion

In addition to its significance in identifying and documenting *A. perpunctulata* as a causative agent of leaf spot disease in *Alternanthera philoxeroides*, the identification of *Alternaria perpunctulata GVKNSV7* (A.no-OQ073752). in India, as first reported instance, underscores the significance of collaborative efforts in mycology, expanding our understanding of fungal diversity

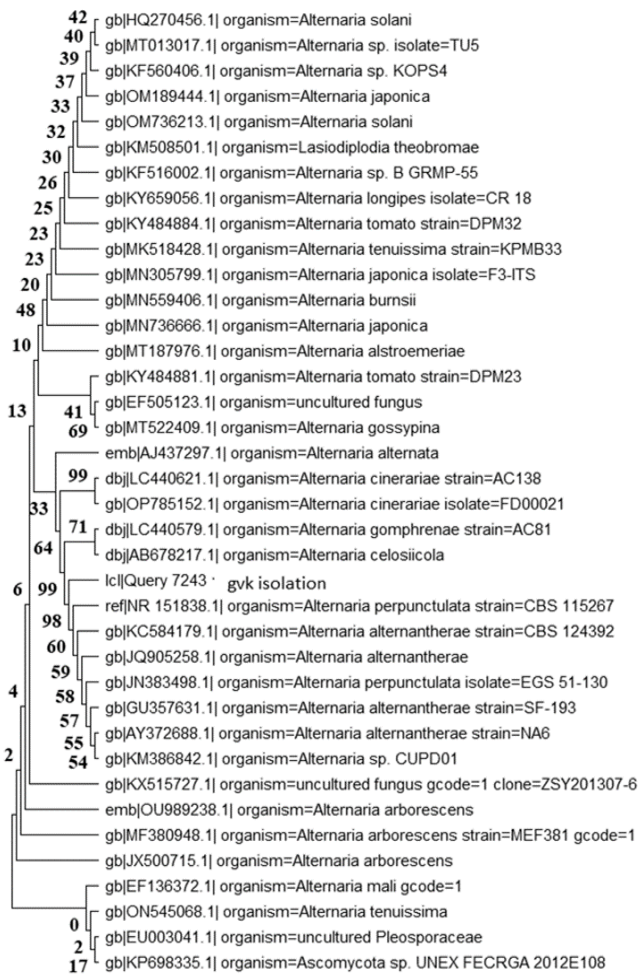

Fig. 4. *Phylogenetic Analysis of Alternaria perpunctulata GVKNSV7 Based on Molecular Data, Constructed Using MEGA Software*

and emphasizing the need for continued global exploration it emphasizing the importance of effective weed management strategies for protecting crop yields and ensuring the availability of a potential leafy vegetable resource in certain communities. Further research and integrated pest management approaches may be necessary to safeguard both agricultural production and the use of *A. philoxeroides* as a leafy vegetable, ensuring food security and agricultural sustainability in the affected regions.

Conflict of interest

The author declare that they have no conflict of interest.

REFERENCES

1. Akbar, M., Amin, A., Khalil, T., Iqbal, M. S., Nazir, A. & Taswar, A. (2021). Antibacterial activity of *Alternanthera philoxeroides* (Mart.) Griseb. against bacterial phytopathogens: *Erwinia carotovora, Ralstonia solanacearum* and *Xanthomonas axonopodis*. *Allelopathy Journal*, *53*(1), 83–92. https://doi.org/10.26651/allelo.j/2021-53-1-1329
2. Akhtar, K. P., Sarwar, N. & Ul-Haq, I. (2012). *Nimbya alternantherae* reported for the first time to cause leaf and stem necrosis of *Alternanthera philoxeroides* (alligatorweed) in Pakistan. *Tropical Plant Pathology*, *37* (6), 428–430. https://doi.org/10.1590/s1982-56762012000600009.
3. Barreto, R. W. & Tôrres, A. N. L. (1999). *Nimbya alternantherae* and *Cercospora alternantherae*: two new records of fungal pathogens on *Alternanthera philoxeroides* (alligatorweed) in Brazil. *Australasian Plant Pathology*, *28* (2), 103. https://doi.org/10.1071/ap99018.
4. Bailey, K. L. (2014). “The bioherbicide approach to weed control using plant pathogens,” In: *Integrated Pest Management: Current Concepts and Ecological Perspective*, ed. D. P. Abrol. (San Diego, CA: Elsevier), 245–266.
5. Chauhan, B. S., Jabran, K., Mahajan, G. & Kumar, V. (2012). Allelopathy for weed control in rice-wheat cropping system. Journal of Crop Improvement, 26(2), 213-242.
6. Daniel, J. T., Templeton, G. E., Smith, R. J., & Fox, W. T. (1973). Biological control of northern jointvetch in rice by an endemic fungal disease. *Weed science*, *21*(4), 303-307.
7. Dixit, A. & Varshney, J.G. (2009). Herbicide Use in Field Crops. Directorate of Weed Science Research, Jabalpur, India.
8. Simmons, E. G. (2004). Novel dematiaceous hyphomycetes. *studies in mycology* 50, 109–118.
9. Gera, V. K., Bhavana, G., Deviprasanna, D., Devi, A., Teja, K. D. & Kumar, P. (2023). Isolation, identification, and enzyme screening of fungi infested various vegetables in the local markets of Visakhapatnam, Andhra Pradesh. *World Journal of Biology Pharmacy and Health Sciences*, *15*(1), 160–174. https://doi.org/10.30574/wjbphs.2023.15.1.0319.
10. Hall, B. G. (2013). Building Phylogenetic Trees from Molecular Data with MEGA. *Molecular Biology and Evolution*, *30*(5), 1229–1235. https://doi.org/10.1093/molbev/mst012.
11. Harding, D. P., & Raizada, M. N. (2015). Controlling weeds with fungi, bacteria and viruses: a review. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00659.
12. Khatun, M., Hassan, A., Islam, S. N. & Oliur, M. (2013). Taxonomy of the leafy vegetables in Bangladesh. *Bangladesh Journal of Plant Taxonomy*, *20*(1), 95–123. https://doi.org/10.3329/bjpt.v20i1.15469
13. Lawrence, D. P., Park, M. S., & Pryor, B. M. (2011). *Nimbya* and *Embellisia* revisited, with nov. comb for *Alternaria celosiae* and *A. perpunctulata*. *Mycological Progress*, *11*(3), 799–815. https://doi.org/10.1007/s11557-011-0793-7.
14. Li, X., Song, J., Xin, T., Zhu, Y., Shi, L., Xu, X., Pang, X., Yao, H., Li, W. & Chen, S. (2013). DNA barcoding the commercial Chinese caterpillar fungus. *FEMS Microbiology Letters*, n/a. https://doi.org/10.1111/1574-6968.12233.
15. Li, Y., Sun, Z., Xiao-Feng, Z., Xu, L., Chen, S. & Li, M. (2003). Research progress on microbial herbicides. *Crop Protection*, *22*(2), 247–252. https://doi.org/10.1016/s0261-2194(02)00189-8
16. Long, Y. Q., Hsiang, T. & Huang, J. (2009). First report of leaf spot of *Smilax china* caused by *Alternaria longipes* in China. *Plant Pathology*, *58*(4), 800. https://doi.org/10.1111/j.1365-3059.2009.02045.x.

17. Menaria, B. L. (2007). Bioherbicides: an eco-friendly approach to weed management. *Current Science*, *92*(1),10-11.
18. Mortensen, K. (1988). The potential of an endemic fungus, *Colletotrichum gloeosporioides*, for biological control of round-leaved mallow (*Malva pusilla*) and velvetleaf (*Abutilon theophrasti*). *Weed Science*, *36*(4), 473-478.
19. Nahar, L., Nath, S. & Sarker, S. D. (2022). "Malancha" [*Alternanthera philoxeroides* (Mart.) Griseb.]: A Potential Therapeutic Option against Viral Diseases. *Biomolecules*, *12*(4), 582. https://doi.org/10.3390/biom12040582.
20. Olofsdotter, M., Navarez, D. & Rebulanan, M. (1999). Weedy Rice—Its Roles and Its Management. In: Weed Science: Principles and Practices (pp. 75-92). John Wiley & Sons.PMRA. (2006). "Re-evaluation of *Colletotrichum gloeosporioides* f. sp. *malvae*
21. PMRA. (2010). "Sclerotinia minor Strain IMI 344141" RD2010-08. Health Canada.
22. Rao, A., Singh, R. K., Mahajan, G. & Wani, S. P. (2020). Weed research issues, challenges, and opportunities in India. *Crop Protection*, *134*, 104451. https://doi.org/10.1016/j.cropro.2018.02.003.
23. Reis, J. B. a. D., Lorenzi, A. S. & Vale, H. M. M. D. (2022). Methods used for the study of endophytic fungi: a review on methodologies and challenges, and associated tips. *Archives of Microbiology*, *204*(11). https://doi.org/10.1007/s00203-022-03283-0.
24. Savary, S., Castilla, N. P., Elazegui, F. A. & Teng, P. S. (2005). Multiple effects of two drivers of agricultural change, labour shortage and water scarcity, on rice pest profiles in tropical Asia. *Field Crops Research*, *91*(2–3), 263–271. https://doi.org/10.1016/j.fcr.2004.07.017.
25. Suthari, S., Kiran, B. R. & Prasad, M. N. V. (2017). Health risks of leafy vegetable *Alternanthera philoxeroides* (Alligator weed) rich in phytochemicals and minerals. *The Eurobiotech Journal*, *1*(4), 293–302. https://doi.org/10.24190/issn2564-615x/2017/04.06.
26. Suwannarach, N., Khuna, S., Kumla, J., Cheewangkoon, R., Suttiprapan, P. & Lumyong, S. (2022). Morphology characterization, molecular identification, and pathogenicity of fungal pathogen causing kaffir lime leaf blight in northern Thailand. *Plants*, *11*(3), 273. https://doi.org/10.3390/plants11030273.
27. Walker, L. C., LeVine, H. & Jucker, M. (2006). Koch's postulates and infectious proteins. *Acta Neuropathologica*, *112*(1), 1–4. https://doi.org/10.1007/s00401-006-0072-x.
28. Woudenberg, J. H. C. (2015). Restyling *Alternaria. Wageningen University*, (pp. 250). Wageningen, The Netherlands.
29. Zhang, X., Li, P., Guo, S., Wang, S. & Liu, D. (2018). Quantitation of β-carboline and quercetin in alligator weed (Alternanthera philoxeroides (Mart.) Griseb.) by LC-MS/MS and evaluation of cardioprotective effects of the methanol extracts. *Drug Discoveries and Therapeutics*, *12*(6), 341–346. https://doi.org/10.5582/ddt.2018.01070