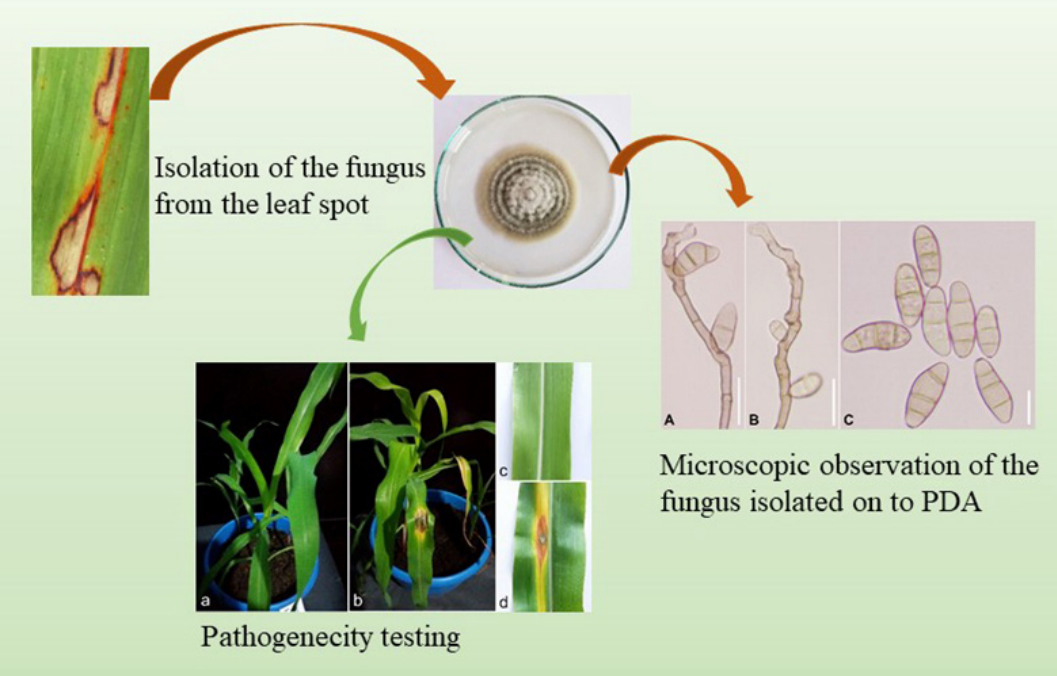
RESEARCH ARTICLE

First report of *Curvularia dactylocteniicola* causing leaf spots on *Zea mays* and *Sorghum* in Sri Lanka

H. S. Ferdinandez, D. S. Manamgoda* and D. Udayanga



Highlights

- A noteworthy leaf spot disease was observed on *Zea mays* and *Sorghum* cultivated in Sri Lanka during the period of 2018–2021.
- The causative agent was identified as *Curvularia dactylocteniicola*.
- This is the first report of *Curvularia dactylocteniicola* causing leaf spots on *Zea mays* and *Sorghum* (traditional - 'Swayanjatha') in Sri Lanka.

Ceylon Journal of Science 53 (1) 2024: 39-43
DOI: http://doi.org/10.4038/cjs.v53i1.8315

RESEARCH ARTICLE

First report of *Curvularia dactylocteniicola* causing leaf spots on *Zea mays* and *Sorghum* in Sri Lanka

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Received: 20.05.2023; Accepted: 16.10.2023

Abstract: The genus *Curvularia* includes pathogens, endophytes and saprobes associated with a diverse range of plants belonging to the family Poaceae. These species are responsible for diseases on cereal crops such as rice, maize, wheat, millet, sorghum and poaceous weedy hosts. During a field survey of cereal pathogens conducted in Galle, Hambanthota, and Matale districts of Sri Lanka, diseased leaf samples were collected randomly from *Zea mays* and an indigenous cereal landrace of *Sorghum* sp. traditionally known as 'Swayanjatha'. Morphology and molecular phylogeny based on combined nuclear ribosomal internal transcribed spacers 1 and 2 with 5.8S region (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and translation elongation factor 1-α (TEF1) revealed that the causative agent of the leaf spots is *Curvularia dactylocteniicola*. Results of the pathogenicity tests confirmed cause of the disease on *Zea mays* and *Sorghum* sp. To the best of our knowledge, this is the first record of *C. dactylocteniicola* causing leaf spots on *Zea mays* and *Sorghum* sp. in Sri Lanka.

Keywords: hyphomycete; molecular phylogeny; phytopathogen; Pleosporaceae

INTRODUCTION

Cereal crops and their crop wild relatives are the most common hosts for taxa in the genus *Curvularia* (Pleosporales, Dothideomycetes). The species of *Curvularia* have reported as saprobes, endophytes and pathogens with worldwide distribution (Manamgoda et al., 2012; Marin-Felix et al., 2017, 2020; Tan et al., 2014, 2018). Pathogenic species of *Curvularia* have caused significant damage specifically on cereal crops (Kusai et al., 2016; Manamgoda et al., 2015; Qostal et al., 2019). However, prior to the molecular era the taxonomy of this genus was confusing and it was solely based on morphological characters. The current species recognition criteria of the genus has established based on multi-locus phylogeny. The most commonly used loci to construct multi-locus phylogenies of the genus are nuclear ribosomal internal transcribed spacers 1 and 2 with 5.8S region (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and translation elongation factor 1-α (TEF1) (Manamgoda et al., 2012; 2015; Marin-Felix et al., 2017; 2020; Tan et al., 2014; 2018).

Curvularia dactylocteniicola was first reported on *Dactyloctenium aegyptium* in Thailand causing oval or fusiform, brown to brown-red leaf spots often surrounded by yellow halo and was first described by Marin-Felix et al. (2017). However, there were no available reports of this species from *Zea mays* and *Sorghum* from Sri Lanka. The aims of the present study were; i) to isolate the causal agents associated with the leaf spot disease of cereal crops cultivated in Sri Lanka; ii) to test the pathogenicity of isolates by fulfilling Koch's postulates; and iii) to characterize the isolated fungi using morphological, molecular and phylogenetic analyses.

MATERIALS AND METHODS

Sample collection, Isolation and Morphological studies

During the period of 2018–2021, a noteworthy leaf spot disease was encountered on *Zea mays* and *Sorghum* sp. cultivated in Sri Lanka. Samples were collected during the field surveys conducted in Galle, Hambanthota and Matale districts. Four (04) fungal isolates were obtained following the single spore isolation method (Chomnunti et al., 2011) and cultures were maintained on potato dextrose agar (PDA, HiMedia-India) at 25 °C, with a light regime of 12 h in light and 12 h in dark. In order to determine colony characters, cultures were in triplicate on three different media: PDA, corn meal agar (CMA, HiMedia-India) and malt extract agar (MEA, Criterion-USA), at 25 °C, with a light regime of 12 h in light and 12 h in dark. Standard color chart by Rayner (1970) was used to record colony colors. Digital images of fungal structures were captured using a Carl Zeiss compound light microscope equipped with an AxioCam digital camera and ZEN lite software (Carl Zeiss Microscopy, Thornwood, NY, USA). The statistical data (mean, minimum, maximum, and standard deviation) for each micro-morphological measurement utilized in the morphological descriptions were recorded.

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The specimens collected were deposited in USJ-H (University of Sri Jayewardenepura Herbarium) and the living fungal cultures are maintained in USJCC (University of Sri Jayewardenepura Culture Collection).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fungal isolates according to the protocol mentioned in Ferdinandez et al. (2021). Amplification of fungal ITS region and TEF loci were carried out according to the conditions and primers described in Manamgoda et al. (2012). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) locus was amplified using the PCR conditions mentioned in Ferdinandez et al. (2021). DNA sequences generated in this study were deposited in NCBI GenBank (Table 1).

Sequence alignment, phylogenetic analyses and species recognition

Raw sequences generated for fresh isolates were assembled with BioEdit v7.0.5 for Windows. To confirm the identity, phylogenetic analyses were performed using both Maximum Parsimony (MP) and Maximum Likelihood (ML) criteria (Ferdinandez et al., 2020) along with ex-type and reference DNA sequences of the genus *Curvularia* (Ferdinandez et al., 2021).

Pathogenicity tests

To confirm the pathogenicity of the fresh isolates, healthy plants of *Zea mays* and *Sorghum* sp. were inoculated with conidial suspension of 1 x 10^6 conidia/mL of respective pathogen isolates (Kaspary et al., 2019). A similar amount of distilled water droplets was applied to the control plants. Both inoculated and non-inoculated plants were then covered to maintain high humidity and left in a plant house at 25 °C under a 12 h photoperiod. The same fungus was recovered from inoculated plants after five days of inoculation confirming Koch's postulates.

RESULTS AND DISCUSSION

The combined multi-locus phylogeny consisted of a total of 46 taxa and the alignment statistics for the parsimony analysis revealed 2246 total characters, with 1649 constant, 392 parsimony-informative, and 205 variable characters were parsimony uninformative. The most parsimonious tree resulting from the MP analysis of the combined dataset is presented here. Resulted phylogram revealed that the three isolates from *Zea mays* and a single isolate from *Sorghum* sp. were similar to *C. dactylocteniicola* which forms a monophyletic group with the ex-type isolate (CPC 28810) of the same species (Figure 1). Therefore, we provide a modern description based on the fresh collection of the species below with full illustrations, notes on habitats and recorded hosts and geographic distribution.

Taxonomy

Curvularia dactylocteniicola Y. Marín, Senwanna & Crous [as 'dactyloctenicola'], in Marin-Felix, Senwanna, Cheewangkoon & Crous, Mycosphere 8(9): 1567 (2017) Figure 2.

Leaf spots (0.5-2 cm), oval or fusiform, brown to brown-red often surrounded by yellow halo which gradually increased up to 4-5 cm long as dark brown patches. Asexual morph: On CMA *Hyphae* 3–4 µm, hyaline, septate, branched. *Conidiophores* up to 316 µm long, simple to branched, septate, straight or flexuous, pale brown to brown. *Conidiogenous cells* hyaline to pale brown, smooth-walled, terminal or intercalary, monotretic to polytretic. *Conidia* (16)19–24(26) × 7–9(10) µm (av. = 21, SD = 3, n = 50; av. = 8, SD =1, n = 50), hyaline to pale brown, usually straight, sometimes curved, smooth-walled, ellipsoid, usually 3-distoseptate; *hila* 1–2 µm flat or slightly protruding, darkened. Chlamydospores formation is observed. Sexual morph: Not observed.

Culture characteristics: Colonies on PDA reaching 50 mm diameter after 7 d at 25 °C, colonies from above: convex, cottony appearance with moderate aerial mycelia, margin entire, mouse grey centre, olivaceous grey at the margins, reverse: dark brown at the margin, black in the centre. Colonies on CMA reaching 67 mm diameter after 7 d at 25 °C, colonies from above: flat, hairy appearance, circular, margin entire, olivaceous grey at the margin, pale brown in the centre, concentric ring growth; reverse: pale brown centre to periphery. Colonies on MEA reaching 67 mm diameter after 7 d at 25 °C, colonies from above: convex, circular, velvety appearance, margin entire, olivaceous green at the margin, grey in the centre; reverse: black from centre to margin (Figure 2 b, c, d).

Materials examined: Sri Lanka, Southern Province, Galle District, Imaduwa, N 6.008556 E 80.373444, leaf spots on *Zea mays*, 30 August 2018, H.S. Ferdinandez, USJ-H-007, living culture USJCC–0061; Sri Lanka, Central Province, Matale District, Palapathwela, N 7.556333 E 80.610611, leaf spots on *Sorghum* sp. (traditional-'Swayanjatha'), 12 November 2018, D. Udayanga, USJ-H-024, living culture USJCC–0052; Sri Lanka, Central Province, Matale District, Palapathwela, N 7.556333 E 80.610611, leaf spots on *Zea mays*, 08 November 2018, D.S. Manamgoda, USJ-H-028, living culture USJCC–0066; Sri Lanka, Southern Province, Hambantota District, Mamadala, N 6.163880 E 80.957413, on leaf of *Zea mays*, 07 August 2019, H. S. Ferdinandez, USJ-H-085, living culture USJCC–0094.

Known hosts and distribution: *Dactyloctenium aegyptium* in Thailand (Marin-Felix et al., 2017); *Saccharum officinarum* in China (Raza et al., 2019); *Sorghum* sp. in Indonesia (Hidayat and Ramadhani, 2019); *Sorghum* sp. (traditional-'Swayanjatha') and *Zea mays* in Sri Lanka (this study).

GenBank Accessions: Table 1

Notes: Isolates USJCC–0052, USJCC–0061, USJCC–0066 and USJCC–0094 were identified as *Curvularia dactylocteniicola*. The fresh isolates were collected from *Sorghum* sp. (traditional-'Swayanjatha') and *Zea mays* during this study.

Pathogenicity tests

Characteristic leaf spots developed after five days of inoculation on test plants while non-inoculated control plants remained asymptomatic (Figure 3). In order to fulfill Koch's postulates, the pathogen was re-isolated

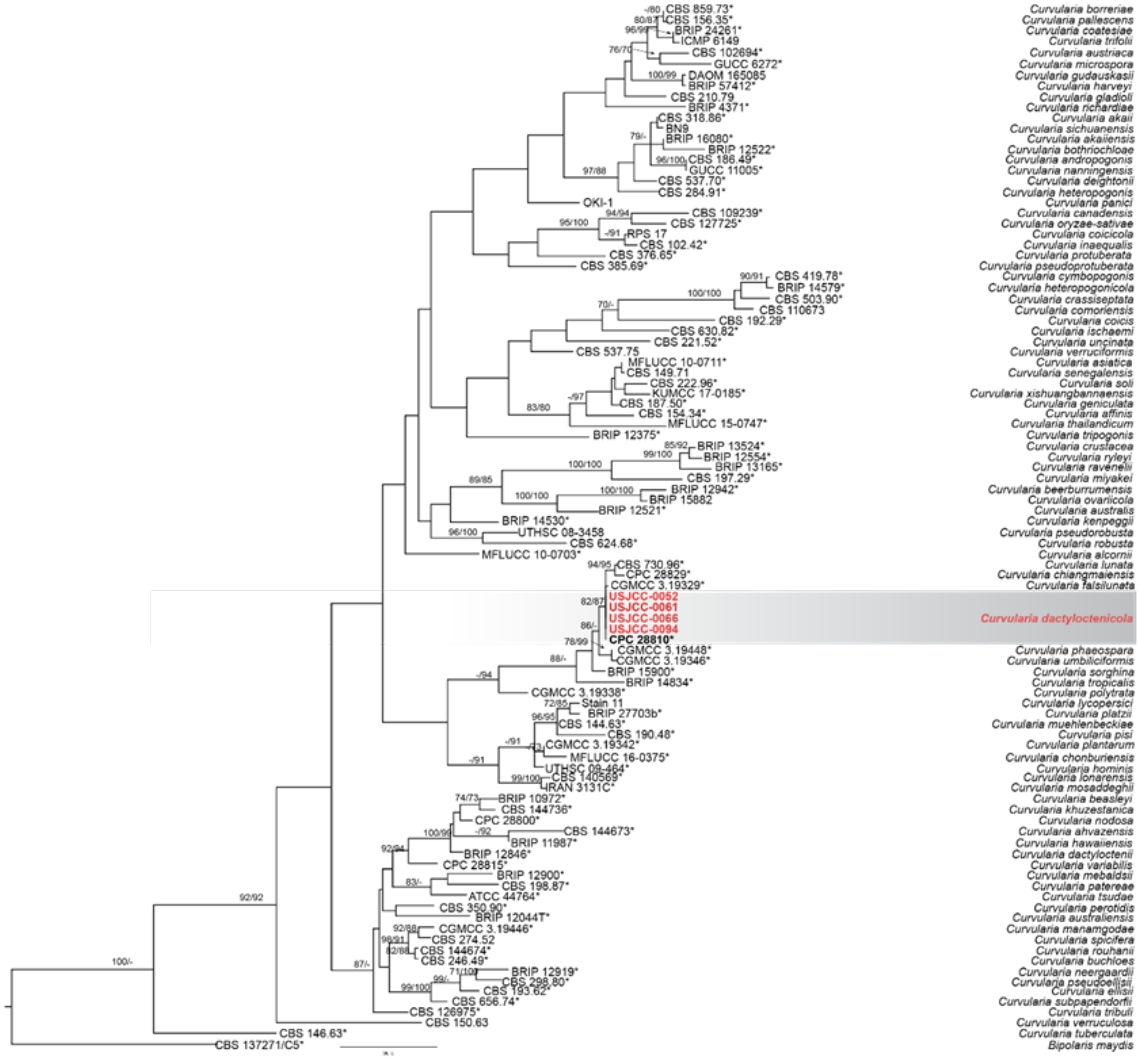

Figure 1: Maximum Parsimony phylogram for *Curvularia* spp. based on the combined ITS, GAPDH, and TEF1 alignment. MP and ML bootstrap support values above 70% are shown at the nodes respectively. Ex-type cultures are marked with an asterisk. New records from the current study are indicated in bold red. The tree is rooted with *Bipolaris maydis* CBS13727/C5.

Figure 2: *Curvularia dactylocteniicola* (USJCC–0052). a Host: leaf spots on *Sorghum* sp.; b–d 7-day-old colony on PDA, CMA, MEA respectively; e–g conidia attached to conidiophores; h–j conidia. Scale bars: a = 0.5 cm; e–g, j = 18 µm; h= 10 µm; i= 8 µm.

Table 1: *Curvularia dactylocteniicola* isolates identified and the DNA sequences generated in this study.

Isolate/Culture Collection	Host/Substratum	GenBank accessions		
		ITS	GAPDH	TEF1
USJCC–0052	*Sorghum* sp. Traditional indigenous cereal landrace-"Swayanjatha"	MN044756	MN053039	MN053008
USJCC–0061	*Zea mays*	ON514021	ON561891	–
USJCC–0066	*Zea mays*	MZ948819	MZ971265	–
USJCC–0094	*Zea mays*	ON514022	ON561892	ON532721

from symptomatic leaves of the inoculated plants. The colonies developed on PDA and the microscopic features of the emerged fungus were similar with those of the isolate initially obtained from diseased leaf spots.

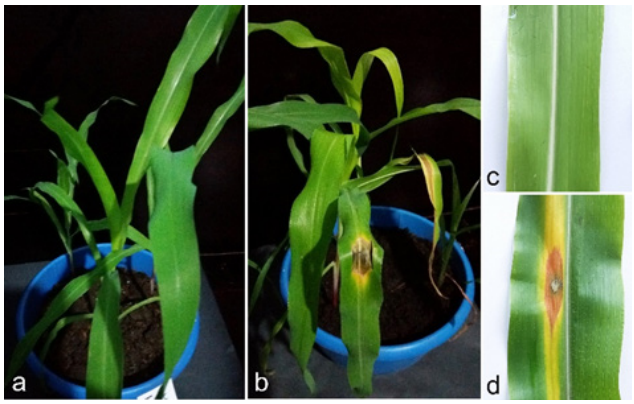

Figure 3: Disease symptom development in the pathogenicity test: a. Control for USJCC–0061; b. leaf spots on *Zea mays* resulted from the inoculation of USJCC–0061; c. control for USJCC–0052; d. leaf spot on *Sorghum* sp. (traditional-'Swayanjatha') resulted from the inoculation of USJCC–0052.

Following the first report from Thailand, *Curvularia dactylocteniicola* has been recorded as a pathogen on *Saccharum officinarum* in China (Raza et al*.,* 2019) and on *Sorghum bicolor* in Indonesia (Hidayat and Ramadhani, 2019). *Zea mays* and *Sorghum* sp. have reported to be infected with several species of *Curvularia* including, *C. australiensis* (Chang et al*.,* 2016), *C. geniculata* (Manzar et al*.,* 2021), *C. lunata* (Akram et al., 2014; Garcia-Aroca et al*.,* 2018). Nevertheless, *C. dactylocteniicola* has not been recorded as a pathogen on *Zea mays* before. Defining the species limits of the genus *Curvularia* is confusing as nomenclatural changes and refinements have occurred frequent within last three decades. There is no clear morphological boundary between the close associated genera *Bipolaris* and *Curvularia* (Manamgoda et al*.*, 2012). Thus, establishment of generic boundaries from molecular phylogenetic assessments have given away the problems in species identification within the genus *Curvularia* with several refinements as well (Manamgoda et al*.,* 2012; Marin-Felix et al*.,* 2017; Tan et al*.,* 2014, 2018). Therefore, accurate identification of the causative agent of the leaf spot disease observed during this study was accomplished with the aid of morphological data and DNA sequence data incorporated multi-locus phylogenetic analyses.

CONCLUSION

According to the morphological and molecular data obtained, the fungal isolates recovered from diseased leaves were identified as *Curvularia dactylocteniicola*. To our knowledge, this is the first report of *C. dactylocteniicola* on *Z. mays* and *Sorghum* sp. (traditional). Thus, the current study contributes the records of host–fungal association and emphasizes the potential risk posed to commonly consumed cereals in Sri Lanka.

ACKNOWLEDGMENTS

This project was supported by the University of Sri Jayewardenepura under the research grant No. ASP/01/RE/SCI/2021/14.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Akram, W., Anjum, T., Ahmad, A. & Moeen, R. (2014). First report of *Curvularia lunata* causing leaf spots on *Sorghum bicolor* from Pakistan. *Plant Disease*, **98**(7), 1007-1007.

Chang, J. Y., Zhang, H. J., Shi, J., Guo, N., Hu, Q. Y., Ma, H. X. & Yang, S. (2016). First report of *Curvularia australiensis* causing leaf spot on maize (*Zea mays*) in China. *Plant Disease*, **100**(8), 1780-1780.

Chomnunti, P., Schoch, C.L., Aguirre-Hudson, B., Ko-Ko, T.W., Hongsanan, S., Jones, E.B., Kodsueb, R., Phookamsak, R., Chukeatirote, E., Bahkali, A.H. & Hyde, K.D. (2011). Capnodiaceae. *Fungal Diversity*, **51**(1), 103-134. https://doi.org/10.1007/s13225-011-0145-6

Ferdinandez, H.S., Manamgoda, D.S., Udayanga, D., Deshappriya, N. & Munasinghe, M.L.A.M.S. (2020). Morphological and molecular characterization of two graminicolous *Exserohilum* species associated with cultivated rice and early barnyard grass from Sri Lanka. *Ceylon Journal of Science*, **49**(5), 381-387. http://doi.org/ 10.4038/cjs.v49i5.7805

Ferdinandez, H.S., Manamgoda, D.S., Udayanga, D., Deshappriya, N., Munasinghe, M.S. & Castlebury, L.A. (2021). Molecular phylogeny and morphology reveal three novel species of *Curvularia* (Pleosporales, Pleosporaceae) associated with cereal crops and weedy

grass hosts. *Mycological Progress*, **20**(4), 431-451. https://doi.org/10.1007/s11557-021-01681-0

Garcia-Aroca, T., Doyle, V., Singh, R., Price, T. & Collins, K. (2018). First report of Curvularia leaf spot of corn, caused by *Curvularia lunata*, in the United States. *Plant Health Progress*, **19**(2), 140-142.

Hidayat, I. & Ramadhani, I. (2019). Phylogenetic study of *Curvularia* on sorghum from Indonesia based on ITS rDNA sequence. *Jurnal Mikologi Indonesia*, **3**(2), 118-124. http://doi.org/10.46638/jmi.v3i2.64

Kaspary, T.E., Bellé, C., Rigon, C.A.G., Cutti, L., Casarotto, G., Gallon, M. & Merotto Junior, A. (2019). *Bipolaris oryzae* causing brown leaf spot on *Echinochloa crus-galli* in Southern Brazil. *Plant Disease*, **103**(5), 1038. https://doi.org/10.1094/PDIS-11-18-2057-PDN

Kusai, N. A., Mior Zakuan Azmi, M., Zulkifly, S., Yusof, M. T. & Mohd Zainudin, N. A. I. (2016). Morphological and molecular characterization of *Curvularia* and related species associated with leaf spot disease of rice in Peninsular Malaysia. *Rendiconti lincei*, **27**, 205-214.

Manamgoda, D.S., Cai, L., McKenzie, E.H., Crous, P.W., Madrid, H., Chukeatirote, E., Shivas, R.G., Tan, Y.P. & Hyde, K.D. (2012). A phylogenetic and taxonomic re-evaluation of the *Bipolaris-Cochliobolus-Curvularia* complex. *Fungal Diversity*, **56**(1), 131-144. https://doi.org/10.1007/s13225-012-0189-2

Manamgoda, D.S., Rossman, A.Y., Castlebury, L.A., Chukeatirote, E. & Hyde, K.D. (2015). A taxonomic and phylogenetic re-appraisal of the genus *Curvularia* (Pleosporaceae): human and plant pathogens. *Phytotaxa,* **212**(3), 175-198. https://doi.org/10.11646/phytotaxa.212.3.1

Manzar, N., Kashyap, A. S., Sharma, P. K. & Saxena, A. K. (2021). First report of leaf spot of maize caused by *Curvularia geniculata* in India. *Plant Disease*, **105**(12), 4155.

Marin-Felix, Y., Senwanna, C., Cheewangkoon, R. & Crous, P. W. (2017). New species and records of *Bipolaris* and *Curvularia* from Thailand. *Mycosphere,* **8**(9), 1556-1574. https://doi.org/10.5943/mycosphere/8/9/11

Marin-Felix, Y., Hernandez-Restrepo, M. & Crous, P. W. (2020). Multi-locus phylogeny of the genus *Curvularia* and description of ten new species. *Mycological Progress*, **19**, 559-588. https://doi.org/10.1007/s11557-020-01576-6

Rayner, R.W. (1970). A mycological colour chart. Commonwealth Mycological Institute, UK

Raza, M., Zhang, Z.F., Hyde, K.D., Diao, Y.Z. & Cai, L. (2019). Culturable plant pathogenic fungi associated with sugarcane in southern China. *Fungal Diversity,* **99**(1), 1-104. https://doi.org/10.1007/s13225-019-00434-5

Qostal, S., Kribel, S., Chliyeh, M., Selmaoui, K., Ouazzani, A., Serghat, S. & Douira, A. (2019). *Curvularia spicifera*, a parasite of the fungal complex of root rot of wheat and barley in Morocco. *Plant cell biotechnology and molecular biology*, **20**(9-10), 354-365.

Tan, Y. P., Madrid, H., Crous, P. W. & Shivas, R. G. (2014). *Johnalcornia* gen. et. comb. nov., and nine new combinations in *Curvularia* based on molecular phylogenetic analysis. *Australasian Plant Pathology*, **43,** 589-603. https://doi.org/10.1007/s13313-014-0315-6

Tan, Y. P., Crous, P. W. & Shivas, R. G. (2018). Cryptic species of *Curvularia* in the culture collection of the Queensland Plant Pathology Herbarium. *MycoKeys*, **35**, 1-25. https://doi.org/10.3897/mycokeys.35.25665.