



RESEARCH ARTICLE

Morpho-molecular characterisation of *Colletotrichum siamense* causing leaf blight disease of *Acorus calamus* from West Bengal, India

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Abstract

The present study investigated the leaf blight disease of Sweet flag (*Acorus calamus* L.) during the cropping seasons of 2019–20 and 2020–21. Field observations showed that all five tested varieties (Nagireddigudem, Munipalli, Gaddipalli, Aihagaripalli and Symbolia) were susceptible, with maximum disease severity recorded between February and March. The highest disease severity was recorded on Nagireddigudem (37.87 %) in 2019–20 and Munipalli (39.54 %) in 2020–21. The pathogen associated with the disease was identified morphologically as a *Colletotrichum* species, producing white to grey cottony mycelium, hyaline cylindrical conidia, melanised septate setae and dark brown appressoria. Pathogenicity tests confirmed symptom reproduction within 10–12 days post-inoculation. Molecular characterisation through Internal Transcribed Spacer (ITS) sequencing and phylogenetic analysis further confirmed the pathogen as *Colletotrichum siamense* (GenBank Accession: MT672519). For the management of disease, five isolates of *Trichoderma* as a bioagent and five botanical extracts were tested against the pathogen, along with a check. *In vitro* evaluations demonstrated significant inhibition of the pathogen by all *Trichoderma* isolates, with T5 (TriK5) showing the highest suppression (53.5 %). Among botanical extracts, *Withania somnifera* exhibited the maximum inhibition (57.4 %), whereas the fungicides carbendazim and copper oxychloride provided the highest suppression at 100 % and 67.4 %, respectively. The study confirms *C. siamense* as the causal agent of leaf blight in *A. calamus* and identifies potential biocontrol, botanical and chemical options for disease management.

Keywords: *Acorus calamus*; biocontrol agents; botanical extracts; *Colletotrichum siamense*; ITS sequencing; leaf blight; *Trichoderma*

Introduction

Sweet flag (*Acorus calamus* L.), belonging to the family Acoraceae, is a semi-aquatic, perennial, aromatic plant with creeping rhizomes (1). The plant is considered to have originated in India and is currently distributed across Sri Lanka, Myanmar, China, Japan, Europe, southern Russia and North America (2). In India, the crop is found under both wild (Himalayan tracts of Kashmir, Himachal Pradesh and in the evergreen forest of South India, Manipur and the Naga hills) and cultivated lands in plains. The crop is cultivated in several Indian states, including Andhra Pradesh, Karnataka, Chhattisgarh, West Bengal, Odisha and Bihar, with Karnataka contributing nearly 70 % of the cultivated area. Approximately 77 % of the total production originates from cultivated fields, while about 23 % is collected from wild populations (3).

Sweet flag has a long history of medicinal uses in Asia (4). The plant has been reported to treat epilepsy, neurological disorders, chronic diarrhoea, diabetes, dysentery and certain tumours (2). It also has many other properties, including antifungal, antibacterial, anti-ageing, antioxidant, anti-cholinesterase, memory enhancing, tranquillising, wound healing, carminative, neuroprotective, spasmolytic, hypotensive and as a vascular modulator (5, 6). In addition to pharmaceutical properties, it is also valuable in food (as flavouring of pipe tobacco and in tea and wine) and perfumery (2).

Several biotic and abiotic factors affect the successful cultivation of the crop. Among biotic constraints, several fungal diseases affect the crop. These include leaf rust caused by *Uromyces acori*, leaf spot caused by *Nigrospora oryzae*, leaf spot caused by *Curvularia pseudobranchyspora* and collar rot caused by *Sclerotium rolfsii*. (4, 7–9). *Colletotrichum* species exhibit high genetic and

morphological variability, complicating species identification. Reliance on morphological and cultural characteristics alone is often unreliable due to overlapping traits, phenotypic plasticity and cryptic species (10, 11). Molecular tools, particularly ITS rDNA sequencing, are therefore essential for accurate identification and phylogenetic analysis, with recent studies emphasising multimarker approaches to resolve species complexes. In India, species such as *Colletotrichum gloeosporioides*, *C. capsici*, *C. truncatum* and *C. siamense* infect a wide range of crops, causing significant losses in yield and quality. These pathogens can remain latent and become aggressive under favourable conditions, especially high humidity and moderate temperatures, leading to disease outbreaks (11–13).

During field surveys conducted at Kalyani, West Bengal, India, a severe leaf blight disease of *Acorus calamus* was observed, characterised by the progression of marginal chlorotic lesions to extensive necrosis of the leaf lamina. As this disease had not been previously reported in the region, the present investigation was undertaken to identify the causal agent using morphological and molecular approaches and to explore sustainable management strategies. The present study was therefore conducted to determine the aetiology and assess the severity of leaf blight disease of *Acorus calamus*, to characterise the associated *Colletotrichum* species through morphological and ITS-based molecular approaches and to evaluate eco-friendly disease management strategies using antagonistic microorganisms and plant-derived botanical extracts. Morphological characterisation remains essential for initial identification and understanding of pathogen traits, such as colony growth and spore structures, which are relevant for developing effective management strategies. This study is significant for farmers as it provides reliable pathogen identification, which can guide timely and targeted disease control measures, reducing crop loss and improving yield.

Materials and Methods

Sampling and isolation of the causal agent

Infected leaf samples from each variety (5 samples) were collected from the medicinal plant garden, C Block farm, BCKV, Kalyani, West Bengal, India (22°59'21"N latitude and 88°27'18" E longitude) during the 2019-20 season and brought to the laboratory for further study. The whole isolation process was performed under aseptic conditions in a laminar air flow. Before the isolation process, diseased leaves showing typical symptoms were washed in tap water for a few minutes to remove dirt. Leaf segments (3–5 mm) containing both diseased and healthy tissues were excised using a sterile blade. The segments were surface sterilised with 0.1 % mercuric chloride (HgCl₂) for 1 min and rinsed three times with sterile distilled water. Excess moisture was removed using sterile blotting paper. Four segments were placed on water agar supplemented with streptomycin (0.05 g L⁻¹) using sterile forceps. Plates were incubated at 25 °C ± 1°C. After 3 to 4 days of incubation, emerging hyphal tips were transferred to potato dextrose agar (PDA) plates for purification.

Field screening of sweet flag varieties against leaf blight disease

To find out the natural incidence and severity of leaf blight disease, five varieties of sweet flag (*Acorus calamus* L.), viz., Aihagaripalli, Gaddipalli, Munipalli, Nagireddigudem and Symbolia were used in the present study. Single-node rhizome cuttings of each variety were planted in a plot of 4 × 4 m² in RCB design with 4 replications.

Ten randomly selected plants from each replicated plot of the variety were used to study the incidence and severity of leaf blight disease during the cropping season. Periodic supervision of disease symptoms was done at 15-day intervals. The % disease index (PDI) of leaf blight was assessed using 0-5 scoring scale where 0= no or a few lesion on leaf; 1= Up to 10 % leaf area affected; 2= 11-25 % leaf area affected, 3= 26-50 % leaf area affected, 4= 51-75 % leaf area affected and 5 = above 75 % leaf area affected (14). The % Disease Index was computed based on the formula as described below (15).

$$\% \text{ disease index (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves} \times \text{Maximum score}} \times 100 \quad (\text{Eqn.1})$$

Pathogenicity tests

A representative fungal isolate was tested for pathogenicity on healthy mature leaves under controlled conditions. Leaves were surface sterilised with 0.1 % mercuric chloride (HgCl₂) solution, followed by spraying sterile distilled water using a spray bottle. The disinfected leaves were then covered with plastic bags and allowed to dry before inoculation. Conidial suspension with a spore concentration of 10⁵ conidia/mL was prepared using a 10-day-old culture in sterile distilled water. The sterilised plant leaves of ten plants were inoculated with conidial suspension by the pin-prick method. Sterile water was sprayed onto the leaves of two plants with the help of a hand-held sprayer, which served as a control. These artificially inoculated plants were covered with polythene bags to maintain the humidity above 80 %.

Symptoms and pathogen morphological

Diseased symptoms were studied visually on the standing plants in the field and also with the help of a Zeiss stereo microscope (Model- Discovery V8). For morphological identification of the pathogen, microscopic slides were prepared from a 10-day-old culture using lacto-phenol and observed under a Zeiss light microscope (Axio Scope.A1) and Nikon Trinocular Research Microscope (Model: Ci-L). Photographs of the specimen were taken with the Nikon Digital CMOS (Model- DSFi3), a fitted camera on a microscope and measurements were taken with Carl Zeiss Vision (Axio Vision LE Rel. 4.3), an Image Analyzing Software.

DNA extraction, PCR amplification and Sequence analysis

The mycelium (0.5 g–1 g) was taken from potato dextrose broth and ground in 500 µL of CTAB buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2 % cetyltrimethylammonium bromide, 2 % β mercaptoethanol) to a fine paste with the help of a sterile mortar pestle. Total genomic DNA was extracted from the mycelium using the CTAB method (16). Quality of DNA was estimated through agarose (1 %) gel electrophoresis with Ethidium bromide (EtBr) stain and visualised with UV light using Gel Doc (Bio Rad). The Internal transcribed spacer (ITS) regions of the 18S rDNA were amplified using universal primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (17). A 25 µL total volume PCR reaction was performed using 12.5 µL of PCR master mix (Xcelris Labs), 2 µL of genomic DNA 1 µL of each primer and 8.5 µL of nuclease-free water. The PCR amplification procedure was: 94 °C for 3 min, 35 cycles of 94 °C for 30 sec., 55 °C for 45 sec., 72 °C for 1.5 min and a cycle of 72 °C for 8 min. The consensus sequence was prepared using BioEdit 7.2.5 (18) and analysed through the Blastn test in the NCBI database for putative identification of the causal agent (18). To understand the evolution of the fungal pathogen

characterised in our study, multiple sequences were aligned using Clustal Omega and a phylogenetic tree was constructed using MEGAX software (19).

Inhibitory effect of biocontrol agents and botanicals on the pathogen

The biocontrol agents were isolated from the rhizosphere soil of healthy plants collected from different locations. Five different *Trichoderma* isolates were isolated using the serial dilution technique. The biocontrol potential against the leaf blight pathogens was evaluated by a dual culture test. The degree of antagonisms between each bioagent and test pathogen in dual culture was scored on a scale of 1-5 (20). To find out the antifungal activity of plants' crude extracts against pathogens, five medicinal plants viz., *Withania somnifera* (Aswagandha), *Calotropis gigantea* (Akanda or Madaar), *Azadirachta indica* (Neem), *Rauvolfia serpentina* (Sarpagandha) and *Ocimum tenuiflorum* (Tulsi), were tested at 10 % concentration through the poisoned food technique. The antifungal activity was evaluated by the poisoned food technique on PDA medium in three replications using a completely randomised design (CRD). Two fungicides, Copper Oxchloride 50WP at 4g/L and Carbendazim 50 % WP at 1g/L were also taken as positive control for comparison. During the present study, all the cultures were maintained on PDA medium and incubated at 25 °C ± 1 °C. The inhibition percentage of the pathogen was calculated by the following formula (21):

$$\% \text{ age of radial growth inhibition (\% RGI) } = \frac{(C - T)}{C} \times 100 \quad (\text{Eqn.2})$$

Where, C= growth in control; T= growth in treatment

Statistical analysis

In vitro experiments were conducted using a completely randomised design (CRD), while *in vivo* experiments followed a randomised block design (RBD). Data were analysed by analysis of variance (ANOVA) and treatment means were compared at the 5 % significance level using appropriate post hoc tests. Molecular characterisation was performed through sequence analysis and phylogenetic methods to confirm species identity.

Results

Disease symptoms and seasonality

Initial symptoms appeared on the leaf margins or tips as small chlorotic lesions. These lesions first extended to cover the entire leaf

margin and gradually spread to the midrib, eventually killing the entire leaf lamina. The affected leaves became blighted and withered. Older leaves were generally more susceptible to the disease (Fig. 1). The seasonality and incidence of disease were recorded during the two-cropping season, i.e. 2019–20 and 2020–21. During 2019–20, the highest % disease index (PDI) was recorded in Nagireddigudem (37.87 %), followed by Munipalli (36.69 %), Gaddipalli (30.39 %), Aihagaripalli (26.82 %) and Symbolia (36.69 %). The seasonality of disease was also recorded during the year 2020–21 (Table 1 and Fig. 2). The maximum severity of the disease was recorded on Munipalli (39.54 %), followed by Nagireddigudem (38.21 %), Aihagaripalli (34.25 %), Gaddipalli (32.29 %) and the least was on Symbolia (25.38 %). Therefore, all five varieties were susceptible to the disease. The disease was noticed in the month of September, whereas the peak period of leaf blight disease was during the month of February to March (Table 1 and Fig. 2). Significant differences in disease severity were observed among varieties ($p \leq 0.05$).

Morphological characterisation of the associated pathogen

The isolated fungus belongs to the genus *Colletotrichum*, producing white to grey cottony mycelium with a smooth colony margin on PDA medium at 25 ± 1 °C. The underside of the colony was white with dark grey patches (Fig. 3). The conidia were single-celled, hyaline and cylindrical with obtuse ends. The average size of conidia was 12.56-16.67 × 3.91-5.60 µm (Mean: 14.43 ± 1.05 × 4.80 ± 0.41). Conidiophores were hyaline, cylindrical, straight to flexuous, septate, sometimes branched, 5.37-14.04 × 1.61-3.87 µm (9.87 ± 2.04 × 2.89 ± 0.49). Setae were thick-walled, melanised, multi-septate (2-4), 30.67-78.29 µm (mean: 52.33 ± 13.00) in length and 3.00-6.25 µm (mean: 4.09 ± 0.88) in width. Appressoria were brown to dark brown in colour, with various shapes such as ovoid, ellipsoidal, clavate or irregular, 4.74-9.48 µm (mean: 6.84 ± 0.90) in length and 3.71-5.91 µm (mean: 4.88 ± 0.52) in width (Fig. 3).

Pathogenicity tests

Inoculated plants were examined regularly for typical leaf blight symptoms development as observed in the field. Symptom development took place after 10 to 12 days after the inoculation, whereas no symptoms were observed on the control plants. Symptoms first appeared at the margin of leaves as dark blighted lesions with a yellow halo and gradually spread to the middle. The pathogen was re-isolated from infected leaves and its morphology matched that of the original isolate.

Molecular characterisation and phylogenetic analysis

Molecular analysis was conducted to confirm the identity of the fungal isolate. The nucleotide sequences of the ITS region were



Fig. 1. Leaf blight symptoms on *Acorus calamus* observed under field conditions.

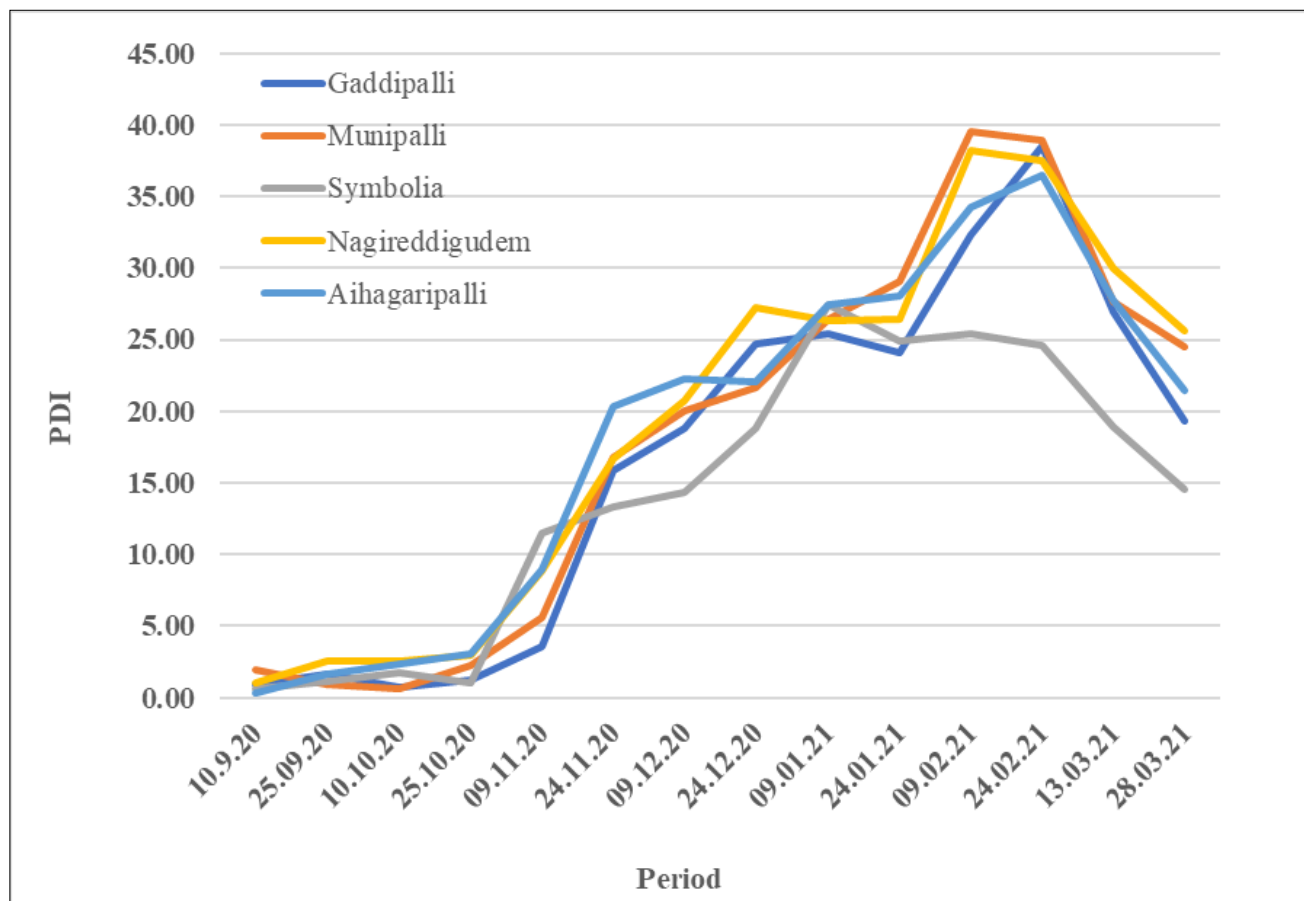


Fig. 2. Seasonal progression of leaf blight severity (% disease index) in five *Acorus calamus* varieties during the 2020–2021 cropping season at Kalyani, West Bengal, India.

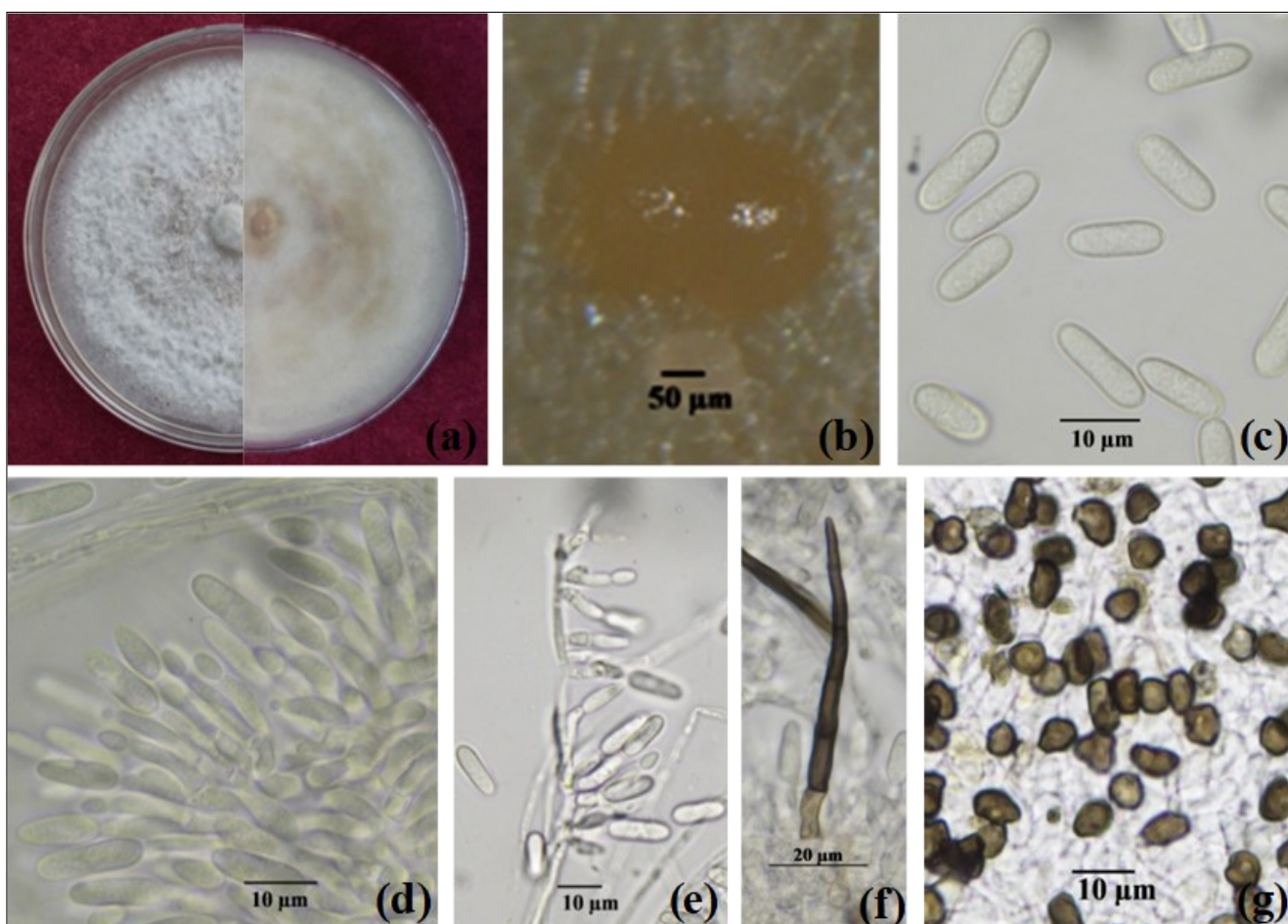


Fig. 3. Morphological characteristics of *Colletotrichum siamense* (Kalyani isolate-1). a. Colony morphology on potato dextrose agar and the reverse side of the culture plate; b. conidiomata; c. hyaline cylindrical conidia; d.–e. conidiophores bearing conidia; f. melanised septate setae; g. Dark brown appressoria were observed under light microscopy.

Variety	Period													
	10.9.20	25.09.20	10.10.20	25.10.20	09.11.20	24.11.20	09.12.20	24.12.20	09.01.21	24.01.21	09.02.21	24.02.21	13.03.21	28.03.21
Saddipalli	0.96 (5.61)	1.59 (7.24)	0.70 (4.64)	1.26 (6.45)	3.58 (10.89)	15.86 (23.47)	18.83 (25.71)	24.73 (29.82)	25.37 (30.22)	24.07 (29.36)	32.29 (34.62)	38.50 (38.35)	26.96 (31.28)	19.37 (26.06)
	1.96 (8.04)	0.89 (5.41)	0.61 (4.46)	2.27 (8.63)	5.61 (13.69)	16.77 (24.17)	20.02 (26.58)	21.69 (27.75)	26.41 (30.92)	29.05 (32.60)	39.54 (38.96)	38.91 (38.59)	27.66 (31.72)	24.45 (29.64)
	0.59 (4.39)	1.16 (6.19)	1.73 (7.54)	1.06 (5.90)	11.48 (19.80)	13.37 (21.44)	14.37 (22.28)	18.80 (25.69)	27.49 (31.59)	24.90 (29.93)	25.38 (30.25)	24.63 (29.75)	18.87 (25.75)	14.57 (22.43)
Nagireddigudem	1.06 (5.84)	2.59 (9.24)	2.55 (9.19)	2.95 (9.88)	8.82 (17.28)	16.63 (24.07)	20.75 (27.09)	27.28 (31.49)	26.31 (30.85)	26.43 (30.93)	38.21 (38.17)	37.49 (37.72)	29.98 (33.19)	25.64 (30.42)
	0.36 (3.41)	1.64 (7.34)	2.30 (8.71)	3.07 (10.09)	8.99 (17.44)	20.37 (26.83)	22.31 (28.18)	22.11 (28.04)	27.48 (31.61)	28.06 (31.98)	34.25 (35.82)	36.54 (37.19)	27.74 (31.78)	21.49 (27.61)
	0.34	0.38	0.50	0.36	0.40	0.26	0.55	0.31	0.98	0.76	0.66	1.23	0.61	0.74
SEm ±	0.75	0.83	1.09	0.78	0.87	0.58	1.21	0.68	2.15	1.65	1.45	2.68	1.33	1.61
CV (%)	8.89	7.64	10.28	6.21	3.58	1.56	3.01	1.54	4.49	3.46	2.64	4.79	2.82	3.83

Average of 4 replications. Values in the parentheses angular transformed values used for statistical analysis.

In vitro evaluation of biocontrol agents and botanicals

The results of the dual culture test presented in Table 2 show that all the *Trichoderma* isolates significantly reduced the mycelium growth of the test fungus under *in vitro* conditions over the control treatment. Among the tested *Trichoderma* isolates, the highest inhibition on radial growth of the pathogen was by T₅ (53.5 %), followed by T₄ (52.6 %) and T₃ (44.4 %). Whereas, the %age inhibition by the remaining two isolates, T₁ and T₂, was found statistically at par, i.e. 40.8 %. It was also noted that all the *Trichoderma* isolates were effectively hyperparasitized on the pathogen at the latter stage (Fig. 5). Additionally, five different medicinal plant extracts were evaluated against the test fungus, using the food poisoning technique (Table 3). Among them, only Ashwagandha had shown better results with %age inhibition of the test fungus by 57.4 %. However, the %age of inhibition of radial growth was highest by the two fungicides, i.e. carbendazim (100 %) followed by copper oxychloride (67.4 %) (Table 3).

Discussion

The present study confirmed *Colletotrichum siamense* as the causal agent of leaf blight in *Acorus calamus* based on morphological, pathogenicity and molecular evidence. In the Indian traditional medicine system, the roots and rhizomes of this plant are used as a brain and nervous system rejuvenator (22). Plant diseases are one of the crucial biotic factors which are responsible for the reduction of production of several crops, including Sweet flag. Among plant-pathogenic fungi, *Colletotrichum* species are the most common, causing diseases on a variety of plant hosts worldwide (23, 24). It can cause anthracnose and leaf blight of aerial plant parts, post-harvest rots and also occur as symptomless endophytes in living plants (19, 25).

The isolate exhibited morphological features typical of *Colletotrichum*, including cottony mycelium, hyaline cylindrical conidia, septate conidiophores, melanised setae and variably shaped brown appressoria. ITS sequencing confirmed the identity as *Colletotrichum siamense* (GenBank Acc. MT672519) and phylogenetic analysis clustered it closely with other *C. siamense* sequences, clearly distinguishing it from related species. Identification of *Colletotrichum* species based on morphological characters (including the size and shape of conidia, appressoria and cultural features) is also crucial but has limitations due to very few differences in anamorphs and rare development of the teleomorphic stage (26-28). Currently, morphological characterisation of plant pathogens combined with

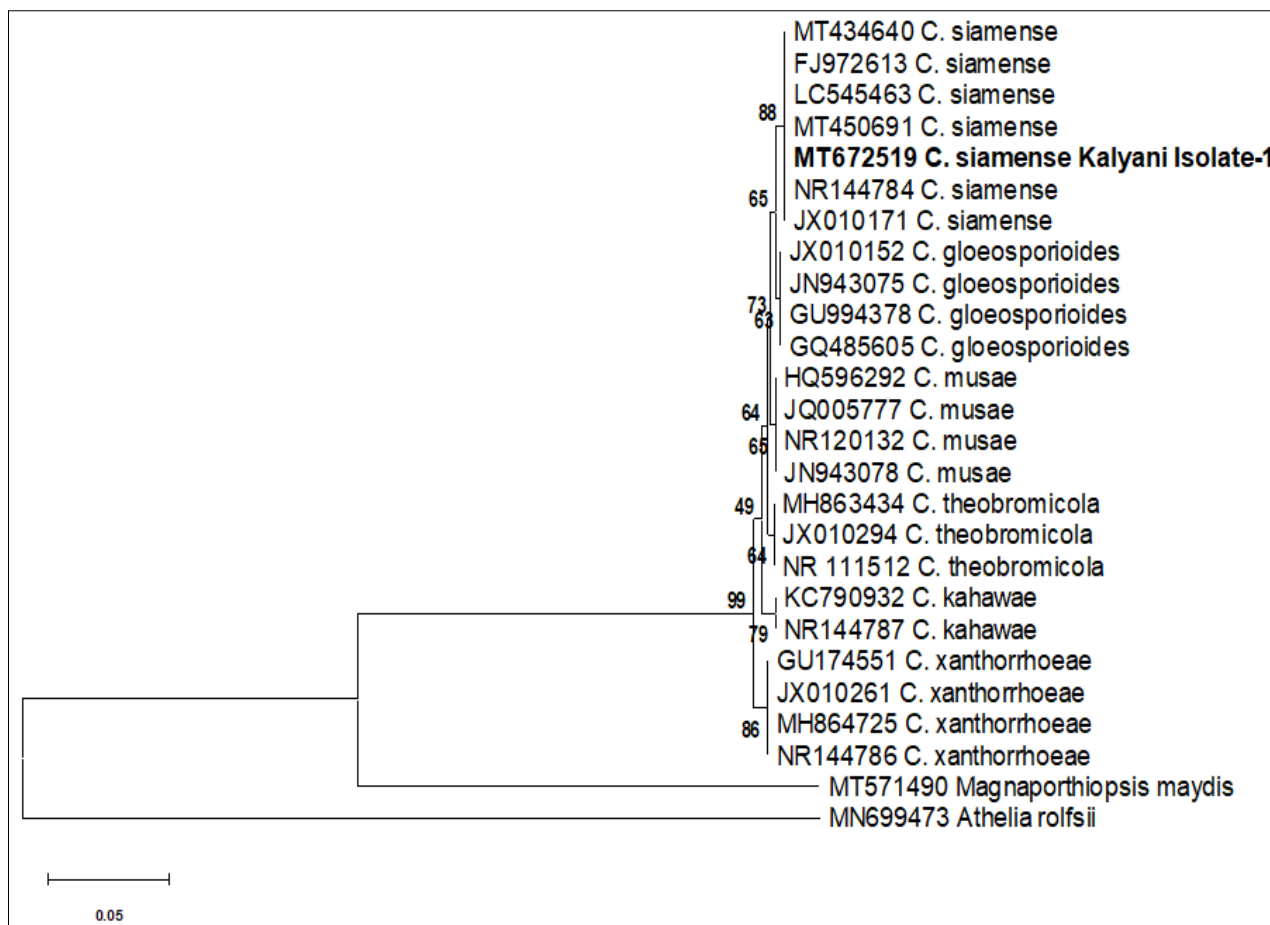


Fig. 4. Neighbour-joining phylogenetic tree illustrating the relationship of different *Colletotrichum siamense* isolates based on the partial sequences of internal transcribed spacer (ITS) regions. The sequence of *Colletotrichum siamense* (Kalyani Isolate-1) from this study is indicated by bold font. The evolutionary history was inferred by the Neighbour-joining method based on the Jukes-Cantor method and bootstrap support values with 1000 replicates are shown at the nodes of the branches. The branches with bootstrap values less than 50 % were collapsed. The scale bar indicates the number of substitutions per site.

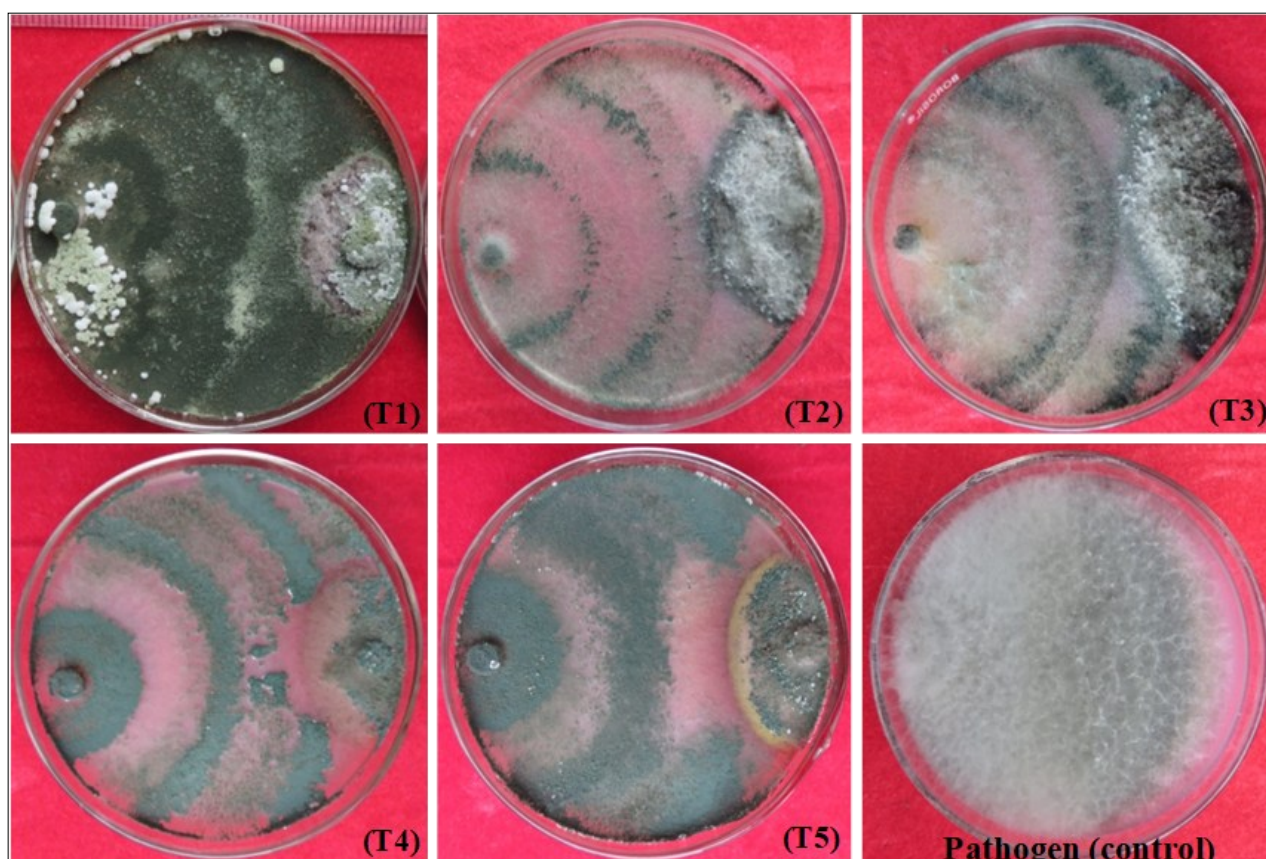


Fig. 5. Antagonistic interaction between *Trichoderma* isolates and *Colletotrichum siamense* in dual culture assay on potato dextrose agar showing inhibition of pathogen growth and overgrowth of the pathogen colony by the antagonist.

Table 2. *In vitro* efficacy of biocontrol agents against *Colletotrichum siamense*

Biocontrol agents	Mycelial growth (in cm) *	% inhibition	Bell's scale
T ₁ (TriK1)	2.5	40.8 (39.7)	2
T ₂ (TriK2)	2.5	40.8 (39.7)	2
T ₃ (TriK3)	2.3	44.5 (41.8)	1
T ₄ (TriK4)	2.0	52.6 (46.5)	1
T ₅ (TriK5)	2.0	53.5 (47.0)	1
Pathogen (Check)	4.2	-	
Sem±	0.1	2.5	
CD (5 %)	0.3	5.5	
CV (%)	6.5	7.0	

*Average of 3 replications. Values in the parentheses indicate the angular transformed values; Tri: Trichoderma; K: Kalyani (Native isolates)

Table 3. Antifungal activity of plant extracts against *Colletotrichum siamense* under *in vitro* conditions.

Medicinal plants	Mycelial growth (in cm)*	% inhibition
Aswagandha	3.8	57.4 (49.3)
Calotropis	8.4	6.3 (14.5)
Neem	7.9	11.9 (20.1)
Sarpagandha	9.0	0.0 (0.0)
Tulsi	8.7	3.7 (11.1)
Blitox (check)	2.9	67.4 (55.2)
Bavistin (check)	0.0	100.0 (90.0)
Control	9.0	-
Sem±	0.07	0.8
CD (5 %)	0.16	1.6
CV (%)	1.47	2.7

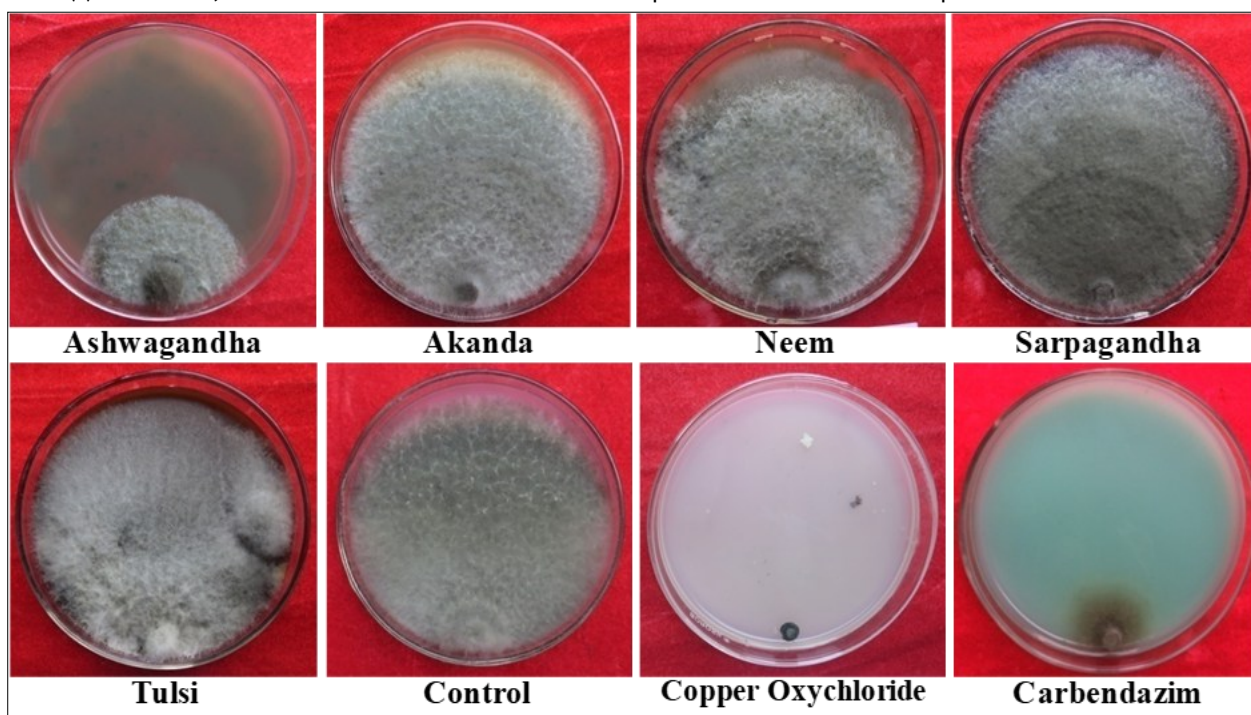
* Average of 3 replications. Values in the parentheses indicate the angular transformation

DNA sequence-based methods is recommended for species-level identification (29, 24). Molecular identification using ITS sequences plays an important role in the identification of the *Colletotrichum* species (30).

Leaf blight disease of sweet flag caused by *Colletotrichum siamense* was first observed during a field survey at Kalyani, West Bengal, India (22°59'21"N latitude and 88°27'18"E longitude) in the year 2019 (1). Since then, this disease has been known to occur

regularly, showing the characteristic symptoms as mentioned above, with disease severity ranging between 25 to 40 % (Table 1 and Fig. 2). The severity of five different varieties, viz. Aihagaripalli, Gaddipalli, Munipalli, Nagireddigudem and Symbolia were recorded during the years 2019–20 and 2020–21. Maximum severity was recorded on Munipalli (39.54 %), followed by Nagireddigudem (38.21 %), Aihagaripalli (34.25 %), Gaddipalli (32.29 %) and minimum PDI was on Symbolia (25.38 %). All tested sweet flag varieties were found susceptible to leaf blight diseases. Initially, the disease appeared in the month of September; however, the peak period was during February and March. The associated pathogen *Colletotrichum siamense* was isolated and its pathogenicity was confirmed to fulfil Koch's postulates. The accurate identification of the pathogen, both morphological and molecular characterisation, was done. The morphological characters were found to be similar as previous research (30-32). Molecular characterisation done using nucleotide sequences of the Internal Transcribed Spacer (ITS) region was analysed using the NCBI database, which showed 100 % sequence similarity with *Colletotrichum siamense* (LC545463, MT434640, MT450691, etc.). The assembled sequence of *Colletotrichum siamense* (Kalyani Isolate-1) characterised in the present study was submitted to the NCBI GenBank with the accession number of MT672519.

The in-vitro evaluation of tested bio-control agents and botanicals significantly inhibited the radial growth of the pathogen (Table 2–3). All tested *Trichoderma* isolates significantly inhibited the radial growth of the pathogen however the maximum inhibition was recorded by T₅ (53.5 %) and the minimum by T₁ and T₂ (40.8 %), when compared with control plate (Table 2 and Fig. 5). All *Trichoderma* isolates had high growth and sporulation rate and also showed hyperparasitism on tested fungal pathogen i.e. *Colletotrichum siamense*. Among the tested botanicals, only Ashwagandha showed antifungal activity and reduced the radial growth by 57.4 % as compared to the control (Table 3 and Fig. 6). The antifungal activity of *W. somnifera* may be related to the presence of bioactive compounds such as withanolides and

**Fig. 6.** Antifungal activity of plant extracts against *Colletotrichum siamense* evaluated by the poisoned food technique on potato dextrose agar, showing reduction in mycelial growth compared with the untreated control.

alkaloids. Although the present study demonstrated promising inhibitory effects under laboratory conditions, field evaluation is required to confirm their effectiveness under natural conditions. The combined morphological and molecular analyses confirmed *Colletotrichum siamense* as the causal agent of leaf blight of *Acorus calamus*. The pathogen showed considerable susceptibility to antagonistic *Trichoderma* isolates and to extracts of *Withania somnifera* under laboratory conditions. These findings provide a basis for developing integrated disease management strategies for sweet flag cultivation.

Conclusion

The fungal genus *Colletotrichum*, consisting of more than 189 species are cosmopolitan plant pathogen distributed throughout tropical and temperate regions, causing several devastating diseases of valuable crops worldwide. Presently, identification of this pathogen must include morphological and molecular characterisation. In the present study, it is evident from the phylogenetic tree that the ITS sequence of the present fungal isolate (Kalyani Isolate-1) was genetically nearest to *Colletotrichum siamense*, which forms a distinct cluster in phylogeny analysis based on the rDNA-ITS sequences. In conclusion, the results revealed that leaf blight disease of sweet flag is caused by *Colletotrichum siamense*. To the best of our knowledge, this is the first report of leaf blight caused by *Colletotrichum siamense* on *Acorus calamus* in India and worldwide, as reported by our previous study (1). The *in vitro* evaluation of biocontrol agents and crude extract of Ashwagandha significantly suppresses the % radial growth of the *Colletotrichum siamense*. Therefore, it is recommended that farmers and researchers adopt biocontrol agents such as *Trichoderma* and botanical extracts like Ashwagandha as effective, eco-friendly and cost-efficient alternatives to chemical pesticides.

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Authors' contributions

SS performed the experiments. SC and SM designed the research. DKC and PK wrote the manuscript. SO, BM revised and corrected the manuscript. GM, MM, BSM and PP have helped me during my research experiments. All authors have contributed to different sections of writing, reviewing, correcting and statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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References

1. Singh S, Mondal G, Mondal B. First report of leaf blight disease

caused by *Colletotrichum siamense* on *Acorus calamus*. New Dis Rep. 2021;44(2):212046. <https://doi.org/10.1002/ndr2.12046>

2. Haghighi SR, Asadi MH, Akrami H, Baghizadeh A. Anti-carcinogenic and anti-angiogenic properties of extracts of *Acorus calamus* on gastric cancer cells. Avicenna J Phytomed. 2017;7(2):145–56.
3. Shetty GR, Shruthi AM. A review on pharmacology of *Acorus calamus* - An endangered medicinal plant. Int J Pharma Bio Sci. 2015;6(1):605–21.
4. Singh S, Mondal G. A new report of rhizome rot of Sweet flag (*Acorus calamus* L.) caused by *Sclerotium rolfsii* from West Bengal, India. J Pharmacogn Phytochem. 2018;7(3):2417–9.
5. Kour GD, Sharma AK, Dash S, Bal N. Vacha (*Acorus calamus* Linn.): A valuable medicinal plant. Int J Ayurveda Pharma Res. 2014;2(8):1–11.
6. Shah AJ, Gilani AH. Aqueous-methanolic extract of sweet flag (*Acorus calamus*) possesses cardiac depressant and EDHF-mediated coronary vasodilator effects. J Nat Med. 2012;66:119–26. <https://doi.org/10.1007/s11418-011-0561-7>
7. Nirmalkar VK, Lakepale N. Symptomatology, characterisation and morphological variation of *Uromyces acori*: A destructive pathogen of sweet flag (*Acorus calamus* L.). J Soils Crops. 2008;18(1):59–61.
8. Paul PC, Dasgupta B. A new report of tip blight and leaf spot of *Acorus calamus* caused by *Nigrospora oryzae* from West Bengal, India. Bioscan. 2014;9(1):355–7. <https://doi.org/10.1071/APP9820009>
9. Srivastava AK, Saroj A, Nishad I, Samad A. First report of *Acorus calamus* leaf spot caused by *Curvularia pseudobrachyspora* in India. Plant Dis. 2019;103(4):767. <https://doi.org/10.1094/PDIS-08-18-1399-PDN>
10. Damm U, Cannon PF, Woudenberg JHC, Crous PW. The *Colletotrichum acutatum* species complex. Stud Mycol. 2019;92:45–126. <https://doi.org/10.1016/j.simyco.2018.04.001>
11. Talhinhas P, Baroncelli R. *Colletotrichum* species and complexes: Geographic distribution, host range and conservation status. Fungal Divers. 2021;110:109–98. <https://doi.org/10.1007/s13225-021-00491-9>
12. Raja HA, Miller AN, Pearce CJ, Oberlies NH. Fungal identification using molecular tools. Nat Prod Rep. 2017;34(7):1–19.
13. Sharma P, Sharma M, Raja M. Advances in molecular identification of fungal pathogens. J Appl Microbiol. 2022;132(5):1–12.
14. Goswami BK, Zahid MI, Haq MO. Screening of *Colocasia esculenta* germplasm to *Phytophthora* leaf blight. Bangladesh J Plant Pathol. 1993;9(1–2):21–4.
15. Wheeler BEJ. An introduction to plant diseases. London: John Wiley and Sons Ltd; 1969. p. 301.
16. Sharma R, Graeser Y. *Microsporium*. In: Molecular detection of human fungal pathogens. Boca Raton: CRC Press; 2011. p. 279–92.
17. White TJ, Bruns TD, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols. New York: Academic Press; 1990. p. 315–22. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>
18. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–8.
19. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35:1547–9. <https://doi.org/10.1093/molbev/msy096>
20. Bell DK, Wells HD, Markham CR. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology. 1982;72:379–82. <https://doi.org/10.1094/Phyto-72-379>
21. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature. 1947;150:850. <https://doi.org/10.1038/159850b0>

22. Pattanaik J, Kumar Y, Khatri RS. *Acorus calamus* Linn.: A herbal tonic for central nervous system. J Sci Innov Res. 2013;2(5):950–4.
23. Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, et al. The top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol. 2012;13:414–30. <https://doi.org/10.1111/j.1364-3703.2011.00783.x>
24. Huang R, Sun W, Wang L, Li Q, Huang S, Tang L, et al. Identification and characterisation of *Colletotrichum* species associated with anthracnose of banana. Plant Pathol. 2021;70(8):1827–37. <https://doi.org/10.1111/ppa.13426>
25. Samarakoon MC, Peršoh D, Hyde KD, Bulgakov TS, Manawasinghe IS, Jayawardena RS, et al. *Colletotrichum acidiae* sp. nov. from northern Thailand and a new record of *C. dematium* on *Iris* sp. Mycosphere. 2018;9(3):583–97. <https://doi.org/10.5943/mycosphere/9/3/9>
26. Hyde KD, Cai L, McKenzie EHC, Yang YL, Zhang JZ, Prihastuti H. *Colletotrichum*: A catalogue of confusion. Fungal Divers. 2009;39:1–17.
27. Cannon PF, Damm U, Johnston PR, Weir BS. *Colletotrichum*: Current status and future directions. Stud Mycol. 2012;73:181–213. <https://doi.org/10.3114/sim0014>
28. Jayawardena RS, Hyde KD, Damm U, Cai L, Liu M, Li XH, et al. Notes on currently accepted species of *Colletotrichum*. Mycosphere. 2016;7(8):1192–260. <https://doi.org/10.5943/mycosphere/si/2c/9>
29. Xiang MM, Zhang YX, Liu X. Research progress on taxonomy of *Colletotrichum*. J Zhongkai Univ Agric Eng. 2016;29:1–8.
30. Zhang Y, Long D, Wang J, Li Q, Wang Z, Lin W, et al. Morphological and molecular identification of *Colletotrichum siamense*, a novel leaf pathogen associated with *Sterculia lanceolata* in China. J Phytopathol. 2020;168(7–8):451–9. <https://doi.org/10.1111/jph.12909>
31. Prihastuti H, Cai L, Chen H, McKenzie EHC, Hyde KD. Characterisation of *Colletotrichum* species associated with coffee berries in northern Thailand. Fungal Divers. 2009;39(1):89–109.
32. De Silva DD, Groenewald JZ, Crous PW, Ades PK, Nasruddin A, Mongkolporn O, et al. Identification, prevalence and pathogenicity of *Colletotrichum* species causing anthracnose of *Capsicum annuum* in Asia. IMA Fungus. 2019;10(1):8. <https://doi.org/10.1186/s43008-019-0001-y>

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