

Assessment of the *Colletotrichum* Leaf Spot Disease of Turmeric in West Bengal, India: A Comprehensive Report on the Current Situation

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Abstract: The low productivity in turmeric in West Bengal is due to severity of the foliar diseases. The leaf spot disease survey was conducted and the report of Cooch Behar and Nadia districts of West Bengal reveals that highest percentage of disease severity (PDI) are shown by locally cultivated varieties such as Malsari (53.12%), Goma (57.49%), Pragati (55.35%) and Sonajuli (57.49%) with higher AUDPC value i.e. 4125.33, 3906.37, 4079.02 and 3237.89 respectively. On the other hand Resistance against leaf spot disease are shown by some varieties i.e. Uttar Rangini, Suranjana, Uttar Rupanjana, Lakadong, Megha etc. These varieties have shown lower PDI and lower AUDPC value compared to local varieties. The symptoms of leaf spot disease in turmeric, caused by *Colletotrichum* are characterized by variable-sized elliptical or oblong spots on the leaf blades forming greyish or brownish center with a yellow halo surrounded by the spots. Both leaf surfaces exhibit numerous black dot-like structures called acervuli, which are arranged in concentric rings. The spots are varied in size, ranging from 3.50 to 7.00 cm in length and approximately 3.40 to 4.60 cm in breadth. The pathogen is isolated from total eight locations during experimental findings. Code name of different collected isolates are given as CCMI-1(Dinhata), CCMI-2(Mondouri), CCPI-3(Pundibari), CCTI-4(Tufanganj), CCRI-5 (Rakhalmari Village), CCGI-6 (Gayeshpur Farm), CCBI-7 (Basantapur Village), CCLI-8 (CTRI Farm Dinhata). Morphological variations among the eight isolates are observed based on the size of conidia and setae. Isolate CCMI-2 have highest conidial length where as CCTI-4 has lowest size of conidia.

Keywords: Turmeric, *Colletotrichum*, PDI, Leaf spot

1. Introduction

Turmeric (*Curcuma longa*) is a perennial herbaceous crop, is a member of the zingiberaceae family. It is native to Southeast Asia and it has been used for centuries in traditional medicines for its many health benefits. In addition to its health benefits, turmeric is also a popular culinary spice. India is the world's leading producer and exporter of turmeric, with a production of over 1 million tonnes in 2021. (FAOSTAT, 2022). The West Bengal is the 7th largest producer of turmeric in India. In 2021-22, the state produced 45,900 tonnes of turmeric, which is about 3.9% of the country's total production. (National Horticulture Board, 2022). One of the factors for this low productivity in West Bengal is the severity of the foliar diseases. *Colletotrichum* leaf spot disease of turmeric is an important disease of turmeric can cause yield loss vary from 20% to more than 60% in some cases (Nair and Ramakrishnan, 1973). *Colletotrichum curcumae* is a causal agent of Leaf spot disease in turmeric. The fungus belongs to the phylum Ascomycota. The disease cause severe foliar damage. The symptoms are characterized by variable-sized elliptical or oblong spots on the leaf blades. These spots have greyish or

brownish center surrounded by a yellow halo. They can range from 1 to 3 cm in width and 2 to 6 cm in length (Li *et al.* 2015).

2. Materials and Methods

2.1 Survey on Leaf spot disease of Turmeric

In the turmeric growing season, August to December months are considered to be the peak period for the occurrence of turmeric leaf spot disease. An intensive fixed plot survey of leaf spot disease of turmeric was carried out during the months of July to November 2022 in some turmeric growing areas of Cooch Behar and Nadia districts (**Plate 1**).



Plate 1: Two Districts Covered under survey in West Bengal

For the survey, representative areas within the Cooch Behar and Nadia districts of West Bengal were chosen, considering factors such as intensity of turmeric cultivation among the areas, geographical diversity of the land areas, and accessibility of the planting materials. The village areas and farmer's fields were the first priority and institutional farms were also visited during this survey. In Cooch Behar five locations were selected for survey i.e. Dinhata CTRL, TufanganjFulbari, Putimari Village under Dinhata block 1, Rakhalmari Village under Dinhata block 2, and the fields under the AICRP Spices of UBKV, Pundibari. Where as in Nadia district the survey was conducted on three locations, i.e. Mondouri Farm BCKV, Gayeshpur village and Basantapur Village. The disease severity of leaf spot in turmeric plants were measured and recorded during data collection.

A standardized disease rating scale was used for this purpose presented in **Table 1**. [Mayee and Datar (1986)].

Percent disease index (PDI)

$$= \frac{\text{Sum of individual numerical disease ratings}}{\text{No. of units examined} * \text{Maximum disease scale}} \times 100$$

* (Unit = Leaves) (Wheeler. 1969)

Table 1: Description of diseased rating scale:

Scale	Disease severity
0	No symptoms on the plant
1	Small spots on leaves; less than 1 % area was infected
3	Medium spots on leaves; covering 1-10 % area of leaf
5	Bigger coalescing spots, covering 11 % to 25 % area of leaf
7	Large coalescing spots, covering 26 % to 50 % area of leaf
9	Spots covering more than 51 % to 75 % area of the leaf

Disease severity, name of the variety, location information (Latitude Longitude), date of survey, plant age, cultural practices, and any other relevant factors that might influence in the disease development were taken into consideration

accordingly. After completion of the survey period, the varieties under survey were categorized based on their resistance towards leaf spot disease. The characterization was done based on the following **Table 2**.

Table 2: Varietal Categorization

Percentage Disease Index (%)	Resistance category
0 – 10	Immune
Nov-20	Resistant
21-30	Moderately Resistant
31-50	Susceptible
> 51	Highly susceptible

The area under disease progress curve (AUDPC) was estimated by from two data points i.e. the initial and final assessments by using the following equation of **Shaner and Finney (1977)**:

$$AUDPC = \sum_{i=1}^{n-1} \left[\frac{(X_{i+1} + X_i)}{2} \right] \times (t_{i+1} - t_i)$$

Where,

X_i = the portion of host tissue damaged at i^{th} day

t_i = the time in days after appearance of the disease at the i^{th} day

n = the total number of observation

2.2 Isolation, Identification, Purification and maintenance of pathogen

First of all collected leaf samples were washed thoroughly by water. Next the leaf samples were cut into small pieces with half diseased portion (showing visible signs of infection) and half healthy portion by using sterile tools. In the laminar air flow chamber the cut pieces were surface sterilized by dipping in 1% sodium hypo-chloride (NaOCl) solution for 1 minutes. After that the cut pieces were washed by sterile distilled water for three times and kept on filter paper for absorbing the excess surface water. Then the pieces of the leaves were inoculated in PDA containing petri plates. The petri plates were kept in BOD incubator at $27 \pm 1^\circ\text{C}$. After 4-5 days of inoculation fungal colony and mycelial growths were observed. For pure culture, a single fungal spore or hyphal fragment was selected from a well-developed colony using a sterile needle or loop. The selected spore or fragment was then transferred to a new culture plate containing fresh medium (i.e. Potato Dextrose Agar). This step ensured that the resulting culture contained only a single strain of the pathogen. After obtaining pure culture the fungal pathogen was inoculated in test tubes and preserved inside refrigerator for future use.

2.3 Microscopic examination

Various features, such as spores, conidiophores, hyphae, acervuli, and setae, were observed using a compound microscope. Microscopic pictures were captured at 40X and 10X zoom and size of conidia, acervuli, setae (i.e. length and breadth) were measured by Axio Vision Software.

2.4 Pathogenicity tests

For *in-vivo* pathogenicity test turmeric plants of lakadong variety were grown in pot culture inside poly house during 15th June 2022 under the Dept. of Plant Pathology BCKV. After 2 months of planting the crop reached 3 to 4 leaf stage. Healthy turmeric plants with uniform growth and no visible disease symptoms were selected for the tests. Fungal spores were taken from actively growing cultures on PDA plates. Spores were then suspended in sterile distilled water, and the spore concentration was adjusted at 1×10^6 spores/ml (Adhipathiet *al.*, 2013) using a Haemocytometer. Sterilization was achieved by washing the leaves with a suitable sterilizing agent (e.g., 70% ethanol) followed by rinsing with sterile distilled water. Small wounds or punctures were made on the sterilized turmeric leaves using a sterile pin or needle. A spore suspension of *Colletotrichum* was applied onto the punctured area by carefully placing droplets onto the wounded site. Control turmeric leaves were treated similarly but with sterile distilled water. The inoculated plants were covered with polythene to maintain humidity for successful disease development. After an appropriate incubation period (5-6 DAI), disease symptoms, such as leaf spots or lesions, were visually assessed and recorded. To confirm the association of *Colletotrichum* with the observed disease symptoms, the pathogen was re-isolated from the infected turmeric leaves. Small pieces of symptomatic tissues were excised and placed onto PDA plates. The plates were incubated until the characteristic fungal colonies of *Colletotrichum* appeared. Microscopic observation of re-isolated fungal cultures were carried out to find the conidia of *Colletotrichum*. After microscopic observation it was concluded that Koch's postulates was successful.

3. Results and Discussions

3.1 Survey on Leaf spot disease of Turmeric

In the surveyed locations of Cooch Behar and Nadia districts fixed plot survey was conducted and in every location turmeric leaf spot disease was quantified (Plate 2). The Fields were visited in monthly intervals from July to November and PDI data were recorded accordingly (Figure 1). The varieties were categorized from immune to susceptible on the basis of PDI (Percent Disease Index) observed at the end of final survey. AUDPC had been

calculated from fortnight interval PDI data (From July to November) The variety fall under immune category (PDI <10%) is Uttar Rangini with PDI value of 9.44% and also exhibited lowest AUDPC value of 829.05. Suranjana and Uttar Rupanjana varieties were fall under resistant category (PDI= 11-20%) with percentage disease severity of 13.89% and 15.56% respectively with AUDPC value of 1219.80 and 1336.05 respectively. Whereas, some varieties i.e. Lakadong, Megha, Tur-1, Sugana and ACC-1025 showed moderately resistance (PDI= 21-30%) with PDI value of 24.40%, 22.80%, 27.15%, 28.72% and 25.78% respectively and the resultant AUDPC values were 2099.25, 1733.1, 1887.75, 1946.72 and 1724.32 respectively. Susceptible reactions showed by D-2154 and CL-276 line with AUDPC Value of 2207.77% and 2446.75% respectively. Higher disease severity (PDI >50%) was observed in some highly susceptible varieties i.e. Malsari, Goma and Pragati with 53.12%, 57.49% and 55.35% of PDI respectively. High AUDPC values were also shown by these local varieties such as Pragati (4079.02), Malsari (4125.33), Goma (3906.37). The details of survey presented in Table 3.

From the results, it was found that most of the local varieties showed highest leaf spot disease severity and higher AUDPC value. This might be happened due to lack of proper management practices and susceptibility of the host. Most of the local varieties (i.e., Goma, Malsari, Sonjuli, Pragati etc) exhibited higher PDI (>50%) percentage. On the contrary, in the experimental plots lower rate of PDI values were observed. This might be happened due to proper management practices and resistance of the cultivars. Among the surveyed varieties, Uttar Rangini, Suranjana, Uttar Rupanjana, Lakadong and Megha showed lowest rate of PDI percentage as well as these varieties provided significant amount of yield. Hence farmers may be suggested to use these varieties by replacing their locally available varieties, which were being used by them for a long time. Similar type of survey was conducted by Gorawar *et al.* (2006) where they found 50% disease severity of leaf spot disease of turmeric in Gulbarga district in North Karnataka. Study of Koche *et al.* (2009) reveals that the most prevalent foliar disease of turmeric was leaf spot and they found "Kanti" and "Sudharsana," cultivar exhibited higher resistance to the disease compared to other 16 turmeric cultivar under study in the Vidarbha region of Maharashtra.

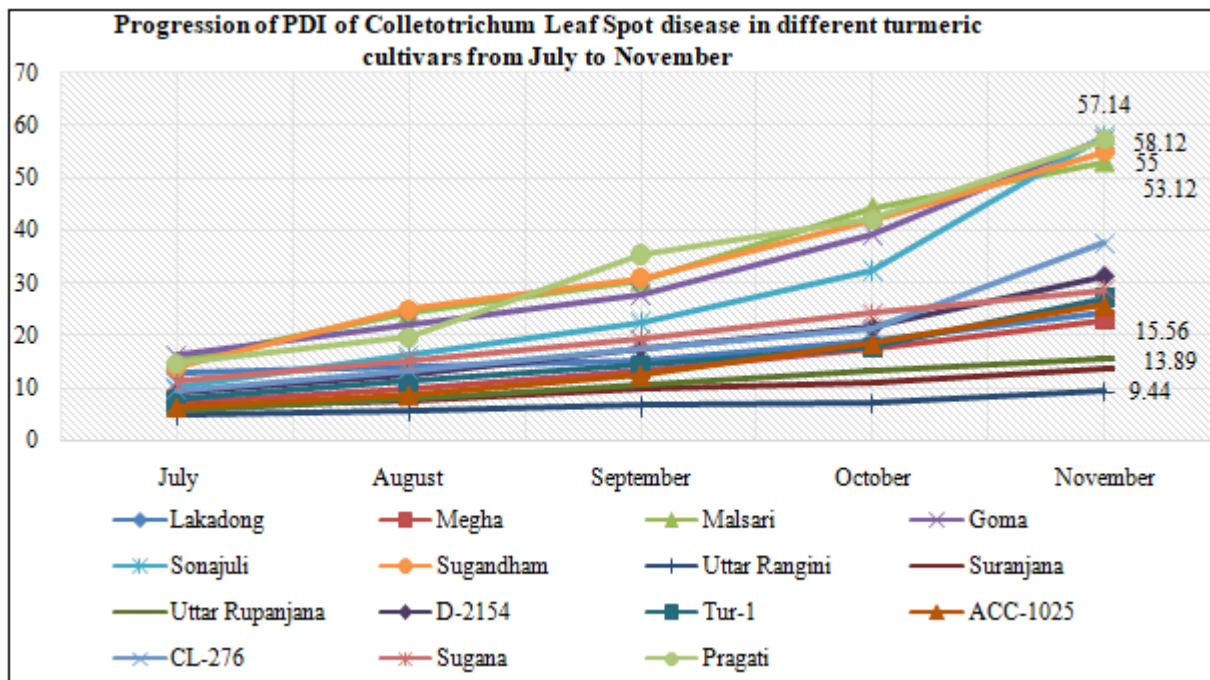


Figure 1: Progression of PDI of *Colletotrichum* Leaf Spot disease in different turmeric cultivars from July to November

Table 3: Details of fixed plot survey on *Colletotrichum* leaf spot during the period of July to November, 2022

District	Places of survey Villages	Latitude & Longitude	Total number of field surveyed	Name of Turmeric variety /line surveyed	Categorization	PDI (%)					AUDPC**	Yield (t/ha)
						July	Aug.	Sept.	Oct.	Nov.		
Cooch Behar	Dinhata CTRI	Lat : 26°12'27"1N Long :89°45'46"E	4	Lakadong	Moderately resistant	12.82 (20.96)	14.07 (22.54)	15.21 (22.95)	18.88 (25.70)	24.40 (29.60)	2099.25	26
		Lat : 26°12'21"N Long : 89°45'44"E	3	Megha	Moderately resistant	7.36 (15.68)	9.87 (18.24)	13.47 (21.47)	17.51 (24.73)	22.80 (28.53)	1733.10	28
	Putimari, Village	Lat: 28°12'92 N Long: 89°46'68"E	3	Malsari	Highly susceptible	15.27 (22.95)	24.36 (29.53)	30.64 (33.60)	44.49 (41.78)	53.12 (46.78)	4125.33	15
	Tufanganj, Fulbari	Lat: 26°18'48"N, Long: 89°39'37"E	3	Goma	Highly susceptible	16.25 (23.73)	22.12 (28.04)	27.83 (31.82)	39.33 (38.82)	57.49 (49.31)	3906.37	12
	Rakhalmari village	Lat: 26°14'11" N, Long: 89°54'50" E	3	Sonajuli	Highly susceptible	10.25 (18.53)	16.23 (23.73)	22.54 (28.32)	32.45 (34.70)	58.12 (49.66)	3237.89	13
	UBKV Pundibari	Lat: 26°23'58"N Long: 89°23'11"E	3	Uttar Rangini	Immune	4.86 (12.66)	5.56 (13.39)	6.58 (14.89)	7.13 (15.45)	9.44 (17.85)	829.05	29
		Lat: 26°23'57"N Long: 89°23'12"E	5	Suranjana	Resistant	6.54 (14.77)	7.67 (16.00)	9.87 (18.22)	11.27 (19.55)	13.89 (21.89)	1219.80	22
Lat: 26°23'59"N Long: 89°23'13"E		3	Uttar Rupanjana	Resistant	5.82 (13.94)	8.24 (16.64)	10.78 (19.19)	13.37 (21.39)	15.56 (23.28)	1336.05	27	
Nadia	Mondouri Farm, BCKV	Lat: 22°58'42" N Long: 88°31'25"E	3	D-2154	Susceptible	8.95 (17.30)	12.26 (20.44)	17.56 (24.73)	21.45 (27.56)	31.22 (33.96)	2207.77	22
		Lat: 22°56'47"N Long: 88°30'59 E	3	Tur-1	Moderately Resistant	7.88 (16.39)	11.45 (19.73)	14.28 (22.14)	17.71 (24.88)	27.15 (31.37)	1887.75	24
		Lat: 22°56'43"N Long: 88°30'56"E	3	ACC-1025	Moderately Resistant	6.41 (14.65)	8.69 (17.15)	12.58 (20.70)	18.49 (25.62)	25.78 (30.53)	1724.32	21
		Lat: 22°56'21"N Long:	4	CL-276	Susceptible	9.64 (18.05)	13.47 (21.56)	17.53 (24.73)	21.22 (27.35)	37.55 (37.76)	2446.75	20

		88°30'43"E										
Gayeshpur	Lat: 22°97'27"N Long: 88°49'51"E	4	Sugana	Moderately Resistant	11.25 (19.55)	15.21 (22.95)	19.24 (25.99)	24.31 (29.53)	28.72 (32.39)	1946.72	25	
Basantapur	Lat: 22°96'90"N Long: 88°52'39"E	5	Pragati	Highly susceptible	14.87 (22.71)	19.69 (26.35)	35.41 (36.51)	42.14 (40.45)	55.35 (48.04)	4079.02	14	
				CD at 5% SEm(±)	1.376 0.471	0.099 0.034	0.633 0.217	0.122 0.042	0.351 0.120			

**AUDPC had been calculated from fortnight interval PDI data (From July to November)







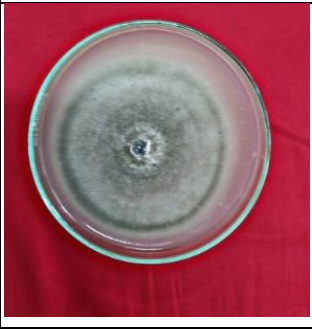

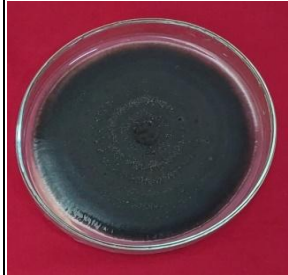
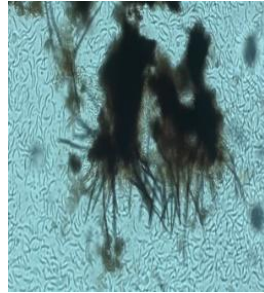





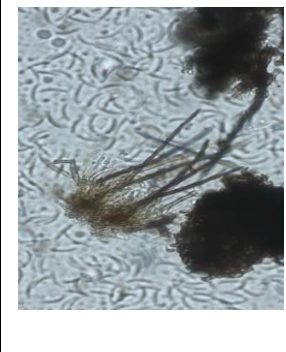


Plate 2: Different locations visited during Survey Period

3.2. Isolation of the Pathogen

The pathogen was isolated from total eight locations. Thus, eight isolates were collected. The descriptions of the eight isolates are presented in the Table 4 below.

Table 4: Details of eight isolates collected from different turmeric growing areas of West Bengal

Isolate information	Colony Morphology	Microscopic observation
<p>Isolate Name: CCDI-1 Location: Dinhata, Block 1, Cooch Behar, West Bengal. Lat.: 26°14'11"N Long: 89°54'50"E Isolated from: Phyllosphere Variety: Sonajuli local Pathogenicity test: Successful</p>		
<p>Isolate Name: CCM1-2 Location: Mondouri, Nadia, West Bengal Lat: 22°56'47"N Long: 88°30'59"E Isolated from: Diseased leaf variety : Pragati local Pathogenicity test: Successful</p>		
<p>Isolate Name: CCPI-3 Location: Pundibari Cooch Behar. Lat: 26°23'58"N Long: 89°23'11"E Isolated from: Diseased leaf Variety: Uttar Rangini Pathogenicity test: Successful</p>		
<p>Isolate Name: CCTI-4 Location: Tufanganj Block II, Cooch Behar, West Bengal Lat : 26°18'48"N Long: 89°39'37"E Isolated from: Diseased leaf Variety : Suranjana Pathogenicity test : Successful</p>		
Isolate information	Colony Morphology	Microscopic observation
<p>Isolate Name: CCRI-5 Location: Rakhalmari village, GP Sahebanj, Cooch Behar, West Bengal Lat : 26°10'32" Long: 89°28'23" Variety : Megha Pathogenicity test : Successful</p>		

<p>Isolate Name:CCGI-6 Location:Gayeshpur, Nadia, West Bengal Lat :22°97'27"N Long: 88°30'59"E Variety :Pragati local Pathogenicity test : Successful</p>		
<p>Isolate Name: CCBI-7 Location:Basantapur, Nadia, West Bengal Lat : 22°96'90"N Long: 88°52'39"E Isolated from: Phyllosphere Variety :Sugana Pathogenicity test : Successful</p>		
<p>Isolate Name:CCLI-8 Location: CTRI farm , Dinhata Block I, West Bengal Field Lat: 26.12'27"N Long: 89.45"46"E Isolated from:Phyllosphere Variety :Lakadong Pathogenicity test : Successful</p>		

3.3 Morphological variations among the isolates

The fungus *Colletotrichum* produce numerous acervuli. Inside acervuli dark brown coloured needle like setea can be seen

and huge number of conidia are formed in the tip of conidiophores. The following **Table 5** showing variation of size of conidia and setea in eight isolates of *Colletotrichum*.

Table 5: Morphological variations among the isolates

Isolate Name	Mean spore length (µm)	Mean Spore Breadth (µm)	Mean Setae length (µm)	Mean Setae breadth (µm)
CCDI-1	20.45	4.10	153.44	4.83
CCMI-2	25.16	4.25	169.50	5.45
CCPI-3	19.28	3.10	118.58	4.72
CCTI-4	16.17	4.09	92.55	3.11
CCRI-5	21.15	3.70	133.25	5.11
CCGI-6	17.34	2.95	94.73	3.64
CCBI-7	23.18	3.52	128.10	3.81
CCLI-8	22.12	3.84	139.66	3.41
CD at 5%	0.561	0.046	0.035	0.235
Sem (±)	1.6832	0.138	1.568	1.654
C.V.	4.760	2.165	2.132	3.520

3.4 Symptoms produced by the pathogen

The symptoms of leaf spot disease in turmeric, caused by *Colletotrichum* is characterized by variable-sized elliptical or oblong spots on the leaf blades. These spots have greyish or brownish center with a yellow halo surrounded by the spots. Both leaf surfaces exhibit numerous black dot-like structures called acervuli, which are arranged in concentric rings. These acervuli are asexual fruiting bodies and characteristic features of the disease caused by

Colletotrichum on turmeric leaves. The spots are varied in size, ranging from 3.50 to 7.00 cm in length and approximately 3.40 to 4.60 cm in breadth. Initially, the spots form small brown lesions, as they grow, multiple spots may merge, creating irregular patches that often cover a significant portion of the leaf. Eventually, the affected leaves dry up. Patel *et al.* (2005) also documented this kind of characteristics of the leaf spot disease symptoms on turmeric plants (**Plate 3**).



Plate 3: Symptoms of *Colletotrichum* Leaf spot disease in Turmeric

3.5 Pathogenicity test

The pathogen had been inoculated in the healthy leaves of the turmeric plants grown in the earthen pots kept inside polyhouse. After 4-5 days of inoculation brown dot like symptom with yellow halo can be seen on pin pricked area. These spots enlarge and become greyish brown with concentric ring which is the typical symptom of the leaf spot disease by *Colletotrichum*. Another method for *in-vitro* pathogenicity test healthy leaves which were pin pricked with pathogen kept inside the B.O.D. After 7-8 days leaf spot symptoms overserved in those leaves. Re-isolation was done from this diseased leaves and isolated pathogen culture shows typical morphological characteristics of *Colletotrichum* (i.e., Conidia, setae, acervuli etc.) when observed under microscope. Hence Koch's postulates were successfully established (Plate 4). Similar type of methods and observations were recorded by Guji *et al.* (2019) when they conducted Koch's postulates to determine the pathogenicity of *Colletotrichum capsicica* using leaf spot disease of turmeric.

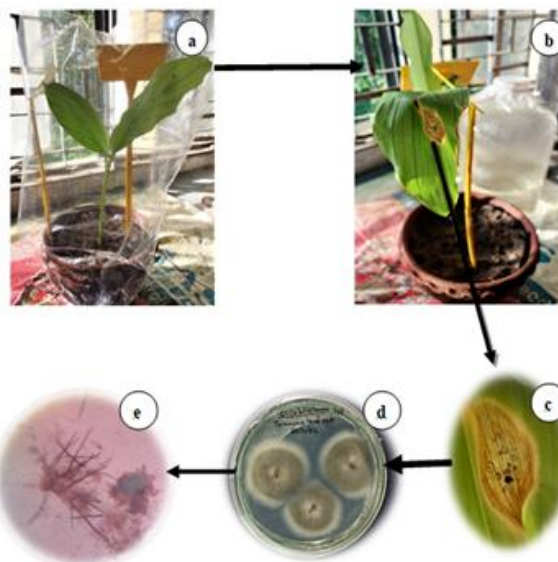


Plate 4. Steps of pathogenicity test; a. inoculated turmeric plant; b-c disease symptoms appeared on inoculated leaves; d. re-isolation of the pathogen; e. microscopic observation

3.6 Weather conditions prevalent during severe disease development:

During the period of July to November the weather conditions i.e. temperature (21-31°C), relative humidity (67-82%) and cumulative rainfall (1246mm) had direct impact on the severe progress of the disease in all the surveyed locations of Northern part of west Bengal and average PDI of 29% was recorded during the survey months, year 2022.

Whereas, in southern part of West Bengal, the weather variables which favored severe disease progression between July to November were temperature (24-34°C), relative humidity (78-91%), cumulative rainfall 504 mm, which resulted 33% PDI during the survey months, year, 2022.

3.7. Molecular detection of isolated pathogens:

Though eight isolates were collected from four locations of North Bengal as well as four locations of south Bengal, but two representative isolates i.e., CCDI-1 from north Bengal and CCMI-2 from south Bengal respectively were selected for molecular identification. The genomic sequences for CCDI-1 and CCMI-2 isolates were deposited in the GeneBank of NCBI blast and accession numbers are already obtained. The accession number for CCDI-1 is OR158054 and for CCMI-2 it is OR150435.

4. Summary and Conclusion

The studies reveal that local varieties which are widely cultivated by farmers i.e. Goma, Malsari, Pragati, Sonajuli are highly susceptible to *Colletotrichum* leaf spot disease. These local varieties should be replaced by resistant varieties which are also high yielder such as Uttar Rangini,

Lakadong, Suranjana, Megha etc. The fungus produce oblong to elliptical leaf spot forming greyish to brown centre surrounded by yellow halo. The isolates are found to be the genus *Colletotrichum* based on their cultural and morphological characteristics. However among the eight isolates mentioned two isolates i.e. CCDI-1 and CCMI-2 are found to be *Colletotrichum curcumae*. The pathogenicity tests of the eight isolates confirms successful establishment of Koch's Postulates.

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