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Research Article

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Identification of *Colletotrichum* species associated with anthracnose disease of chilli in major chilli growing area of India

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ABSTRACT
Anthracnose fruit-rot samples were randomly collected from 95 chilli farms grown in different hotspot regions from following states. Survey was conducted during Kharif 2020 from Andhra Pradesh, Karnataka, and Tamil Nadu, while during Kharif 2021 from Maharashtra, Madhya Pradesh, and Chhattisgarh. Samples were analyzed based on morphological characters. After morphological characterization of collected samples, it was confirmed that 80 fields were infected alone with *C. capsici*, 7 fields were infected alone with *C. gloeosporioides* while 8 fields were mixed infected with both *C. capsici* and *C. gloeosporioides*. Anthracnose infection mainly affects chilli at red fruit stage. Morphological characterization of collected samples from the surveyed region revealed that *Colletotrichum capsici* and *C. gloeosporioides* were the major species infecting chilli while *Colletotrichum capsici* was the predominant species. As *C. capsici* is predominant species causing Anthracnose, we need anthracnose resistant source at least against *C. capsici*. Resistant source against both C, capsici and *C. gloeosporioides* will be a boon for breeders to strengthen disease resistance
Keywords: Anthracnose, Fruit rot, Anthracnose Die-back, *Colletotrichum*, *C. capsici*, *C. gloeosporioides*, *C. acutatum*,

INTRODUCTION

Chilli, *Capsicum* annum (L.) is one of the most important spices and vegetable crop throughout the world including India. It is one of the most widely cultivated among *Capsicum* species comprises of both pungent chilli as well as sweet (bell pepper) of numerous shapes and sizes. It is a good source of Vitamin A and C, potassium, and folic acid (Pathirana 2012). Chilli is widely used as vegetable, spice, and condiments, it is also used in medicines and beverages.

India is the world's largest producer of dried chillies and in 2021 India produced 2.05 million tons, out of 4.84 million tons produced worldwide (FAOSTAT 2021). India is not only the largest producer but also the largest consumer and exporter of chilli in the world. Chilli alone contributes 36.4 % to the total spice export quantity of the country (Spice Board, 2022) and contributes 42.34% of world chilli production followed by Bangladesh, Thailand, China, Ethiopia (FAOSTAT 2021). In India during year 2021-22, Andhra Pradesh tops the list in dry chilli production of 7 lakh tonnes covered under 1.6 lakh hector with 4375 kg/ha productivity followed by Telangana, Madhya Pradesh, Karnataka, Odisha, Tamil Nadu and Maharashtra. Guntur district in Andhra Pradesh produces 15% of all the chillies produced in India and the state of Andhra Pradesh individually contributes 43.71% of India's chilli production (Spice Board, 2022).

The sustainability of chilli is affected by various abiotic and biotic stresses poses hurdle for successful large-scale commercial cultivation to farmers and seed industries. Chilli is susceptible to various viral, bacterial, and fungal diseases. Among these Anthracnose (fruit rot) is most widespread and economically important fungal disease and is known to cause by one of the four species of *Colletotrichum* viz. *C. capsici*, *C. gloeosporioides*, *C. acutatum* and *C. coccodes* in solo or as disease complex (Simmonds, 1965; Johnston and Jones, 1997; Kim et al., 1999; Nirenberg et al., 2002; Voorrips et al., 2004; Sharma et al., 2005; Pakdeevaraporn et al., 2005; Than et al., 2008). The disease was reported for the first time in India from Coimbatore of Madras Presidency (Sydow, Butler & Bisby 1913). *Colletotrichum capsici* can cause disease on almost all parts of the Chilli plant during any stage of plant growth. Anthracnose has been observed to infect chilli crop in three phases viz. (i) seedling blight / damping off stage, prevalent in the nursery, (ii) die back stage which is initiated at different growth stages and (iii) fruit rot stage where majorly ripe fruits are infected.

Chilli anthracnose usually develops under high humid conditions especially in the rainy season. The disease is mainly a problem on mature fruits, causing severe losses up to 84% (Thind and Jhooty, 1985) due to both pre- and post-harvest fruit decay (Hadden and Black, 1989; Bosland and Votava, 2003). The severity of the disease varies depending on cultivars grown and the weather conditions prevailing in a particular region. The disease is both seed borne and air borne and affects seed germination and vigour to a greater extent (Ahmed, 1982; Perane and Joi, 1988; Mesta, 1996 and Asalmol et al., 2001). Economic losses caused by the disease are mainly attributed to lower fruit quality and marketability.

The disease is more severe in India because of its complex nature. In the recent climate change situation, there is a need to investigate the disease thoroughly, as epidemics vary in different regions giving scope for understanding the extent of variability in pathogen population. To identify the resistant germplasm for Anthracnose, the geographical mapping along with Cataloguing and assortment of *Colletotrichum* species using morphological characterization is of prime importance. Further, their confirmation using molecular markers is a more reliable technique and can give a logical conclusion regarding the genetic variability existing among isolates. Hence, an extensive survey was conducted in Kharif 2020 and Kharif 2021 at South and Central India respectively to assess the incidence of anthracnose of chilli.

MATERIALS AND METHODS

The research activities and laboratory experiments were carried out during 2019 to 2022 in the Department of Plant Pathology, Ankur Breeding Support Centre, Ankur Seeds Pvt. Ltd., Nagpur. Nagpur is situated at centre of India and of Maharashtra state at 21°9′N 79°5′E and at an altitude of 370.0 Ft. above mean sea level.

Survey

Major red chilli growing states in India are Andhra Pradesh, Madhya Pradesh, Telangana, Karnataka, Tamil Nadu, and Maharashtra where it was grown in 5.23 lac hectors in year 2021-22 (Data Source: Spice Board 2021-22) which contributes approximately 75.25% red chilli growing area in India. Thus, an extensive survey was conducted in Central and Southern part of India to for geographical mapping of *Colletotrichum* Species causing chilli anthracnose. Anthology of different *Colletotrichum* isolates causing Anthracnose fruit rot was done during Kharif 2020 from Andhra Pradesh, Karnataka, and Tamil Nadu while in Kharif 2021 from Maharashtra and Madhya Pradesh. Chilli samples infected with Anthracnose fruit-rot were randomly collected from 95 locations grown in different hotspot regions from above states.

Isolation

Infected chilli fruits showing typical anthracnose symptoms, sunken necrotic tissues, with concentric rings of conidial masses on chilli fruits were selected for isolation. Five individual fruits per location were subjected for pathogen isolation. Isolation was carried out using infected tissue and, in some cases, (where saprophytic infection was more on outer tissue) by Blotter method (ISTA,2005). Surface sterilised (0.1% $HgCl_2$ for 1 min) infected tissue (~3-5 mm) was cultured on Potato Dextrose agar plates. After incubation for 3-4 days fungal hyphae grown were subcultured onto PDA plates (PDA, Himedia). In Blotter method infected seeds were placed on moist blotter paper microchamber and were incubated at 25°C ± 2 for seven days. Further fungal hyphae grown on filter paper was subcultured onto potato dextrose agar plates. Inoculated plates were incubated at 25°C ± 2 for 8-10 days in alternative 12 hours of darkness and 12 hours of light. Cultures were isolated, maintained and preserved for further study at Plant Pathology Laboratory at Breeding Support Centre of Ankur Seeds Pvt. Ltd. Nagpur (Maharashtra).

Morphological Characterization

Morphological colony characters of the fungus were observed on incubated PDA plates. Cultures developed perithecia on media were selected for microscopic analysis. Individual sample-wise slides were prepared and stained with lactophenol cotton blue. Conidial morphology was examined with the help of binocular microscope (Leica, Germany: DM6000B). Conidial size of each sample was measured using Leica Q-Win Software package. Categorisation of *Colletotrichum* species was done based on conidial shape. However, this result needs to be confirmed using molecular markers to avoid misidentification of the species.

DNA Extraction and Molecular confirmation using PCR

Genomic DNA from all the 103 isolates were extracted using Dellaporta DNA extraction method (Dellaporta et. al. 1983), from fresh mycelial mat grown on Potato Dextrose Broth following the standardized protocol. DNA quality was assessed on a 0.8% (w/v) agarose gel, quantified by comparing with a known amount of Lambda DNA, diluted to 2 ng/µL and then stored at -20 °C until ready for PCR. PCR detection by species-specific primers for *Colletotrichum capsici* (Torres-Calzada et. al. 2011), *Colletotrichum gloeosporioides* (Tapia-Tussell et al 2008) (Talhinhas et al 2002) (Mills et al 1992) (White et al 1990) and *Colletotrichum acutatum* (Freeman et al. 2000) and (Afanador et al. 2003) was conducted using following species-specific Primers (Table 1).

For molecular confirmation, genomic DNA of 103 isolates of *Colletotrichum* species were amplified by PCR separately with 3 species-specific primers of *C. capsici, C. gloeosporioides and C. acutatum* (Table 1). PCR reactions were performed in 96 well plate with 20µl volume. The constituents of reaction mixture are mentioned in Table 2.

Table 1. *Colletotrichum* species and their primer pair sequence with expected amplicon size.

Organism	Sequence		Expected Amplicon
C. capsici	C cap-F	5'-GTAGGCGTCCCCTAAAAAGG-3'	394
	C cap-R	5'-CCCAATGCGAGACGAAATG-3'	
C. gloeosporioides	Cg INT-F	5'-GGCCTCCCGCCTCCGGGCGG-3'	450
	ITS-4-R	5'-TCCTCCGCTTATTGATATGC-3'	
C. acutatum	Ca INT-2-F	5'-GGGGAAGCCTCTCGCGG-3'	490
	ITS-4-R	5'-TCCTCCGCTTATTGATATGC-3'	

Table 2. Constituents of PCR reaction Mixture.

Initial Concentration	Component	Volume	Final Concentration
	Template DNA	1.2 µl	100 ng/µl
10 X	PCR buffer	2 µl	1 X
25 mM	MgCl2	1.6 µl	1.5 mM
10 mM	dNTPs	0.5 µl	200 µM
10 µM	Forward primer	0.5 µl	0.2 µM
10 µM	Reverse primer	0.5 µl	0.2 µM
1 Unit	Taq DNA polymerase	1 µl	1 U/µl
10 X	BSA	1 µl	1 X
	Nuclease Free Water	11.7 µl	

PCR reactions conditions for the amplification were standardized for the primer and thermal cycler. The following amplification program was used for desired amplicon:

Table 3. PCR Conditions for respective species-specific primer pair.

	C. capsici		*C. gloeosporioides*		*C. acutatum*	
Steps	Temp (°C)	Duration (min)	Temp (°C)	Duration (min)	Temp (°C)	Duration (min)
Initial Denaturation	92	3	92	3	92	3
Denaturation	92	1	92	1	92	1
Annealing	56	1	62	1	62	1
Extension	72	1	72	1	72	1
Final Extension	72	10	72	10	72	10
Hold	4	20	4	20	4	20
No of Cycles	30		30		30	

The amplified PCR products (20µl each) to be analyzed were mixed with bromophenol blue and carefully loaded to respective sample wells using micropipette. Samples along with standard 100 bp DNA ladder were electrophoresed for about 1Hr. at 50Vcm^{-1} ensuring Ethidium bromide-stained DNA bands were observed under Gel Doc (Model No) and photographed for documentation.

Pathogenicity test

Isolated 103 fungal isolates were inoculated in healthy susceptible red chilli fruits to prove Koch's postulate of the fungus. Isolates were cultured on PDA media at 28°C for 10-15 days for sporulation in alternative 12 hours of darkness and 12 hours of light. Conidia from sporulating cultures were harvested by adding approx. 10 ml of sterilized distilled water into culture plate and was gently scrapped to dislodge the conidia. The conidial suspension was filtered aseptically through double layered muslin cloth to exclude mycelial mat and media particles. Spore count of the conidial suspension was done using haemocytometer and concentration was adjusted to get 1 X 10^6 conidia/ml. Pathogenicity test was conducted by inoculating spore suspension into the healthy fruits of susceptible chilli variety by detached fruit inoculation bio-assay as per (Kanchanaudomkan et. al. 2004). A set of same number of susceptible chillies were inoculated (as a negative control) with sterile distilled water used for preparation of conidial suspension. Separate sets of chillies were used for inoculation of different isolates to prove pathogenicity.

Inoculation Method

Chilli fruits were surface-sterilized with 0.1 % (v/v) (HgCl2) for 5 minutes and washed twice with sterile distilled water for 5 minutes each. The fruits were wiped dry with a paper towel. Fruits were inoculated with *Colletotrichum* isolate by the modified microinjection method involved pin-pricking the chilli pericarp to ≈1mm depth followed by injecting 10µl of conidial suspension (having concentration 10^6 conidia/ml) using Eppendorf repetitive micropipette in the middle portion of each chilli fruit using repetitive micropipette (Eppendorf: Multipette® plus). The inoculated fruits were incubated in plastic crates at 25°C ± 2 and ≈80% relative humidity for 10 Days. Five fruits per cultivar were taken for inoculation in each treatment. One set of negative/blank control fruits were inoculated with 10 µl of sterilized distilled water. To fulfil the Koch's postulates, the symptoms developed on the inoculated fruits were compared with original symptoms and the fungi were re-isolated from inoculated fruits following the above mention procedure.

Classification and Identification of predominant *Colletotrichum* species

Colletotrichum species was identified from collected infected chilli samples of different states, by analysing conidial morphology and molecular markers. In India mixed infection of *Colletotrichum capsici* and *Colletotrichum gloeosporioides* strains was observed. Out of 95 infected chilli sample locations *Colletotrichum capsici* was isolated and identified from 88 location samples and *Colletotrichum gloeosporioides* was isolated and identified from 15 location samples. However, based on this analysis *Colletotrichum capsici*

is the predominant *Colletotrichum* species infecting chilli in India. *Colletotrichum gloeosporioides* was identified from a smaller number of fields but it may become major threat to chilli crop in future days.

RESULTS AND DISCUSSION

Chilli Anthracnose samples collected from 95 different vulnerable regions in India were characterized by a circular, sunken zone with concentric rings of conidial masses on fruits of chilli. During sample collection, broadly two types of anthracnose lesion symptoms were observed on the fruits. First type of symptoms (Phenotype 1) observed in large number of samples showed dark black coloured colonies grown in concentric rings on chilli fruits (Figure 1A). Second type of symptoms observed in smaller numbers showing cream yellow to pinkish orange-coloured conidial masses grown in concentric rings on chilli fruits (Figure 1B). There were very few locations where infected fruits carrying both type of symptoms was present on the same field. The state wise, isolated isolates with different phenotypes are mentioned in Table 4. The phenotypic data is a representation as defined by (Than et. al. 2008; Mongkolporn et al. 2010; Liu et. al. 2016; de Silva et. al. 2019).

Morphological Characterization of Pathogen

In our study, isolation of pathogen was done from infected tissue or using blotter method (from infected seeds). Fungus isolated from infected fruit showing Anthracnose phenotype 1 (Figure 1A) has shown formation of dark black coloured lesions containing acervuli bearing setae on the PDA as well as moist filter paper (Figure 2A). On the other hand, fungus isolated from fruit showing Anthracnose phenotype 2 (Figure 1B) (with creamy yellow to pinkish orange-coloured lesions) was successfully isolated from infected chilli tissue and produced acervuli without setae on the PDA (Figure 2C).

In vitro cultural characteristics were important for differentiating among *Colletotrichum* species (Prihastuti, H. et al, 2009). Traditionally, species identification is principally based on morphology, host specialization, and mode of parasitism (Bailey et al. 1996; Cannon et al. 2008; Josep et. al 2004; Pratibha, 2009). In our study, the appearance of colony characters on PDA was observed after 10 days of sub-culturing. The mycelia of all isolated fungi formed cottony, fluffy aerial mycelial growths on PDA. The mycelial colony colour from upper side appeared to be greyish-white, while it was appeared pale orange when observed from backside (Figure 2C). *C. capsici* cultures had dense aerial mycelium, were dark brown to black or greyish felt mycelium. Tufting of mycelium was irregular, with honey-coloured or greyish conidial masses. Acervuli were formed with abundant dark setae (Figure 2B). Morphological characters of all the isolates were examined using a Leica DM6000B LED compound microscope. Conidia of *C. capsici* were hyaline, falcate with acute apex and narrow truncate base, aseptate, uninucleate, 16 - 23 × 3 - 5 μm (Figure 2D).

Morphological characters of the spores were matching the previous reports (Liu et. al. 2016.; Than et al., 2008) *C. gloeosporioides* conidia in all isolates were smooth-walled, hyaline, straight, aseptate, cylindrical with both end round, 11 to 18 μm × 3.1 - 4 μm wide (Figure 2E). Mycelial growth of associated cultures observed cottony, fluffy whitish grey mycelial growth on PDA. Microscopic observation of *C. gloeosporioides* isolates did not showed setae formation. Cultural characteristics observed in our study were in line with the earlier studies reported (Kim et. al., 2008; Prihastuti, H. et al, 2009; Ismail et.al., 2015; Liu et. al., 2016). However, conidial morphology and cultural characteristics often overlap between *C. acutatum* and *C. gloeosporioides* (Freeman and Rodriguez 1995; Freeman et al. 2000; Widodo and Hidayat, 2018), consequently, leading to confused diagnosis between *C. acutatum* or *C. gloeosporioides* (Bailey et al. 1996; Sutton 1980; 1992) caused the anthracnose symptom. For this reason, these results need to be confirmed using species-specific molecular markers to avoid misidentification of the species.

DNA Extraction:

Total 103 *Colletotrichum* isolates were subjected to DNA isolation as per method mentioned by (Dellaporta et. al. 1983). Genomic DNA was extracted from 7 days old mycelial mat grown on Potato Dextrose Broth. Proper isolation of genomic DNA and its concentration was confirmed by running on 0.8% Agarose Gel (Figure 3). Molecular confirmation of the *Colletotrichum* species was completed by amplification of 103 isolate's genomic DNA with 3 separate PCR by species-specific primers of *C. capsici, C. gloeosporioides and C. acutatum* (Table 1). After amplification with *C. capsici* markers (C cap-F and C cap-R) 88 samples shown expected amplicon band of 394bp (Figure 4) on separation of agarose gel and confirmed as *Colletotrichum capsici*. The results were matching PCR detection of *C. capsici* (Tapia-Tussell et al. 2008). After amplification with *Colletotrichum gloeosporioides* markers (Cg INT-F and ITS-4-R) 15 samples shown expected amplicon of 450bp (Figure 5) on separation of agarose gel confirming the presence of *Colletotrichum gloeosporioides.* (Kim et. al., 2008) also used the same primer pair for identification of *Colletotrichum gloeosporioides*. In the same manner all the DNA samples were amplified using *Colletotrichum acutatum* markers (Ca INT-2-F and ITS-4-R) but none of our sample showed expected amplicon of 490bp (Figure 6) on agarose gel confirming the absence of *Colletotrichum acutatum* in the samples.

Several molecular techniques have been developed to characterize populations of *Colletotrichum spp.*, and to identify different *Colletotrichum spp.* (Crouch et. al. 2009; Garrido et al. 2008; Johnston and Jones 1997; Lardner et al. 1999). Arbitrarily primed PCR and limited restriction digest analysis of PCR-amplified rRNA were employed to differentiate between *C. gloeosporioides* and *C. acutatum* isolates from a diverse host range

Table 4. State-wise samples collected and their phenotype

States	Total Locations	(Phenotype 1) *C. capsici*	(Phenotype 2) *C. gloeosporioides*	Total isolates
Andhra Pradesh	18	17	4	21
Madhya Pradesh	41	40	2	42
Maharashtra	21	19	4	23
Karnataka	11	11	2	13
Tamil Nadu	3	0	3	3
Punjab	1	1	0	1
Total	95	88	15	103

Figure 1. Anthracnose Fruit rot symptom observed during survey (A) Phenotype 1 (B) Phenotype 2 (C) Image from Zoom Microscope of Anthracnose Infected and Non infected Chilli seeds.

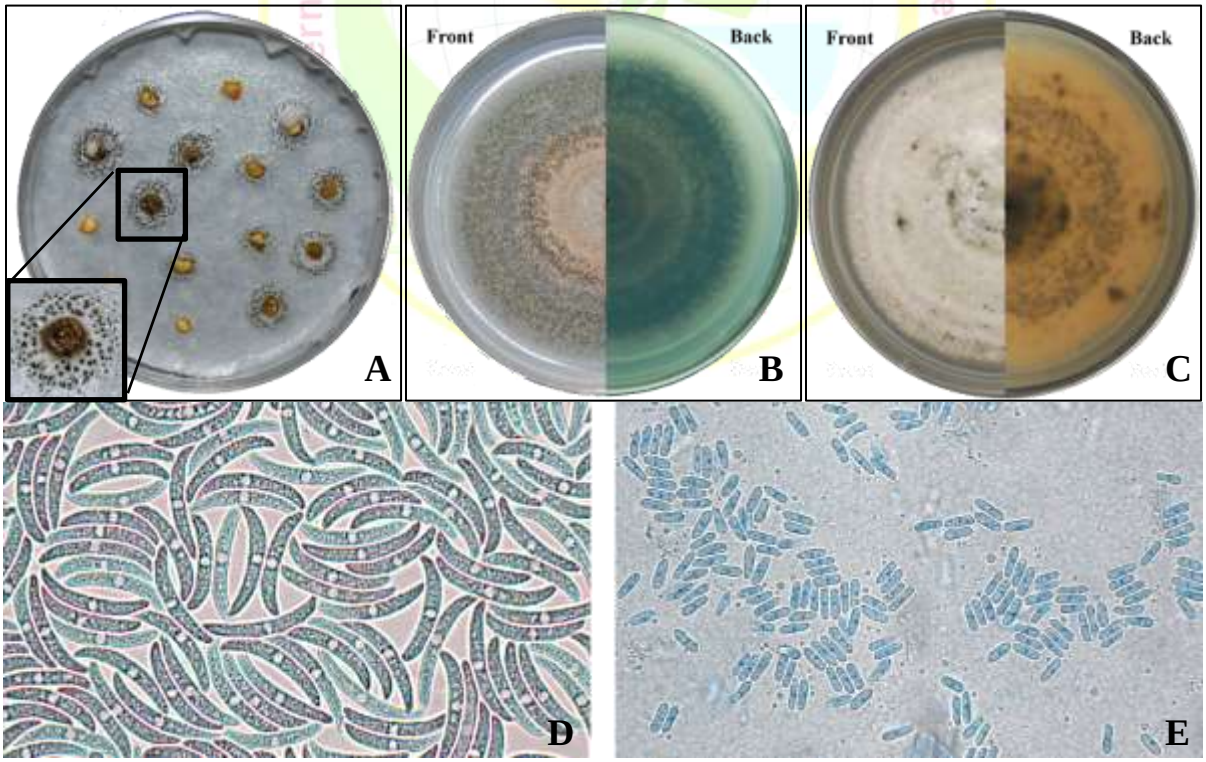

Figure 2. (A) Formation of Acervuli bearing setae on filter paper (B) Colony characteristics of *C. capsici* on PDA (C) Colony characteristics of *C. gloeosporioides* on PDA (D) *C. capsici* conidial morphology observed under microscope (E) *C. gloeosporioides* conidial morphology observed under microscope.

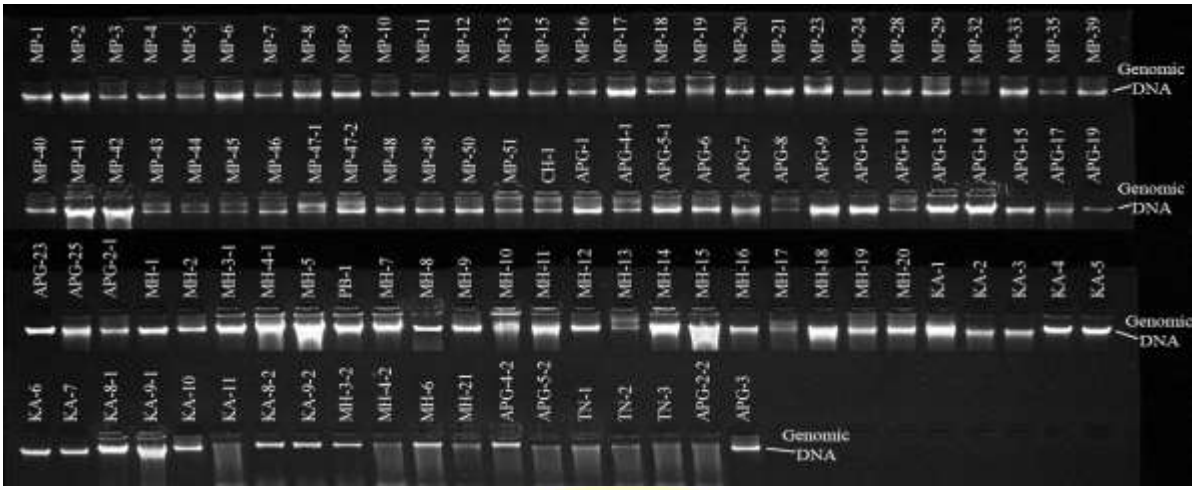

Figure 3. Genomic DNA Observed in Gel Doc after running on 0.8% Agarose Gel.

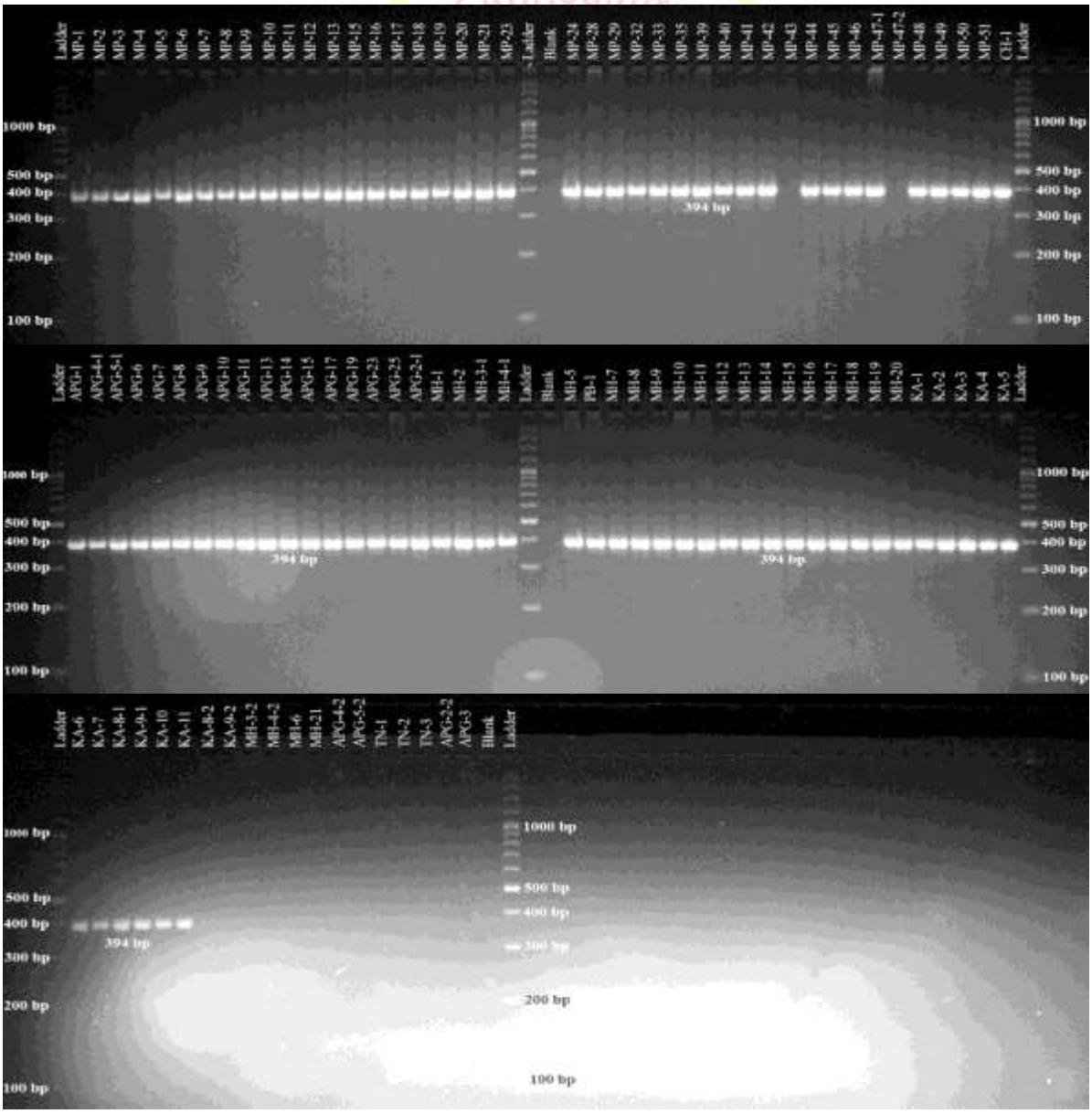

Figure 4. Results of PCR Amplification of 103 isolates with *C. capsici* species specific primer (Expected Amplicon

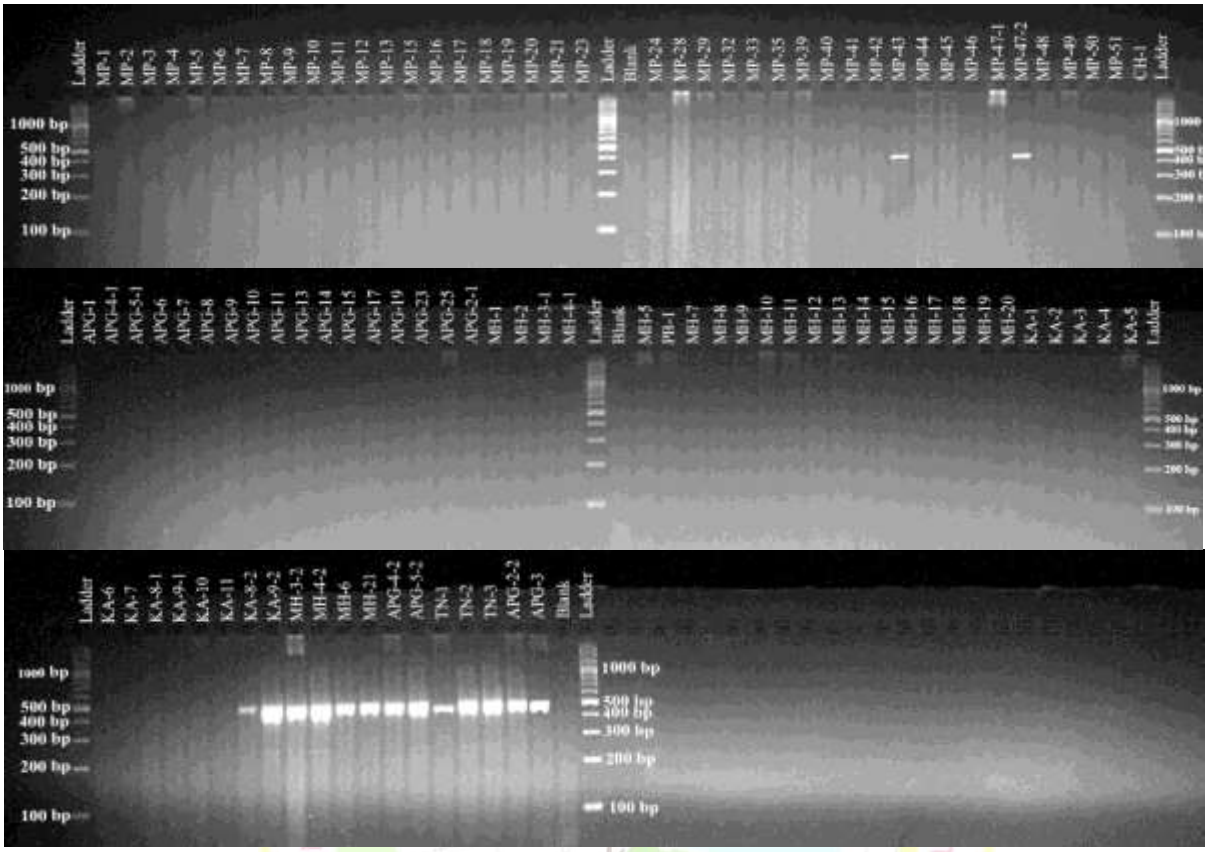

Figure 5. Results of PCR Amplification of 103 isolates with *C. gloeosporioides* species specific primer (Expected Amplicon 450bp).

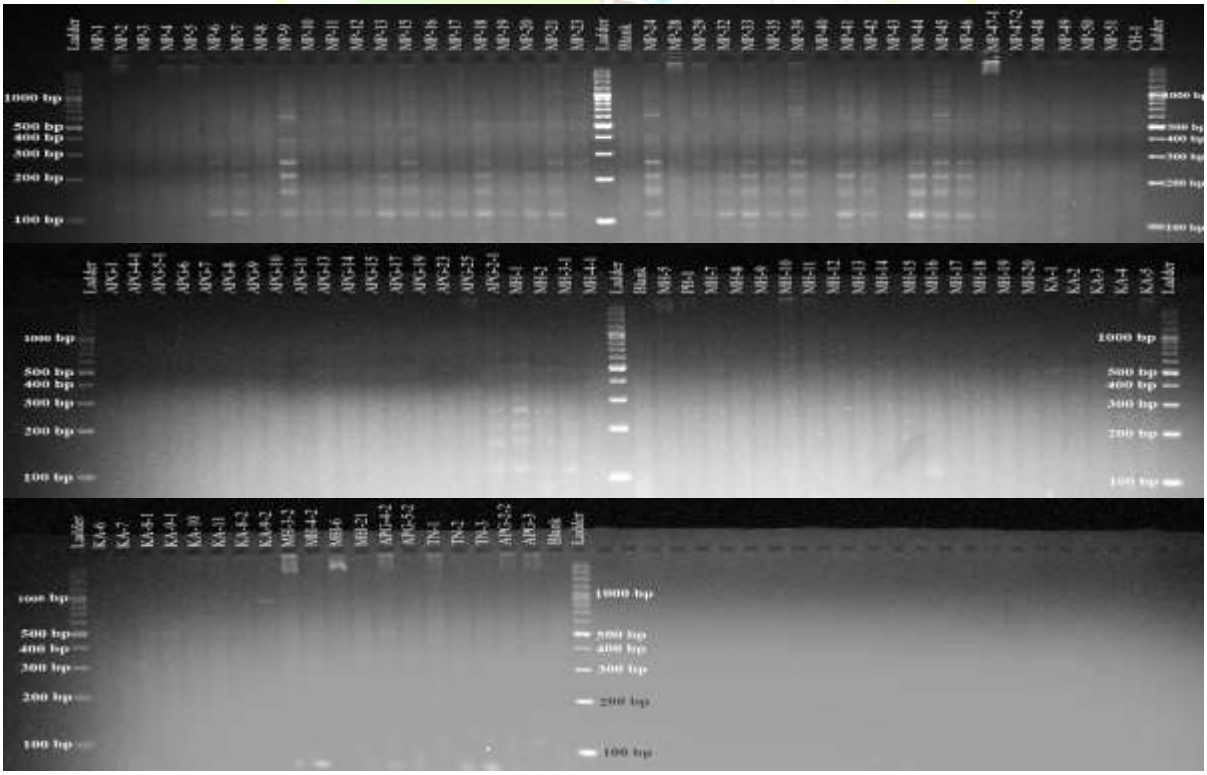

Figure 6. Results of PCR Amplification on 103 isolates with *C. acutatum* species specific primer (Expected Amplicon

Table 5. Details of disease samples collected and *Colletotrichum* species identified

Sr. No.	District	Code	*Colletotrichum sp.*	Setae
Madhya Pradesh (2021)				
1	Khandwa	MP-1	*C. capsici*	Present
2		MP-50	*C. capsici*	Present
3		MP-2	*C. capsici*	Present
4		MP-3	*C. capsici*	Present
5		MP-4	*C. capsici*	Present
6		MP-5	*C. capsici*	Present
7		MP-6	*C. capsici*	Present
8		MP-7	*C. capsici*	Present
9		MP-8	*C. capsici*	Present
10		MP-9	*C. capsici*	Present
11		MP-10	*C. capsici*	Present
12		MP-11	*C. capsici*	Present
13		MP-12	*C. capsici*	Present
14	Khargone	MP-13	*C. capsici*	Present
15		MP-15	*C. capsici*	Present
16		MP-16	*C. capsici*	Present
17		MP-17	*C. capsici*	Present
18		MP-18	*C. capsici*	Present
19		MP-19	*C. capsici*	Present
20		MP-20	*C. capsici*	Present
21		MP-21	*C. capsici*	Present
22		MP-23	*C. capsici*	Present
23	Manawar	MP-24	*C. capsici*	Present
24		MP-28	*C. capsici*	Present
25		MP-29	*C. capsici*	Present
26		MP-32	*C. capsici*	Present
27		MP-33	*C. capsici*	Present
28		MP-35	*C. capsici*	Present
29		MP-39	*C. capsici*	Present
30		MP-40	*C. capsici*	Present
31		MP-48	*C. capsici*	Present
32		MP-49	*C. capsici*	Present
33	Dhar	MP-41	*C. capsici*	Present
34		MP-42	*C. capsici*	Present
35		MP-43	*C. gloeosporioides*	Absent
36		MP-44	*C. capsici*	Present
37		MP-45	*C. capsici*	Present
38		MP-46	*C. capsici*	Present
39		MP-47-1	*C. capsici*	Present
40		MP-47-2	*C. gloeosporioides*	Absent
41	Ratlam	MP-51	*C. capsici*	Present
Chhattisgarh (2021)				
42	Bilaspur	CH-1	*C. capsici*	Present
Andhra Pradesh (2020)				
43	Guntur	APG-1	*C. capsici*	Present
44		APG-2-1	*C. capsici*	Present
45		APG-2-2	*C. gloeosporioides*	Present
46		APG-3	*C. gloeosporioides*	Absent
47		APG-4-1	*C. capsici*	Present
48		APG-4-2	*C. gloeosporioides*	Absent
49		APG-5-1	*C. capsici*	Present
50		APG-5-2	*C. gloeosporioides*	Absent
51		APG-6	*C. capsici*	Present
52		APG-7	*C. capsici*	Present
53		APG-8	*C. capsici*	Present
54		APG-9	*C. capsici*	Present
55		APG-10	*C. capsici*	Present
56	Prakasam	APG-11	*C. capsici*	Present
57		APG-13	*C. capsici*	Present
58		APG-14	*C. capsici*	Present
59		APG-15	*C. capsici*	Present
60	Kurnool	APG-17	*C. capsici*	Present
61		APG-19	*C. capsici*	Present
62		APG-23	*C. capsici*	Present
63		APG-25	*C. capsici*	Present
Karnataka (2020)				
64	Bellari	KA-1	*C. capsici*	Present
65		KA-2	*C. capsici*	Present
66		KA-3	*C. capsici*	Present
67		KA-4	*C. capsici*	Present
68		KA-5	*C. capsici*	Present
69		KA-6	*C. capsici*	Present
70		KA-7	*C. capsici*	Present
71		KA-8-1	*C. capsici*	Present
72		KA-8-2	*C. gloeosporioides*	Absent
73		KA-9-1	*C. capsici*	Present
74		KA-9-2	*C. gloeosporioides*	Absent
75		KA-10	*C. capsici*	Present
76		KA-11	*C. capsici*	Present
Tamil Nadu (2019-2020)				
77	Trichi	TN-1	*C. gloeosporioides*	Absent
78		TN-2	*C. gloeosporioides*	Absent
79		TN-3	*C. gloeosporioides*	Absent
Maharashtra (2017-2021)				
80	Nagpur	MH-1	*C. capsici*	Present
81		MH-2	*C. capsici*	Present
82		MH-3-1	*C. capsici*	Present
83		MH-3-2	*C. gloeosporioides*	Absent
84		MH-4-1	*C. capsici*	Present
85		MH-4-2	*C. gloeosporioides*	Absent
86		MH-5	*C. capsici*	Present
87		MH-6	*C. gloeosporioides*	Absent
88		MH-8	*C. capsici*	Present
89		MH-10	*C. capsici*	Present
90		MH-11	*C. capsici*	Present
91		MH-12	*C. capsici*	Present

92		MH-13	*C. capsici*	Present
93		MH-14	*C. capsici*	Present
94		MH-15	*C. capsici*	Present
95		MH-16	*C. capsici*	Present
96		MH-17	*C. capsici*	Present
97		MH-18	*C. capsici*	Present
98		MH-19	*C. capsici*	Present
99		MH-20	*C. capsici*	Present
100	Yavatmal	MH-21	*C. gloeosporioides*	Absent
101	Wardha	MH-7	*C. capsici*	Present
102	Latur	MH-9	*C. capsici*	Present
Punjab (2021)				
103	Fazilka	PB-1	*C. capsici*	Present

Species-specific primers have also been designed based on dissimilarities in the sequence of the internal transcribe spacer (ITS) regions of representative isolates of *Colletotrichum*. This approach has been demonstrated to be a reliable technique to identify and differentiate *C. acutatum* from *C. gloeosporioides* (Harp et al. 2008; Sreenivasaprasad et al. 1992; 1994; Kamle et. al. 2013; Widodo and Hidayat, 2018).

Pathogenicity of identified *Colletotrichum* species.

After incubation period for 10 days, *Colletotrichum* symptoms were produced by all the isolates. Red chilli sets inoculated with *C. capsici* isolates has developed sunken lesions bearing black coloured setae and acervuli with greyish coloured conidial masses developed in concentric rings. Red Chilli sets inoculated with *C. gloeosporioides* has developed sunken lesions with pinkish to orange coloured spore masses in concentric rings around point of inoculation. The fungi were reisolated and we received satisfactory results to prove Koch's postulates.

Identification of predominant species

In our experiment, infected chilli samples were collected from 95 locations and successfully isolated 103 *Colletotrichum* isolates. *Colletotrichum capsici* was identified and confirmed from 88 location samples while *Colletotrichum gloeosporioides* was identified and confirmed from 15 location samples. Based on current study, the predominant species was *Colletotrichum capsici* in India as identified and confirmed with species specific markers. Sampling details along with identified *Colletotrichum* species is mentioned in Table 5.

The present findings agree with the earlier reports of (Patidar and Tomar, 2022) from Sehore district of Madhya Pradesh and (Harshitha et. al. 2022) from northern part of Karnataka. Additionally, *Colletotrichum acutatum* was also spotted at few locations in North Karnataka (Harshitha et. al. 2022). *C. capsici* and *C. gloeosporioides* were the 2 predominant species in Southern India (Rao et al., 2007). *C. capsici* was prominent in Karnataka, Tamil Nadu and Maharashtra (Rao et al., 2007), Himanchal Pradesh (Sharma et al., 2005), Andhra Pradesh (Rao et al., 2007; Pratibha, 2009; Nanda, 2011) and in Arunachal Pradesh (Selvakumar, 2007). (Anamika et. al., 2014) reported *C. capsici* and *C. gloeosporioides* causing serious problem for chilli cultivation in Fiji. Similarly isolates collected from chili producing areas of Indonesia, Malaysia, Sri Lanka, Thailand, and Taiwan showed predominance of *Colletotrichum capsici* (De silva et. al. 2019). Reports of (Liu et. al. 2016) confirming predominance of *C. capsici* infecting chilli from Sichuan Province, China. However, *C. acutatum* has been reported to be predominant species followed by *C. capsici* and *C. gloeosporioides* particularly in Indonesia (Widodo and Hidayat, 2018) and in Korea (Kim et. al. 2008).

In our study we did not find *C. acutatum* while (Harshitha et. al. 2022) reported it from some locations in North Karnataka. We have collected infected samples from Karnataka region in Kharif 2020 while (Harshitha et. al. 2022) has collected samples in Kharif 2021. It is possible that this species was missed unfortunately during collection. In Kharif 2021 we have received some reports (data unpublished) of outbreak of *C. acutatum* from Karnataka particularly infected chilli on green fruit stage but unfortunately, we were unable to collect samples and include in this study.

Researchers from other countries have already mapped their regional *Colletotrichum* isolates. India contributing very large agricultural area specifically growing chilli and recently anthracnose creating havoc to chilli growers due to climate change and favorable disease conditions. Most of the Indian studies published recently emphasizing on small geographical area and that too in different years. This misleads the by and large scenario of the current anthracnose disease incidence. This study was specifically carried out with Indian *Colletotrichum* isolates which demonstrate the current tentative/approximate contribution of different Anthracnose causing *Colletotrichum* species. To identify the resistant/immune germplasm for Anthracnose, the geographical mapping of *Colletotrichum* species is of prime importance.

CONCLUSION

Anthracnose infection mainly affects chilli at red fruit stage. Morphological characterization of collected samples from the surveyed region revealed that *Colletotrichum capsici* and *C. gloeosporioides* were the major species infecting chilli while *Colletotrichum capsici* was the predominant species. As *C. capsici* is predominant species causing Anthracnose, we need anthracnose resistant source at least against *C. capsici*. Resistant source against both C, capsici and *C. gloeosporioides* will be a boon for breeders to strengthen disease resistance.

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