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Understanding the Etiology of Chilli Fruit Rot Disease in Tamil Nadu

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ABSTRACT

Background: Fruit rot is an age old destructive disease of chilli caused by complex fungal pathogens resulting in severe yield losses both at pre and post-harvest stages. Hence, this study aims to investigate the occurrence of fruit rot disease in Tamil Nadu and to identify the fungal pathogens associated with fruit rot symptoms based on morpho-molecular characters.

Methods: Roving survey was conducted in various districts of Tamil Nadu to determine the prevalence of fruit rot disease and to ascertain its causative agents. The pathogens were identified based on the conidial morphology, cultural characteristics and further confirmed by Polymerase chain reaction (PCR) using ITS 1 and ITS 4 primers.

Result: Maximum disease severity was documented in Dharmapuri followed by Namakkal and lowest in Nagapattinam district. Ten isolates representing two genera *viz., Colletotrichum* and *Fusarium* were recovered from fruit rot infected chilli fruits and validated by pathogenicity test. Based on the morpho-cultural characters, 2 isolates were identified as *Fusarium* sp., with oval/ellipsoidal microconidia and straight/curved macroconidia, 6 isolates as *Colletotrichum scovillei* with fusiform conidia and 2 isolates as *Colletotrichum truncatum* with falcate conidia. The virulent isolates were further confirmed as *C. scovillei* based on PCR amplification of ITS region of genomic DNA.

Key words: Chilli, *Colletotrichum scovillei, Colletotrichum truncatum,* Fruit rot, *Fusarium* sp., Roving survey.

INTRODUCTION

Chillies were cultivated all over the world for their vibrant colour, spicy flavour and medicinal properties (Rahman *et al*., 2011). It is a spice crop of India and plays a significant role in the Indian economy. Although India is a global leader in chilli production (FAOSTAT, 2020), unfortunately its productivity is negatively impacted by more than forty fungal diseases (Rangaswami, 1979). Fruit rot is one of the most threatening pre and postharvest disease of chilli, posing a significant challenge to its profitable cultivation in all the chilli producing regions of the world (Than *et al.,* 2008). It has been reported to be caused by complex pathogens including various species of *Colletotrichum*, *Fusarium* and *Alternaria alternata* (Machenahalli *et al.,* 2014; Parey *et al.,* 2013). These pathogens extensively damage the fruits and significantly lower the quality, appearance, yield and marketability of the fruits (Suresha *et al.,* 2012).

Though there are 24 different species of *Colletotrichum* documented to cause fruit rot worldwide (Mongkolporn and Taylor, 2018), in India, it is predominantly caused by *C. acutatum* (Simmonds), *C. capsici* (Syd.) Butler and Bisby, *C. gloeosporioides* (Penz) Penz. and Sacc. (Saxena *et al.,* 2016). Fruit rot of chilli is also caused by various species of *Fusarium* (Zhu *et al.,* 2021; Datar and Ghule, 1985 and Parey *et al*., 2013). Under congenial environmental conditions, *Colletotrichum* species complicated fruit rot could result in 80 per cent yield loss (Katoch *et al*., 2017). The substantial financial losses due to fruit rot disease is a result of seed borne nature, (Mishra, 1988) pre- and postharvest infection and the complexity of fungal pathogens.

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Thorough identification of pathogens and accurate characterization of species are pre-requisite to understand the epidemiology of the disease and to establish more effective disease management approaches (Deyol *et al.,* 2015). Thus, studies were conducted to determine the state of chilli fruit rot incidence in different districts of Tamil Nadu and to identify the fungal pathogens associated with fruit

rot symptoms using the combination of morpho- cultural and morphological features.

MATERIALS AND METHODS

All the experiments were conducted during 2020-2021 at the Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore, India.

Survey for the incidence of chilli fruit rot disease

During 2020-2021, roving surveys were conducted to assess the severity of chilli fruit rot disease in various chilli growing regions of Tamil Nadu, India. Two taluks were selected in each district and in each taluk, four villages were selected and in each village two fields were randomly selected for assessing the disease severity at fruiting stage. One hundred fruits were randomly collected by walking across the field from south-west to north-east corner and disease severity was assessed according to the disease score chart proposed by Montri *et al.,* (2009) (Table 1) and the percentage disease index (PDI) was computed (Wheeler, 1969).

Isolation of fruit rot pathogens

Pathogens were isolated from the infected fruits collected during the survey by tissue segment method. The diseased portion of the fruits were surface sterilized with 1% NaOCl for 1-2 min and rinsed thrice with repeated changes of sterile distilled water (Pappachan *et al.*, 2020). The surface sterilized tissues were inoculated onto sterile PDA medium supplemented with streptomycin sulphate (0.03 g/l) and incubated for 5 days at 28±2°C. Purified cultures were maintained on PDA slants at 25°C and used for further studies.

Pathogenicity test and virulence assessment

The pathogen cultures were tested for its pathogenicity on chilli fruits by detached fruit assay (De Silva *et al*., 2017). Fresh healthy unripened fruits (hybrid Ganga) were disinfected in 2% sodium hypochloride solution for 2 minutes followed by 70% ethanol for 30 sec and washed thrice with sterile distilled water. Surface disinfected fruits were inoculated with mycelial agar plugs of each individual isolate by pin-pricking chilli fruit pericarp with a sterile needle to a depth of one mm. Fruits inoculated with plain agar plugs was maintained as control. The Petri dishes were incubated in growth chamber at 25°C and 95-98% relative humidity for 5-7 days. The experiment was repeated twice with three fruits per isolate. In order to validate Koch's postulates, pathogens were re-isolated from the infected fruits as mentioned previously. The pathogen identity was confirmed by morpho-molecular characteristics. Disease severity on inoculated fruits were assessed 7 days after inoculation (Montri *et al.,* 2009).

Identification of fruit rot pathogens

The pathogens were identified based on the morpho-molecular characteristics, pathogenicity and by comparison with authentic description given by Booth (1971) and Liu *et al.* (2016). The cultural characteristics of pathogen isolates such as colony color, growth pattern and sporulation were studied on PDA medium (Sharma *et al*., 2014). Semi-permanent slides were made from 10-day-old colonies and the conidial characteristics of various isolates were documented using a phase contrast microscope (Leica DM2000 and DM2000 LED). Three highly virulent isolates *viz.,* TD1, TC2 and TS1 which caused maximum fruit rot infection were confirmed at molecular level by Polymerase chain reaction (PCR) using ITS 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′TCCTCCGCTTATTGATATGC-3′) primer pairs (White *et al*., 1990). The PCR amplicons were sequenced by Sanger dideoxy method in Biokart India Pvt. Ltd, Bangalore, India. The sequences were submitted to the NCBI GenBank to obtain the accession numbers.

Statistical analysis

The data were statistically analyzed following standard methods (Gomez and Gomez 1984) and the significant difference between the treatments was found out by Least Significant Difference (LSD) at 5 per cent in the AGRES. The data showing percentages were transformed in to arc sine values.

RESULTS AND DISCUSSION

Survey for the incidence of chilli fruit rot disease

The survey revealed that fruit rot disease was prevalent in all the surveyed locations with varied levels of incidence ranging from 44.75 to 72.59 per cent. The disease severity was maximum in Dharmapuri (72.59 per cent) followed by Namakkal (61.88 per cent) and lowest in Nagapattinam district (40.49 per cent) (Table 2). During the survey it was observed that symptoms were observed not only on red ripe fruits but also on green fruits (Fig 1). Present results were in accordance with the findings of Raj and Christopher, (2009)

Table 1: Chilli fruit rot disease score chart.

Score	Per cent fruit area infection	Disease reaction
0	No infection	Highly resistant (HR)
1	1-2% of fruit area shows necrotic lesion/a large water-soaked lesion at the infection site	Resistant (R)
3	>2-5% of fruit area shows necrotic lesion/water-soaked lesion upto 5% of fruit surface /acervuli may be present	Moderately resistant (MR)
5	>5-15% of fruit area shows necrotic lesion with acervuli/water-soaked lesion upto 25% of the fruit surface	Moderately susceptible (MS)
7	>15-25% of fruit area shows necrosis with acervuli	Susceptible (S)
9	>25% of fruit area shows necrosis with abundant acervuli	Highly susceptible (HS)

who reported that fruit rot is one of the most devastating disease of chilli with yield loss ranging from 20 to 70 per cent in Tamil Nadu. The highest disease severity may be due to the conducive environmental conditions, cultivation of chilli year after year without crop rotation and susceptibility of cultivar grown in those areas and varying degrees of virulence (Shilpa and Mesta, 2017).

Table 2: Survey for the incidence of chilli fruit rot disease in Tamil Nadu.

District	Crop stage	Per cent disease index (PDI)
Coimbatore	Vegetative and fruiting	59.26^{b} (50.35)
Dharmapuri	Fruiting	72.59^{a} (58.46)
Krishnagiri	Fruiting	54.01^{bc} (47.30)
Nagapattinam	Fruiting	40.49^{d} (39.49)
Namakkal	Fruiting	61.88^{b} (51.91)
Salem	Vegetative and fruiting	44.75^{d} (41.98)
Thoothukudi	Fruiting	47.55^{cd} (43.59)
	CD (0.05)	5.061
	SE(d)	2.297

Values are mean of three replications. Means in a column followed by same superscript letters are not significantly different at 5% level. Values in parenthesis are arc sine transformed.

Pathogenicity on chilli fruits

In the detached fruit assay typical fruit rot lesions were developed in the fruits inoculated with fungal isolates whereas plain agar plug inoculated fruits remain asymptomatic (control). The fungal isolates re-isolated from the injected fruits had identical morphologies to the original isolates, proving Koch's postulates. Among the ten isolates, TD1, TC2 and TS1 isolates obtained from Dharmapuri, Coimbatore and Salem district were found to be highly virulent in causing maximum fruit rot incidence compared to other isolates (Table 4). All the isolates induced fruit rot symptoms in chilli fruits and *C. scovillei* was found to be highly pathogenic. This is in concurrence with the findings of Liu *et al.,* (2016) who stated that *C. scovillei* was the most virulent species of acutatum complex affecting *Capsicum*

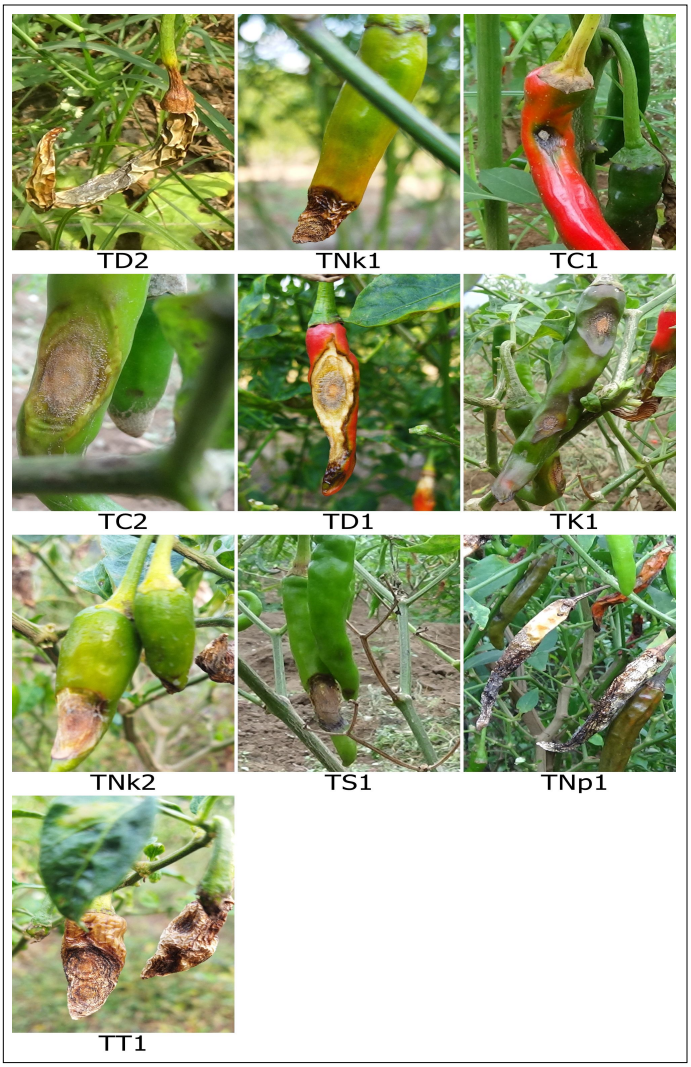

Fig 1: Chilli fruit rot symptoms at various locations of Tamil Nadu.

Table 3: Morphological and cultural characteristics of pathogens causing chilli fruit rot disease.

Isolate code	Morphological identity	Morphological group	Culture character				Conidial character	
			Colony colour	Mycelial growth pattern	Days to full growth	Growth rate	Sporulation	Shape
TD2	*Fusarium* sp	Group 1	White to pale brown, reverse side: brown	Fluffy growth with concentric rings	9	Fast	Excellent	Curved macroconidia, oval microconidia
TNk1	*Fusarium* sp	Group 1	White, reverse side: brown	Fluffy growth	7	Fast	Fair	
TC1	*C. scovillei*	Group 2	White to pale orange, reverse side: pale orange to white	Suppressed growth	15	Slow	Good	Fusiform
TC2	*C. scovillei*	Group 2	Pale orange, reverse side: pale orange	Suppressed growth	16	Slow	Good	
TD1	*C. scovillei*	Group 2	Pale orange to pale grey, reverse side: pale orange	Cottony growth	16	Slow	Excellent	
TK1	*C. scovillei*	Group 2	Pale orange with white patches, reverse: pale orange	Cottony suppressed growth	17	Slow	Good	
TNk2	*C. scovillei*	Group 2	Pale orange and black at periphery, reverse side: black	Suppressed growth	15	Slow	Fair	
TS1	*C. scovillei*	Group 2	Pale orange to white, reverse side: pale orange	Fluffy growth	14	Medium	Excellent	
TNp1	*C. truncatum*	Group 3	Pale grey, reverse side: black	Cottony growth with concentric ring at periphery	8	Fast	Excellent	Falcate
TT1	*C. truncatum*	Group 3	Grey, reverse side: black	Fluffy growth	8	Fast	Good	

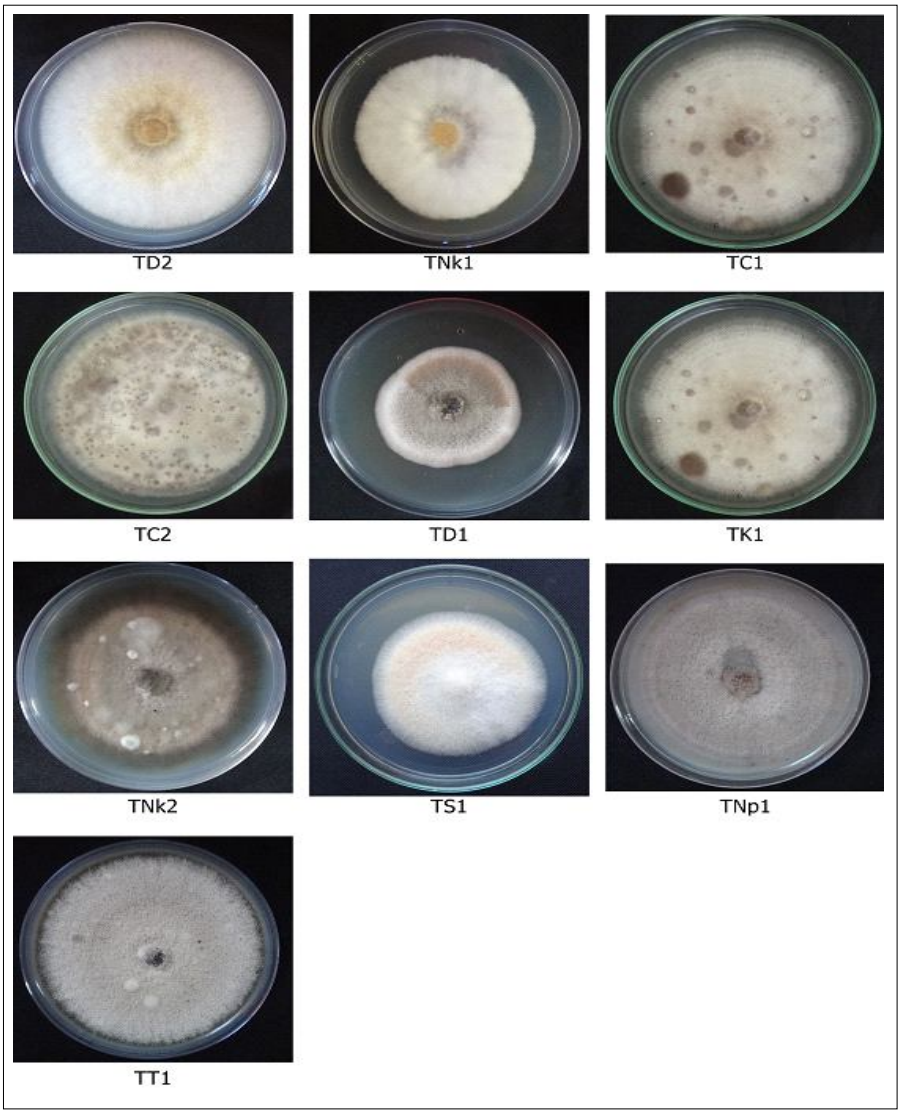

Fig 2: Cultural characters of chilli fruit rot pathogens.

Table 4: Disease severity and aggressiveness of pathogens determined by pathogenicity test.

Isolate code	District	Pathogen identity	Morphological group	Fruit rot incidence (%)	Mean fruit rot incidence (%)
TD2	Dharmapuri	*Fusarium* sp	Group 1	42.44^{ef} (40.64)	38.07
TNk1	Namakkal	*Fusarium* sp	Group 1	33.71^{f} (35.47)	
TC1	Coimbatore	*C. scovillei*	Group 2	53.52^{bcd} (47.03)	60.49
TC2	Coimbatore	*C. scovillei*	Group 2	61.86^{ab} (51.88)	
TD1	Dharmapuri	*C. scovillei*	Group 2	70.39^{a} (57.07)	
TK1	Krishnagiri	*C. scovillei*	Group 2	56.96^{bc} (49.00)	
TNk2	Namakkal	*C. scovillei*	Group 2	57.38^{bc} (49.28)	
TS1	Salem	*C. scovillei*	Group 2	62.84^{ab} (52.46)	
TNp1	Nagapattinam	*C. truncatum*	Group 3	45.66^{de} (42.44)	46.78
TT1	Thoothukudi	*C. truncatum*	Group 3	47.89^{cde} (43.79)	
			CD (0.05)	5.906	
			SE(d)	2.812	

Values are mean of three replications. Means in a column followed by same superscript letters are not significantly different at 5% level. Values in parenthesis are arc sine transformed.

spp. Further, Than *et al*. (2008) stated that *C. acutatum* was a highly virulent species capable of infecting the wound-resistant *Capsicum chinense* PBC 932. Current findings of *Fusarium* sp as the casual organism of chilli fruit rot is consistent with the previous reports on the association of *Fusarium solani* (Mart.) Sacc. (Datar and Ghule, 1985), *F. incarnatum* (Zhu *et al.,* 2021), *Fusarium oxysporum* (Yang *et al.,* 2009), *Fusarium moniliforme* and *Fusarium pallidoroseum* (Parey *et al*., 2013) with fruit rot disease.

Morpho-molecular characterization of fruit rot pathogens

Growth rate of fungal colonies, conidial shape and size of a pathogen were the significant characters for distinguishing among *C. gloeosporioides, C. truncatum* and *C. acutatum* (Than *et al*., 2008). According to morpho-cultural characteristics, ten isolates were classified into three morphological groups (Table 3). Each group exhibited distinct morphology on potato dextrose agar (PDA) medium 10 days after incubation. Isolates from Group 1 produced white colonies which gradually become light brown on upper side and dark brown on reverse side (Fig 2). The mycelial colonies of Group 2 isolates varied from white to pale orange to pale grey. The isolates belonging to Group 3 produced pale grey to dark grey to black cottony colonies. Group 1 and group 3 isolates grew faster while Group 2 isolates recorded medium to sluggish growth. Isolates belonging to group 1 reached full growth in 90 mm Petri plates within seven to ten days, whereas group 3 isolates required approximately eight to ten days and group 2 isolates required approximately twelve to eighteen days. Previous studies have shown that *C. acutatum* can be differentiated from *C. gloeosporioides* based on its slower growth rate (Simmonds, 1965). Hence, slow growth of *C. scovillei* conformed to the characteristics of the *C. acutatum* complex.

The isolates also reveal substantial variations in their conidial shape. The isolates belonging to Group 1 produced spherical to oval/ellipsoidal microconidia and straight or curved macroconidia. Isolates of Group 2 and 3 produced fusiform and falcate shaped conidia respectively (Fig 3). On the basis of their conidial characteristics, Group 1 isolates correspond to *Fusarium* sp, Group 2 isolates correspond to *C. acutatum* complex and Group 3 isolates match the description of *C. truncatum*. Hence, on the basis of phenotypic and cultural characteristics, the isolates under group 1 were identified as *Fusarium* sp and group 2 and 3 isolates were identified as *C. scovillei* and *C. truncatum* respectively. *C. scovillei* was reported to be virulent yet slow-growing. Liu *et al.,* (2016) also characterized *C. gloeosporioides* complex, *C. truncatum* isolates and *C. acutatum* isolates in pepper based on the morphological and molecular characters. Mallik *et al*. (2021) also identified *F. solani* as casual organism of fruit rot disease of sweet pepper based on morphological features. The sequences of highly virulent isolates *viz.,* TD1, TC2 and TS1 were provided with accession numbers ON182069, ON178661 and ON178658 respectively which further confirms the identity of the pathogen as *C. scovillei*.

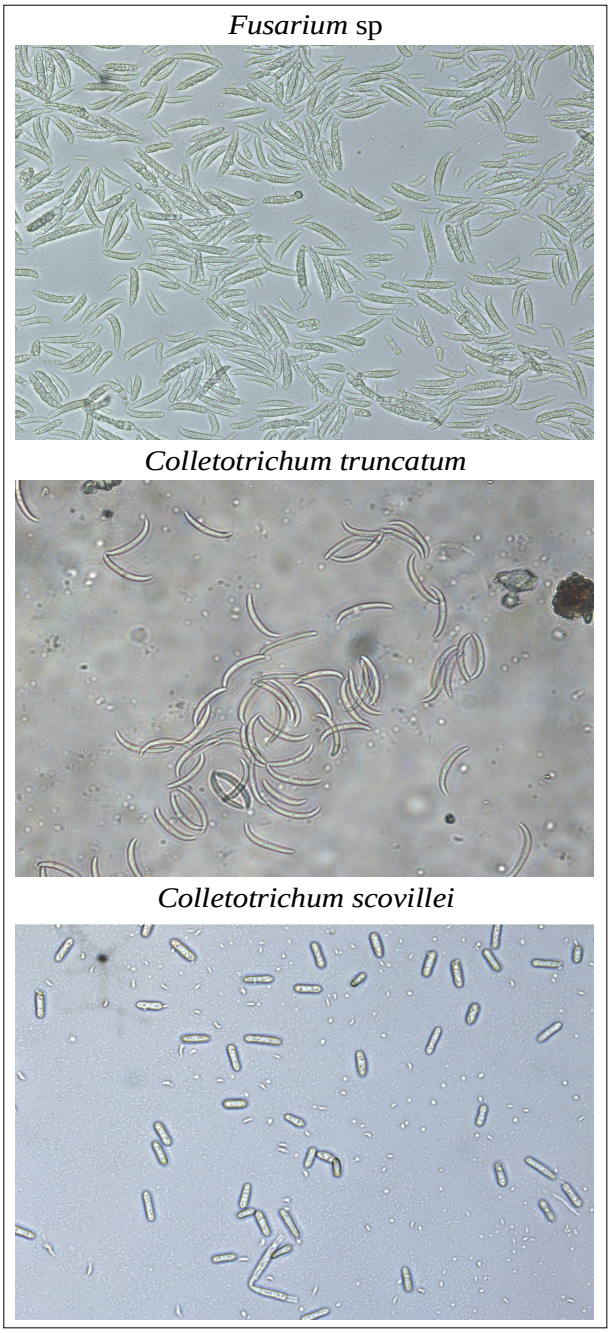

Fig 3: Spore character of chilli fruit rot pathogens.

CONCLUSION

Hence, the fungal pathogens associated with fruit rot disease were identified as Fusarium sp., *C. scovillei* and *C. truncatum* based on morpho-cultural characters and further confirmed at molecular level and by pathogenicity tests. Hence, this finding could be the basis for establishing more effective disease management approaches to combat this complex natured infection in chilli cultivation.

Conflict of interest: None.

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