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# A new report of *Colletotrichum jasminigenum*, an anthracnose rot causing pathogen to *Capsicum annuum* in Kashmir valley, India

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Bell pepper (*Capsicum annuum L.*) is damaged by many fungal pathogens, among which anthracnose rot is one of the economically most important fungal disease. A field survey was carried out throughout 2019-2022 and infected bell pepper samples were collected from many fields across the valley of Kashmir. The fungus was cultured and observed, leading to identification of one isolate on, cultural, microscopic and molecular basis as *Colletotrichum jasminigenum*. The pathogen was re-confirmed by following pathogenicity test. To the best of our knowledge this is the first report of this pathogen causing anthracnose rot of bell pepper in Kashmir Valley, India

**Keywords:** Bell pepper, disease fungus, phytopathogen

## INTRODUCTION

In tropical and subtropical regions, *Capsicum annuum* L. is cultivated as an important vegetable crop. Bell pepper is one of the world's most commercially grown crops, with the green fruit consumed as a vegetable and the dried chilli utilised as a spice in a broad range of dishes and cuisines (Kambar *et al*. 2014). Capsicum preparations are used to treat a number of diseases (Sahitya *et al.* 2014). According to food and agricultural organisation 2020, chilli production throughout world is 41.57 lakh tonnes. The FAO estimates that India has become a major producer and exporter of chillies, accounting for one-fourth of global output. India produces about 1,702,000 metric tonnes of chilli in 2020 followed by Thailand with 322, 886 metric tonnes. It is a significant cash crop farmed in India for both the domestic and international markets. In Kashmir, the area under cultivation is 3200 hectares with an annual production of 64800 tonnes (NHB, 2017).

The production of chilli is getting affected by a lot of factors and fungal diseases being the major constraint. In India, Chilli anthracnose is thought to be the most serious disease caused by several species of *Colletotrichum,* including *C. acutatum, C. capsici*, and *C. gloeosporioides*. Out of them, *C. capsici* is the principal pathogen causing this disease (Susheela, 2012; Chaisemsaeng *et al*. 2013). *Colletotrichum* species are predominant post-harvest pathogens of vegetables and fruit crops causing substantial damage to various economically important crops (De Silva *et al*. 2017). Keeping in view the extent of losses caused by anthracnose pathogens, the study was carried out to isolate the anthracnose rot pathogen infecting bell pepper in Kashmir valley.

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## MATERIALS AND METHODS

### *Survey sites and sample collection*

The survey was conducted at different fields, markets, and go-downs in the five districts of the Kashmir Valley, viz., Anantnag, Kulgam, Ganderbal, Baramulla, and Bandipora during the years 2019–2022 (Fig.1). A total of 30 infected fruits with marks including recessed necrotic tissue and concentric bands of acervuli were taken from multiple locations around the Kashmir valley in labelled polythene bags. The samples were brought to the Plant Pathology, Mycology

and Microbiology Laboratory, University of Kashmir for the further processing.

***Isolation of the pathogen***

Infected samples were carefully cleansed with tap water before being diced into small pieces by sterilised blade. The small pieces have half healthy and half diseased tissue. The infected pieces were surface sterilised using 1% sodium hypochlorite for five mins, followed by two or three rounds of sterile water washing. These infected parts were then aseptically placed on the plates of potato dextrose agar supplemented with lab. grade streptomycin. The plates were then incubated at 26 ± 2 °C in dark and monitored on regular basis. The expanding mycelium from the edges of colonies that seemed to be separate was sub-cultured on new culture plate after 48–72 hours of incubation.

***Identification of fungi***

The identification of the fungus was done based on cultural, morphological, and molecular characteristics.

***Cultural and morphological characteristics***

Culture characters like colony shape, size, colour (upper and reverse), margins, and growth form were observed. A 10-day-old culture was used to prepare a permanent slide. Morphological characters of the fungi were observed, including mycelium characters (hyphae, colour and septation), conidia (size and shape) and appressoria (shape and size).

***Molecular and phylogenetic analysis***

Total genomic DNA was extracted from a 14 day old culture using chloroform isoamyl alcohol method. The fungal specific primers ITS1-F and ITS4-R amplify a wide range of fungal targets and have high efficiency for analysing DNA isolated from individual fungi were used (White *et al.* 1990). The amplified PCR products were sequenced and the quality of the sequences was checked from the chromatogram with Sequence Scanner Software ver. 1. The sequences were then analysed with BLAST against the reported fungi at NCBI site. The phylogenetic tree was also constructed using the sequencing data of the fungi with the help of RAxML software.

***Pathogenicity test***

The pathogenicity test was carried out under both *in-vitro* and *in-vivo* conditions on chilli fruit samples. For pathogenicity, six replicates for every treatment and control were maintained under both *in-vitro* and *in-vivo* conditions. Under *in-vitro* conditions, the pathogenicity of each isolate was examined using wound inoculation techniques. The pathogenicity test was conducted using mature, detached chilli fruits under in vitro conditions. Healthy bell peppers were collected from field and they were surface sterlized with 4% sodium hyphochlorite followed by washing with sterile water. By placing the sterilised fruits on pre-sterilised blotting sheets, they were allowed to air dry in the laminar airflow. A 3mm wound was made in the fruits with the help of cork borer. A 3 mm mycelial plug of *C. jasminigenum* from 10 day old culture was inserted in the wound. The fruits were then incubated for 7 days at 28 ±2°C in moist, airtight chambers maintained on sterilized beakers covered with aluminium foil using wet filter sheets. For *in-vivo* pathogenicity, seeds were procured from SKAUST Kashmir, and were sown in germination trays. After 30 days, the seedling plants of bell pepper were grown in pots under greenhouse conditions. The plants were watered regularly. After 2 months of fruit initiation, the fruits were treated with pathogen *C. jasminigenum*. For this, 10µl of spore suspension maintained at $1x10^6$ conidia were inoculated in the healthy fruits by fruit puncture method (Susheela, 2012). Control plants were inoculated with 10 µl of sterile water. The pots were then incubated at 28 ±2°C for 7 days in moist airtight sterilized poly bags. After 6 days of inoculation, the symptoms developed on the treated fruits were compared with the original symptoms and the symptom expressions were recorded. The pathogen was isolated from the inoculated fruits and identified to validate Koch's postulates.

**RESULTS AND DISCUSSION**

The results revealed that from the total of 30 rotted fruit samples, 12 species of fungi were

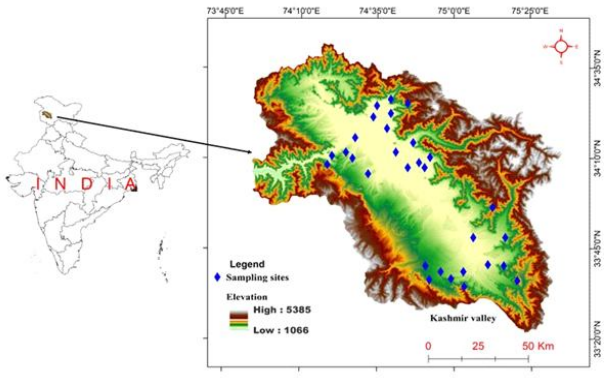


**Fig. 1:** Map showing sampling sites selected for survey and collection of rotted samples of *capsicum annuum* in Kashmir valley

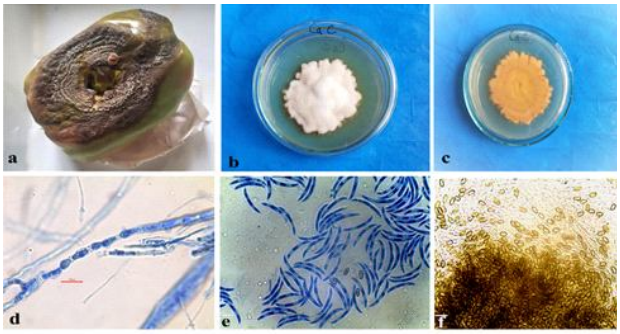

**Fig. 2 :** *Colletotrichum jasminigenum* **a.** Infected fruit samples showing anthracnose symptoms **b.** Colony culture front **c.** Colony culture reverse **d.** Septate mycelium **e.** Curved conidia **f.** Brown appressoria.

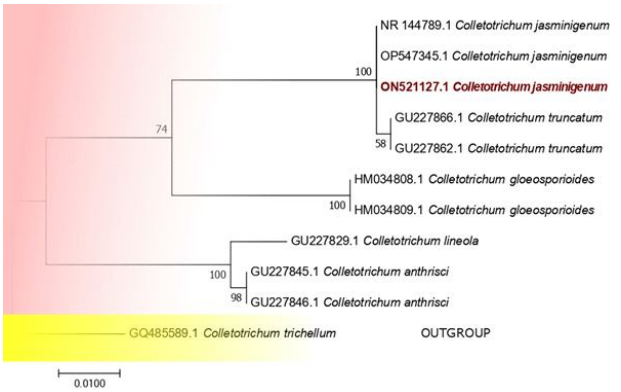


**Fig.3:** Phylogenetic relationship of *C. jasminigenum* as inferred from ITS sequence using maximum likelihood method.

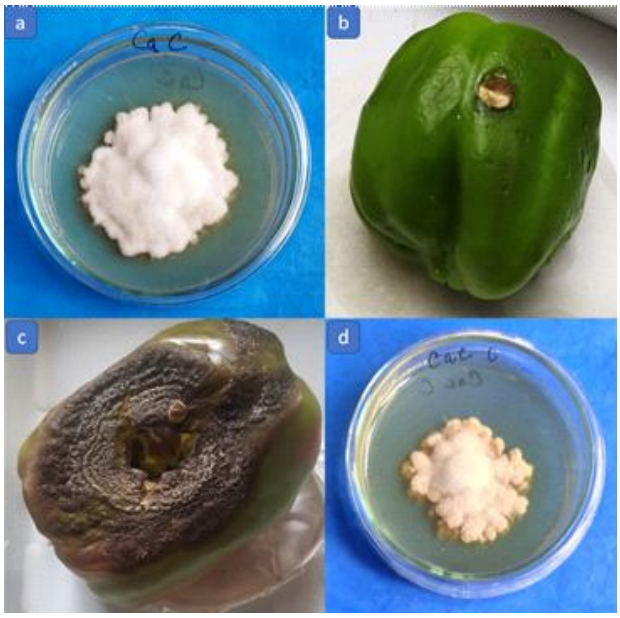

**Fig. 4:** *In-vitro* pathogenicity of *C. jasminigenum* causing anthracnose rot of bell pepper **a.** *C. jasminigenum* isolated from infected bell pepper **b.** Fruits inoculated with *C. jasminigenum* **c.** Inoculated fruit showing symptom expression of anthracnose **d.** Re-isolated fungus from inoculated fruit.

**Fig. 5:** *In-vivo* pathogenicity of *C. jasminigenum* causing anthracnose rot of bell pepper **a.** *C. jasminigenum* isolated from infected bell pepper **b.** Fruits inoculated with *C. jasminigenum* **c.** Inoculated fruit showing symptom expression of anthracnose **d.** Re-isolated fungus from inoculated fruit.

isolated. Among these isolated fungi, *C. jasminigenum was* found to be a new pathogen associated with anthracnose fruit rot. The infected fruits showed symptoms with marks including recessed necrotic tissue and concentric bands of acervuli (Fig.2a)

### *Cultural characteristics*

A freshly prepared culture on PDA was used for morphological analysis. Colonies were raised grey, irregular margins and reverse with zonation. Colonies produce black acervuli from centre to the margin. There were no pigments in the media and colony growth rate is slow measuring 43-47mm in diameter 7 days after incubation at 25±2$^0$C (Fig. 2b, 2c).

### *Microscopic characteristics*

Morphological characters were examined using a Leica DM-750 compound microscope.

Vegetative hyphae branched, hyaline septate and 10-20 μm wide (Fig. 2d). Chlamydospores and setae were not observed. Conidiophores hyaline, not branched and setae absent in all isolates. Conidia are hyaline, curved, one-celled, smooth-walled, broadest in the centre, with a large central guttule and measure 20.5-25 µm x 3-3.5 µm (Fig. 2e). Compared to other species in the truncatum-complex *Colletotrichum jasminigenum* produces much more brown ovoid, clavate to irregular and bigger appressoria as shown in (Fig.2f) (Damm *et al.* 2009).

***Molecular and Phylogenetic analysis***

The identification was confirmed by ITS-rDNA analysis and phylogenetic analysis in addition to morpho- cultural characteristics. By performing molecular analysis with the species-specific primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G -3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC -3'), the identification of the *C. jasminigenum* isolates was established (White *et al.*1990). The resultant ITS sequence was deposited in the NCBI genebank with the accession number ON521127 and had the highest identity (100%) with *C. jasminigenum* MFLUCC 10-0273 (gene bank accession no NR 144789.1).

The phylogenetic analysis was carried out in raxmlGUI ver. 2.0 software. 10 rDNA-ITS nucleotide sequences were retrieved from NCBI based on blast search and relevant published phylogenies. The final dataset consisting of 11 sequences including our sequences and the outgroup were aligned using MAFT alignment and manually edited in Bioedit. The final alignment contained 547 characters including gaps. The phylogenetic analysis based on Maximum Likelihood criteria with the GTRGAMMA substitution model was carried out in RAxML Version 2 software. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analysed. The branches corresponding to partitions reproduced in less than 50% of the bootstrap replicates are collapsed. The obtained phylogenetic tree (showing our species in red font) is presented in Fig.3 Our sequence align with NR 144789.1 and OP 547345.1 with a strong boot support of 100 suggesting its identity as *C. jasminigenum* which further authenticate our identification. *C. trichellum* was used as an outgroup for the present study. Based on cultural, microscopic, molecular and phylogenetic studies, the fungus was identified as *C. jasminigenum* (Fig. 3).

**Pathogenicity test:** Under both *in-vitro* and in vivo circumstances, the pathogenicity of the isolated pathogen was demonstrated in accordance with Koch's postulates. After 6 days, pathogen-inoculated healthy fruits displayed symptoms of necrotic lesion with acervuli under both *in-vitro* (Fig. 4) and *in-vivo* (Fig. 5) conditions. Re-isolating the phytopathogen from the diseased fruits and comparing it to the initial culture revealed that it was identical. Fruits that were injected with sterile water and used as a control did not exhibit any disease symptoms

***DISCUSSION***

The fruit rot of chillies decreases the shelf life and market value of the fruit. The disease anthracnose of chilli was prevalent in all the surveyed locations, posing a new threat to chilli growers. During the present study 30 diseased samples of chilli were collected for isolation of rot causing fungi and pathogens like *Colletotrichum truncatum, Alternaria alternata, Aspergillus flavus, Fusarium solani and Colletotrichum jasminigenum* were identified as rot causing. Among the fungal species identified, *Colletotrichum truncatum, Alternaria alternata, Aspergillus flavus, Fusarium solani* were found previously pathogenic on chilli as well as on other fruits at different places (Parey *et al.* 2013). The pathogens that cause rot diseases include species of *Colletotrichum, Aspergillus, Fusarium* sp. and *Alternaria* sp. Fruit rot and dieback are the most important fungal infections that affect chillies in India (Singh, 2000). Chilli fruit rot is caused by a variety of fungus that may be found in all chilli-growing locations across the world (Parey *et al*. 2013). The morphological and molecular study revealed a new fungus was *Colletotrichum jasminigenum* found associated with anthracnose rot of chilli in Kashmir valley for the first time in India. Though reports of *C. jasminigenum* on jasmine infection in Vietnam has been reported (Wikee *et al*. 2011), to our

understanding and knowledge, this is the first instance of *C. jasminigenum* being reported as causal agent of bell pepper anthracnose in the Kashmir valley, India.

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