Plant Archives Vol. 24, Special Issue (GABELS), 2024 pp.344-355 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.SP-GABELS.049

CHILLI ANTHRACNOSE: SURVEY FOR THE INCIDENCE AND CHARACTERIZATION OF *COLLETOTRICHUM* SPP. ISOLATES IN TELANGANA STATE

R.G. Kavya[1]*, M. Madhavi[2], B. Rajeswari[3], P. Sujatha[4] and S.N.C.V.L. Pushpavalli[5]

[1]Dept. of Seed Science and Technology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Hyderabad , Telangana, India

[2]Seed Research and Technology Centre, P.J.T.S.A.U., Rajendranagar, Hyderabad, Telangana State, India.

[3]Department of Plant Pathology, Agricultural College, Adilabad (Dist.), Telangana, India.

[4]Dept. of Seed Science, College of Agriculture, P.J.T.S.A.U., Rajendranagar, Hyderabad, Telangana State, India

[5]Institute of Biotechnology, College of Agriculture, P.J.T.S.A.U., Rajendranagar, Hyderabad, Telangana State, India.

*Corresponding author E-mail : kavyaguttal8@gmail.com

ABSTRACT

Chilli crop is susceptible to many foliar and soil borne fungal diseases of which fruit rot/ anthracnose caused by the *Colletotrichum truncatum* (syn. *C. capsici)* is significant. Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues with concentric rings of acervuli. Since, the pathogen express the latent infection, in India the crop yield losses ranged from10-80% with losses of up to 80-89% in Telangana. A roving survey was conducted inkharif chilli crop in the month of December, 2023 at fruit ripening stage in Khammam (Konijerala, Wyra mandals) and Warangal (Nallabelli mandal) districts, the major chilli growing areas ofTelangana where the disease incidence was assessed in different popular cultivars such as swarnamukhi, tejitha, keerthiteja, armour and tomato. The study revealed that highest anthracnose incidence was recorded in chilli cultivar, tomato with 72.34 per cent collected from Nagarajpalle village, Nallabelli mandal of Warangal district followed by cv. swarnamukhi and tejitha from Pandillapalli village, Konijerala mandal of Khammam district with 32.69 to 35.74 per cent and keerthiteja and armour with 23.58 to 26.21 per cent Vallapuram village of Wyra mandal, Khammam district. However, the average disease ranged from 25-29% in Khammam district and 15-18%, irrespective of the cultivars. In Warangal district. The collected anthracnose infected chilli seed samples were subjected to different standard seed health tests as given by ISTA, 2022 for detectionof test pathogen.Of the methods followed, standard blotter method was found effective and showed maximum per cent mean recovery of *Colletotrichum* spp. isolates 35.48 per cent followed by Agar plate method with 31.44 per cent. Component plating studies statedof *Collectotrichum* sp. on seed coat with mean per cent of 46.88 compared to the other parts of the seed. A total of five *Colletotrichum* isolates were isolated from the said cultivars and were further studied for their cultural, morphological and molecular characterization which revealed variability in their colony colour ranging from pale gray to dark gray brown, with cottony and fluffy texture and with colony margins varied from regular margins in *Cc*1, *Cc*3 and *Cc*4 isolates. While irregular margins in *Cc*2 and *Cc*5isolates. The isolate *Cc*5 exhibited maximum of 72.1 mm mycelial growth rate at 10 days after incubation and minimum by the isolate *Cc*3with 43.8mm.Morphological characters revealed highest number (+++) of acervuli in isolate, *Cc*1 moderately in isolates *Cc*5, *Cc*4(++) and lowest number in *Cc*3 and *Cc*2(+) isolates. All the isolates have exhibited falcateshape conidia with size ranging from 2.04 to 2.98 µm, conidial mass colour from orange to black in acervuli with setaesize from 4.31 to 6.65µm. Phylogenic analysis using MEGA 11 delineated the three virulent *Colletotrichum* spp. isolates in to two clades, where the isolate Cc4 (E0524 180 002 PCR ARM ITS 1);,*Cc2* (E0524180004 PCR TEJ ITS1) and *Cc1* (E0524 180 003 PCR SWA ITS 1) expressed consistency index with LC 488838.1 *C. truncatum* HCM 321; and MT95064.1 *C. capsici* isolate GK 17A, respectively.

***Keywords*:** Chilli, Anthracnose, *Colletotrichum*

Introduction

Chilli (*Capsicum annum*), which belongs to solanaceae family is a widely used vegetable and spice in tropical and subtropical locations across the globe. The term "Chilli" is derived from the Mexican word "chili" and has acquired the title of "wonder spice," emphasizing its importance as a staple crop. As green and ripe chilli fruits possess the alkaloid capsaicin, which gives food a distinctive spiciness and are used in a wide range of culinary applications, medicines, cosmetics etc. Globally, India is the leading chilli producer, consumer and exporter having maximum cultivable area. In India, chillies are cultivated in 1.69 lakh hectares with a production and productivity of 6.94 lakh tonnes and 4109 kg/ha, respectively (PJTSAU, Chilli Outlook. 2022) with the majority growing in Andhra Pradesh, Telangana, Tamil Nadu, Maharashtra, Karnataka, Orissa and West Bengal. Chilli production in Telangana is expected to be 5.21 lakh tonnes in 2022-23, compared to 7.16 lakh tonnes in 2021-22(ANGRAU, Chilli Outlook. 2022) with Telangana contributing to 22% and 38% of total India area and production, respectively.

Cultivation of chilli crop has become challenge for the past few years due the climatic changes and emergence of different biotic stresses. The crop is known to be infected by a wide range of foliar and soil-borne diseases, including damping off, twig blight, bacterial leaf spot, powdery mildew, wilt, stem rot and anthracnose or fruit rot. Chilli anthracnose disease is one of the most economically important diseases which cause pre- and postharvest fruit decay and thereby reducing marketable yield from 10% to 80% (Poonpolgul and Kumphai, 2007).

Chilli anthracnose, which is both seed and airborne, has a significant impact on seed germination and vigor (Saxena *et al*., 2016). It is a polycyclic disease caused by *Colletotrichum* species and infects the chilli during early crop growth and fruit ripening stages through latent infection by surviving on the seeds as acervuli and micro sclerotia with infection rates higher in mature plants than in young ones (Raj and Christopher, 2009). The spores disperse, deposit and germinate on surface of leaves, twigs and fruits of chilli plants by piercing the cuticle layer and producing the infectious hyphae, which eventually lead to development of lesions. Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues with concentric rings of acervuli. The weather factors such as temperatures of 27°C, relative humidity of 80% and soil pH of 5-6favors the incidence and spread of the disease. Infection occurs during early stages of the crop growth as die back phase while during fruit stage results in fruit rot phase. The symptoms of fruit rot include dark patches and water-soaked lesions on leaves, twigsand fruits which spread rapidly to other parts under favorable conditions.

In all the chilli-growing locations, the disease is prevalent where in the pathogen express the latent infection and causes an estimated yield losses ranging from20% to 100% in the world (Kiran *et al.,* 2020, Mongkolporn and Taylor, 2018). However, the latent infection, affects the crop yield losses of up to 50% (Pakdeevaraporn *et al*., 2005). In India, a calculated loss of 10–54% has been reported in yield of the crop due to the anthracnose disease (Lakshmesha *et al*., 2005; Ramachandran and Rathnamma, 2006). The loss is high owing to the pre and post harvest involvement of the pathogen causing a loss of 10–80% of the marketable yield of chilli fruits (Than *et al*., 2008). In India, more than half of all the losses are caused by pre-harvest and post-harvest losses. Haryana and Punjab (20-60%) as well as Assam (12 to 30%), have reported significant yield reductions (Sahitya *et al*., 2014). However, an estimated annual loss of about 29.5% amounting to 491.67 million US$ has been reported from India alone (Garg *et al*., 2014). In recent past, the disease monitoring survey conducted in major chilli growing areas of Telangana state *viz.,* Khammam, Warangal, Karimnagar and Rangared dy reported highest (54.63) mean per cent incidence of anthracnose in Warangal district followed by Karimnagar (43%) and Khammam districts (40%) (Ramesh *et al.*, 2018).

Association of various fungi with seed is certainly harmful causing loss in viability of seed and pre and post-emergence mortality of seedlings. The seed mycoflora of chilli seeds are responsible for loss in seed viability and seedling mortality in nursery beds. Chilli is a major commercial crop in Telangana state. Since the cultivable area is increasing and with the concern of pathogen affecting the seed quality and longevity with expanding chances of the disease, the present study was taken up by conducting survey which would be helpful for identifying the high-infection anthracnose incidence areas and also the *Colletotrichum* spp. isolates prevailing in these areas, so that timely control measures can be advised to prevent the spread of the disease to the farming community.

Materials and Methods

Survey and collection of seed samples

A roving survey was conducted in kharif crop in the month of December, 2023 in 86 farmers' fields to assess the chilli anthracnose/fruit rot disease at fruit

ripening stage in major chilli growing areas of Khammam and Warangal districts *i.e.* Pandillapalli village of Konijerala and Vallapuram village of Wyra mandals in Khammam district and Nagarajpalle village of Nallabelli mandal in Warangal district of Telangana covering different chilli segments based on pungency such as highly, medium and low pungent cultivars grown in the areas mentioned above, respectively. The per cent disease incidence (PDI) was recorded in one square meter area covering four sides and one in the center of the chilli fields. The per cent anthracnose incidence was calculated using the formula given under.

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{No. of plants infected by anthracnose disease}}{\text{Total no. of plants observed}} \times 100$$

From the surveyed areas, anthracnose-affected chilli pods were collected from various popular cultivars for isolation of *Colletotrichum* sp. isolates randomly from different chilli segments in the mentioned locations in table 1

Standard seed health detection methods and isolation of *Colletotrichum* isolates

Standard detection methods as described by ISTA, 2022 and Agarwal and Sinclair, 1997 were used for testing the seed health of collected chilli seed samples.

Standard blotter method

In a sterile glass Petri plate of 9cm diameter, three wet blotter papers were placed on lower plate. Twenty-five chili seeds in three layers with 16 towards periphery, 9 in the middle region and one in the centre of the plate were placed and covered with the upper lid. The seeds in the plates were cultured at 25 ± 2°C for 7 days with alternating 12-hour UV light and darkness in a BOD incubator. On the eight day, the seeds were observed for mycoflora associated with the seed samples using a stereo binocular microscope by preparing temporary water mounts. The per cent infection and recovery rate of *Colletotrichum* sp. from seed samples were recorded. For each seed sample four replications were performed and *Colletotrichum* sp. isolates were extracted, identified and purified.

Agar plate method

Chilli seeds were surface sterilized with 1% NaOCl followed by rinsing with sterile distilled water thrice and blotter air-dried. Five seeds per Petri plate were placed on streptomycin-amended potato dextrose agar (PDA) medium and incubated at 25 ± 2°C for 7 days. On the 8th day, mycoflora recovered from seed samples were observed and identified using a stereobinocular microscope. Fungal colonies were recorded and expressed as percentages.

Component plating method

To locate *Colletotrichum* sp. in different seed parts, anthracnose-affected chili seeds were soaked in sterile water for 2 hours then using sterile blade and forceps separated into seed coat, embryo and endosperm. These components were surface sterilized with 1% NaOCl for 30 seconds and sterile distilled water and placed on PDA medium. After incubating at 28 ± 2°C for two weeks, the seed components were then examined for development of fungal colonies.

The per cent seed infection, recovery of test fungi and other mycoflora associated with the chilli seed samples recorded from the above methods were calculated using the following formulae

$$\text{Seed Infection (\%)} = \frac{\text{Number of seeds infected by the test fungus/other fungi}}{\text{Total number of seed in Petri plate}} \times 100$$

$$\text{Seed Infection (\%)} = \frac{\text{Number of seeds infected by the test fungus/other fungi}}{\text{Total number of seed in Petri plate}} \times 100$$

Paper towel method

One hundred seeds from each chilli seed sample were randomly selected and placed at equidistant spacing between moistened paper towels, rolled and incubated at 25 ± 0.5°C and 90 ± 3% RH in a walk-in germinator. Four replications were maintained per sample. On14th day, percent germination and seed infection were recorded.

$$\text{Seed Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

$$\text{Seed Infection (\%)} = \frac{\text{Number of seeds infected by } \textit{Colletotrichum} \text{ sp.}}{\text{Total number of seeds}} \times 100$$

Characterization of *Colletotrichum* isolates.

The isolated *Colletotrichum* sp. isolates from different cultivars were characterized for their cultural, morphological and molecular characters for species differentiation.

Cultural characterization:

Cultural variability among isolates was studied according to Tesfaye and Kapoor (2007) standards. Mycelial discs (5mm) from seven-day-old cultures were transferred onto PDA plates and incubated at 25 ± 1°C in a BOD incubator. Colony characteristics

including color, texture, colony margins and mycelial growth rate (mm) were recorded after 14 days of incubation.

Morphological characterization

Morphological variations among *Colletotrichum* sp. isolates were observed and recorded after growing the isolates separately on PDA medium for 15 days. Conidial suspensions were prepared by flooding 5 ml of sterile distilled water and using a sterile scalpel blade and camel hair brush. Short-term water mount slides were prepared and conidial morphology was examined. Microphotographs illustrating the acervulus components of each *Colletotrichum* sp. isolate were taken using a stereo binocular microscope at magnifications of 10X and 40X.

Pathogenicity

Pathogenic variability among *Colletotrichum* sp. isolates was studied on a susceptible chilli cultivar tejitha following pin prick method (Kanchan *et al* 2004). All the five isolates were cultured on PDA medium for 10 days under 12-hour photoperiod cycles. Conidia were harvested and a suspension was prepared @ 10^6 spores/ ml. The mature chilli fruits were surface sterilized for five minutes with sodium hypochlorite then washed twice in sterile distilled water and air dried. The fruits were then given pin prick in the center using a sterile needle and inoculated with 6 µl of conidial suspension. The inoculated fruits were incubated at 25±2°C for 10 days and observed daily for symptom development.

Molecular characterization

From the five *Colletotrichum* sp. isolates total genomic DNA was extracted following a modified Murray and Thompson (1980) protocol. Mycelia were grown in potato dextrose broth for 15 days, harvested and stored at -70°C. The mycelia were ground using liquid nitrogen, mixed with CTAB buffer, incubated and treated with isoamyl alcohol/chloroform. DNA was precipitated with isopropanol, washed with ethanol and dissolved in TE buffer, then stored at -20°C. The isolated DNA was quantified using nanodrop at 260 nm to 280 nm expressed in ng/ul and the developed bands were visualized through Gel Documentation system. The internal transcribed spacer (ITS) region of the fungal isolates' DNA was amplified using the primers ITS1 and ITS4.The ITS region PCR products from five isolates were sent to Eurofins Genomics India Pvt Ltd, Bangalore for purification and sequencing. Sequence analysis was performed using Bio Edit, MEGA11and NCBI-BLAST. Bio Edit generated a consensus sequence from forward and reverse data, which was then BLASTed against the NCBI Gen Bank database. Isolates were identified based on the closest matches in the database.

Results and Discussion

Survey and collection

Roving survey conducted to assess anthracnose incidence in major chilli-growing areas of Telangana stated that among the popular chilli cultivars such as swarnamukhi, tejitha, keerthiteja, armour and tomato, the disease incidence ranged from 23.58 to 72.34 per cent with the highest per cent (72.34%) in cv. tomato from Nagarajpalle village of Nallabelli mandal, Warangal District. Whereas, the cultivars, swarnamukhi and tejitha from Konijerala mandal of Khammam district have reported 32.69 per cent and 35.74 per cent disease incidence, respectively. While, the cv. armour from Wyra mandal of same district had showed 26.21 per cent anthracnose incidence and the lowest incidence of 23.58 per cent anthracnose was recorded in cv. keerthiteja of Vallapuram village in Wyra mandal of Khammam district. However, the survey revealed varying levels of anthracnose incidence across the surveyed locations, with the highest mean incidence of 25-29% in Khammam and lowest of 15 -18 per cent in Warangal district. Irrespective of the cultivars in the study as mentioned in the (Table 1)

In the present study, maximum anthracnose incidence from different locations in Khammam district might be due to varied factors such as continuous mono culturing of the chilli crop by the farmers which aided in survival of pathogen propagules in infected host debris and soil (Ranathunge *et al*., 2012), application of high nitrogen fertilizer dosages, prevalence of favorable weather i.e. humid conditions from vegetative to fruit ripening stage. The pathogen causes die- back phase during early crop growth stage and fruit rot at crop maturity stages, resulting in seed and air borne nature thus helps in continuous availability of inoculum throughout crop growth period. Further, the pathogen infects the fruits and survives as latent infection or post-harvest infection even in storage by affecting the seed quality and seed health attributes.

The results of the study arein agreement with survey studies conducted by Chandini *et al*. (2022) during kharif 2022 who reported chilli anthracnose incidence in different chilli cultivars in Telangana which ranged from 10% to 89% with significant variability among 19 collected *Colletotrichum* isolates. Survey results of Rao *et al.* (2020) during 2019-20 for anthracnose incidence in chilli crop in Khammam district stated that disease incidence ranged from

36.0% to 53.6% with the highest incidence at the second picking stage during December months.

Detection and isolation of *Colletotrichum* sp. isolates

The anthracnose affected chilli pod samples collected from various locations were subjected to the standard seed health methods to detect and isolate the *Colletotrichum* sp. isolates. In the present study, seed samples from different chilli cultivars have showed significant differences in seed health parameters as mentioned in the (Table 2)

Standard Blotter method

Significant differences were observed for per cent seed infection among the seed samples of various cultivars. The results of the study recorded varying percentages of *Colletotrichum* sp. and other mycoflora from the seed samples. Among the five collected chilli seed samples, the seed samples of cv. tomato had significantly highest per cent seed infection of 80.60 per cent followed by the seed samples of tejitha, swarnamukhi and armour with 78.60%, 69.40% and 67.40% infection, respectively and lowest in keerthiteja (58.60%).

Of the seed associated mycoflora, *Colletotrichum* sp. was the most frequently detected fungus across all the cultivars, with per cent seed infection ranging from 20.20 to 52.60 per cent. Cultivar tomato (52.60%) showed highest per cent *Colletotrichum* sp. followed by from seed samples of cv. armour (37.20%), tejitha (35.80%) and swarnamukhi (31.60%) and lowest per cent in the seed samples of cv. keerthiteja (20.20%). However, the recovery of other mycoflora from the different seed samples ranged from 13.20% to 45.80% with highest recovery in keerthiteja (45.80%) which was followed by armour, swarnamukhi and tomato 25.20%, 23.00% and 19.40%, respectively, seed samples. While, the lowest inthe samples of tejitha (13.20%). The other mycoflora included *Alternaria, Fusarium* and *Aspergillus* sp., which are also the post-harvest pathogens, in addition to the field fungi.

The results of the study are in accordance with Singh *et al.* (2009) who reported that standard blotter method was found to be the best for detection of *Colletotrichum* sp. from seed which is seed borne in nature and cause significant losses in chilli. Guldekar *et al*. (2009) who compared screening methods for seed borne fungi in chilli seeds and stated that standard blotter paper method showed high efficiency in detecting*Aspergillus* sp. and *Colletotrichum* sp. Further, the findings of Vinaya *et al*. (2009) from northern Karnataka stated that standard blotter method was identified as the most efficient among the seed health testing methods for rapid and accurate diagnosis of *Colletotrichum capsici* from the seed samples collected from Haveri district. Jogi *et al*. (2010) subjected chilli seed samples of different cultivars of were to standard blotter method and reported recovery of *Aspergillus* sp*., Fusarium oxysporum* and *Colletotrichum capsici* were predominant in all cultivars. Chilli seed sampleswere found to positive forthe presence of *C. truncatum* and *C. coccodes* using blotter and agar plate methods (Dhiman, 2015)

Agar plate method

The percent seed infection (PSI) recorded by the seed samples of five chilli cultivars ranged from 47.00 to 61 per cent with significant variations. Out of five cultivars, cv. tomato seed samples had the highest PSI of 61.00 per cent from Nagarajpalle village, Nallabelli mandal, Warangal District followed by seed samples of tejitha with 57.00 per cent, swarnamukhi (54.00%), armour (52.00%) and the lowest of 47.00 per cent in seed samples of keerthiteja cultivar from Vallapuram village of Khammam District. Significant differences were observed among the interaction studies between cultivars and per cent seed infection of Colletotrichum isolates. Wherein, the highest *Colletotrichum* sp. per cent infection was reported in seed samples of tomato (40.00%), which is on par with seed samples of armour (38.80%) and differed significantly with per cent recovery of *Colletotrichum* isolate from the seed samples of tejitha (30.60%), swarnamukhi (28.60%), and the lowest from the seed samples of cv. keerthiteja (19.28%).The per cent recovery of other seed associated mycoflora among the seed samples of cultivars varied significantly with highest per cent recorded in seed samples of cv. keerthiteja followed by swarnamukhi, armour, tejitha and the lowest in cv. tomato with 35.60, 29.20,22.40,19.20 and 18.80 per cent, respectively.

The present studies are in congruence with Chigoziri and Ekefan (2013) using the standard blotter paper and agar plate methods investigated chilli seeds (varieties GVC101 and GVC111) from five locations. Wherein, the agar plate method detected 16 fungal species, while the blotter paper method identified 8 species. Both approaches revealed that the frequency and diversity of fungal growth associated with the seeds increased with the duration of seed storage periods. Agar plate method as most efficient detection technique and recorded more number of seed associated pathogens (10.1%) with seeds Mukesh Birla 2020 stated that and (Sruthy and Kansara, 2020).

Component plating method

The study confirmed the presence of *Colletotrichum* sp. in all the seed components *i.e* seed

coat, embryo and endosperm with the highest per cent recovery from the seed coat followed by endosperm and embryo. Across different cultivars collected from varied locations per cent infection of *Colletotrichum* isolates from all the seed components ranged from 22.80 to 53.40 per cent. Among five cultivars, cv. tomato seeds from Nagarajpalle village, Nallabelli mandal, Warangal district. had the highest *Colletotrichum* sp. presence on the seed coat (53.40%), followed by swarnamukhi (49.40%), armour (45.6%), tejitha (44.6%), and keerthiteja (41.60%).From the endosperm, seed samples of swarnamukhi Pandillapalli village of Konijerala district showed highest pathogen recovery (46.80%), followed by tomato (39.40%). While, the cultivars, armour, tejitha and keerthiteja showed (35.20%), (34.20%) and (33.60%) per cent infection, respectively. *Colletotrichum* sp. presence in the embryo ranged from 22.80 to 34.20 per cent, with cv. tejitha collected from Pandillapalli village of Konijerala district having highest (34.20%) and was on par with swarnamukhi (33.60%), followed by tomato (32.40%), armour (23.00%) and keerthiteja (22.80%).

However, the interaction studies stated that the per cent infection of *Colletotrichum* sp. from endosperm of cv. tejitha and keerthiteja with embryo of cultivars, swarnamukhi and tomato, respectively were on par with each other. The present study which states that highest colonization of *Colletotrichum* on seed coat and endosperm results in collapse of parenchymatous tissues of seed coat and thereby affects the seed quality attributes. Results arein agreement with Than et al., 2008 and Afutu, 2012. Kumud Kumar *et al*. (2004) who reported presence of *C. dematium* in the seed coat of all the infected seeds tested and embryo with 31.25 per cent infection in chilli seeds of cultivar A-36. Welideniya *et al*. (2019) reported, *C. capsici* and *C. gloeosporioides* in the seed coat, pericarp, and embryo, whereas *Fusarium* and *Aspergillus* were observed only on the seed coat of infected seeds.

Paper towel method

Anthracnose affected natural seed samples from five chilli cultivars of different locations were subjected to germination and seed infection tests. Among the seed samples collected from different cultivars and across the locations, only the seed samples of cultivars, tejitha, swarnamukhi and armourfrom Khammam district had recorded the per cent seed germination above Indian Minimum Seed Certification Standards (>60%). Highest per cent seed germination was recorded by the seed samples of tejitha (87.00%), followed by swarnamukhi (66.50%) and armour (61.50%). While, the cv.tomato (52.75%) and keerthiteja (52.50%) were lowest and on par in recording seed germination. Contrarily, the highest seed infection rates were recorded in tomato (44.25%) and keerthiteja (43.75%), followed by armour (35.25%), swarnamukhi (29.00%), and the lowest in tejitha (11.50%).

The studies are in agreement with Birla *et al* (2020) following rolled paper towel method assessed the germination percentage and presence of seed-borne pathogens in two chilli varieties, Garima-12 and HPH-12 and stated *Aspergillus flavus*, *A. niger*, *Colletotrichum capsici*, *Penicillium citrinum*, and *Fusarium annum* as predominant pathogens associated with the seed samples.

Cultural, morphological and molecular characterization of seed isolates of *Colletotrichum* sp.

The *Colletotrichum* sp. isolates isolated from seed of five different chilli cultivars were designated from *Cc*1 to *Cc*5 and further characterized for their cultural, morphological and molecular characters as mentioned in the (Table 3).

Cultural characteristics

The cultural characteristics of five *Colletotrichum* sp. isolates studied on PDA medium revealed that isolates *Cc*1, *Cc*3 and *Cc*4 showed fluffy colony textures, while *Cc*2and *Cc*5 have exhibited cottony textures with varied colony colours *Cc*1 and*Cc*3 changed from white to pale gray, *Cc*2 and *Cc*5 from pale to dark gray, *Cc*4 from whitish-orange to pale gray. Conidial masses were present in the centre of all isolates exceptfor*Cc*3 where the conidial masses were observed away from the centre of the Petri plate. Colony margins ofthe isolates varied with regular margins in *Cc*1, *Cc*3 and*Cc*4 isolates. While irregular margins in the isolates *Cc*2 and *Cc*5. Mycelial growth rate at 10 days after incubation stated that isolate *Cc*5 exhibited maximum of 72.1 mm growth rate followed by the isolates,*Cc*4, *Cc*1, *Cc* 2,*Cc*3 recorded63.2mm, 58.3mm, 50.7mm and 43.8mm, respectively.

Morphological characters

The morphological characteristics of *Colletotrichum* sp. were studied, focusing on acervulus, setae and conidial spores stated thatisolate, *Cc*1 showed highest number (+++) of acervuli followed by moderately in isolates *Cc*5, *Cc*4(++) and lowest number in isolates *Cc*3 and *Cc*2(+). setae size varied from 4.31µm to 6.65µm, with isolate *Cc*1 having the longest setae (6.65µm) and *Cc*5 the shortest (4.31µm). Conidial size ranged from 2.04µm to 2.98µm, with the largest spores in *Cc*1 (2.98µm) and the smallest in *Cc*5 (2.04µm). Falcate-shaped conidia

were observed in the isolates *Cc*1 to*Cc5.* (Table 4 and plate 2)

Pathogenicity test

Thefive *Colletotrichum*sp. isolates isolated from different cultivars were tested for the pathogenicity on pods of chilli cultivar tejitha through artificially inoculated conditions using pin prick method. All the test isolates have developed typical anthracnose symptoms which included necrotic lesions which are sunken with acervuli in concentric rings but at varied incubation periods. However, the isolates *Cc1, Cc2* and*Cc4* have exhibited the anthracnose symptoms in 7-8 days and the remaining in 10-11 days after inoculation. From the infected pods, respective Colletotrichum isolates were reisolated which have showed similar characteristic features of inoculated original pure culture.

Molecular characteristics

The *Colletotrichum* spp. isolates collected from various cultivars were subjected for their Pathogenicity on susceptible chilli cv. tejitha. Wherein, out of five *C. truncatum* (syn. *Colletotrichum capsici*, Anuradha *et al.*, 2023) isolates, three isolates *viz., Cc1, Cc2* and *Cc4* isolated from the cultivars, swarnamukhi, tejitha and armour collected from different locations have manifested typical anthracnose symptoms in 7-8 days and were stated as virulent isolates in the study were further characterized for molecular characters.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987), the bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The *C. truncatum* isolates along with reference sequences were arranged in a single monophyletic group. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. This analysis involved 8 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1195 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura, 2021).

The results of phylogenic analysis using ITS primers sequences 16 sRNA separated the Colletotrichum isolates into two major clades. Wherein, the isolate Cc4 (E0524 180 002 PCR ARM ITS 1) from cv. armour; and the isolates *Cc2* (E0524180004 PCR TEJ ITS1) from cv. tejitha and *Cc1* (E0524 180 003 PCR SWA ITS 1) from cv. swarnamukhi showed consistency index with LC 488838.1 *C. truncatum* HCM 321 and MT95064.1 *C. capsici* isolate GK 17A, respectively.

Further, the three virulent Colletotrichum isolates were registered in NCBI Gene Bank under accession numbers *Cc*1, (PQ084769), *Cc*2 (PQ069703) and *Cc*4 (PQ084768).

The present study unraveled variations among the *C. truncatum* isolates collected from major chilli growing locations of the state. The five representative isolates from important chilli cultivating areas showed variations in their cultural (colony colour, texture, distribution of conidial masses in culture plate), morphological (acervuli, conidial shape, size, setae length), pathogenic (incubation period for expression of anthracnose symptoms) characteristics including molecular characters for three virulent isolates. Several researchers have also reported variability of cultural, morphological and molecular among the *Colletotrichum* spp. isolates (Sharma *et al*., 2005; Masoodi *et al*., 2013; Kumar *et al.,* 2019; Handiso and Alemu, 2015; Anggrahini *et al*., 2020 and Hema *et al*., 2015).

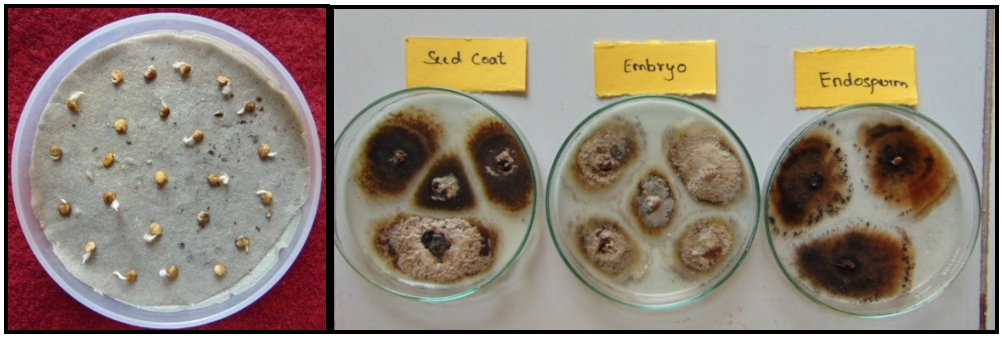


Plate 1 : Standard blotter and component plating method for the detection of *Colletotrichum* sp. pathogen

Table 1 : Survey for anthracnose incidence and collection of Colletotrichum isolates from major chilli growing locations of Telangana state.

S. No	District	Mandal	Village	Latitude	Longitude	Soil type	Cultivar	Crop stage	Per cent disease incidence (PDI)
1	Khammam	Konijerala	Pandillapalli	17.15630 N	80.31140 E	Red soil	Swarnamukhi	Fruit ripening stage	32.69
2	Khammam	Konijerala	Pandillapalli	17.15816 N	80.31090 E	Red soil	Tejitha	Fruit ripening stage	35.74
3	Khammam	Wyra	Vallapuram	17.08330 N	80.30075 E	Clay loam soil	Keerthiteja	Fruit ripening stage	23.58
4	Khammam	Wyra	Vallapuram	17.07888 N	80.30333 E	Clay loam soil	Armour	Fruit ripening stage	26.21
5	Warangal	Nallabelli	Nagarajpalle	18.04261 N	79.89997 E	Red soil	Tomato	Fruit ripening stage	72.34

Table 2 : Detection of anthracnose affected chilli seed samples using standard seed health methods

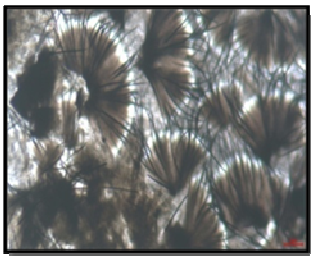
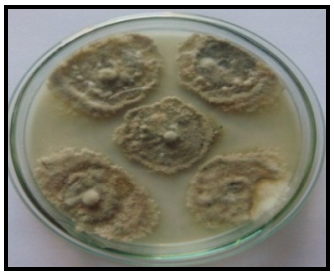
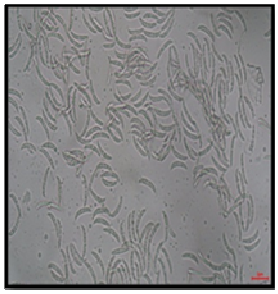
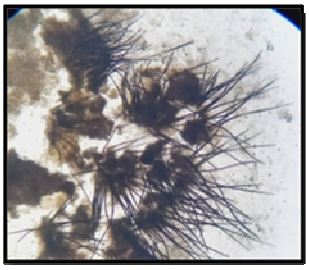
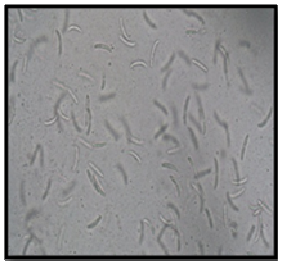
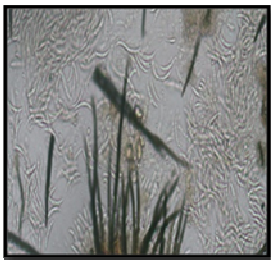
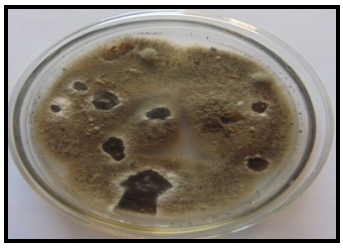
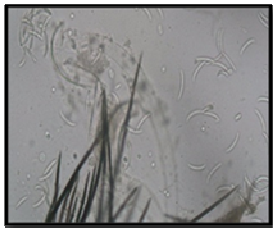
Variety	Blotter method			Agar method			Component plating method			Paper towel method	
	Seed infection	Seed infection of *Colletotrichum* sp (%)	Recovery of other mycoflora (%)	Seed infection	Seed infection of *Colletotrichum* sp (%)	Recovery of other mycoflora (%)	Embryo	Endosperm	Seed coat	Per cent seed Germination	Per cent seed infection
Swarnamukhi	69.40 (56.42)	31.60 (34.32)	23.00 (28.65)	54.00 (47.06)	28.60 (32.32)	29.20 (32.70)	33.60 (35.42)	46.80 (43.16)	49.40 (44.65)	66.50 (54.65)	29.00 (32.58)
Tejitha	78.60 (62.44)	35.80 (36.74)	13.20 (21.29)	57.00 (48.90)	30.60 (33.58)	19.20 (25.98)	34.20 (35.78)	34.20 (35.78)	44.60 (41.89)	87.00 (68.89)	11.50 (19.80)
Keerthiteja	58.60 (49.95)	20.20 (26.70)	45.80 (42.59)	47.00 (43.14)	19.28 (26.08)	35.60 (36.62)	22.80 (28.51)	33.60 (35.42)	41.60 (40.04)	52.50 (46.43)	43.75 (41.41)
Armour	67.40 (55.18)	37.20 (40.40)	25.20 (30.13)	52.00 (46.14)	38.80 (38.52)	22.40 (28.24)	23.00 (28.65)	35.20 (36.38)	45.60 (42.47)	61.50 (51.65)	35.25 (36.42)
Tomato	80.60 (63.87)	52.60 (46.49)	19.40 (26.12)	61.00 (51.23)	40.00 (39.23)	18.80 (25.69)	32.40 (34.69)	39.40 (38.87)	53.40 (46.95)	52.75 (46.58)	44.25 (41.70)
Means	70.92 (57.57)	35.48 (36.93)	25.32 (29.75)	54.20 (47.34)	31.44 (33.92)	25.04 (29.84)	29.20 (32.61)	37.84 (37.92)	46.88 (43.20)	64.05 (53.68)	32.75 (34.38)
Cd (p=0.05)	1.085	0.836		0.829	0.757		0.930			1.61	1.12
Cv	1.428	1.916		1.327	1.859		1.939			1.99	2.17

Table 3 : Cultural and morphological characterization of *Colletotrichum* sp. isolates

S. No	Cultivars	Isolate Designation	Colony Colour	Colony Texture	Colony margin	Mycelial growth rate at 10 days 11 in mm	No. of acervuli/ sporulation	Setae size (µm)	Conidia size (µm)
1	Swarnamukhi	*Cc*1	White to pale gray; Conidial mass in centre	Fluffy	Regular	58.3	+++	6.65	2.98
2	Tejitha	*Cc*2	Pale gray to dark grey; Pink conidial mass in centre	Cottony	Irregular	50.7	+	4.7	2.09
3	Keerthiteja	*Cc*3	White to pale gray ;Conidial mass away from the centre	Fluffy	Regular	43.8	+	4.84	2.38
4	Armour	*Cc*4	Whitish orange to pale gray ; Pink conidial mass in centre	Fluffy	Regular	63.2	++	5.24	2.64
5	Tomato	*Cc*5	Pale gray to dark grey; Conidial mass in centre	Cottony	Irregular	72.1	++	4.31	2.04

Note: Highest (+++), moderate (++), lowest (+)

Plate 2: Cultural and morphological characterization of *Colletotrichum* sp. Isolates from chilli seed

Isolates	Varieties	Colletotrichum isolates cultures	Setae	Conidia
Cc 1	Swarnamukhi			
*Cc*2	Tejitha			
*Cc*3	Keerthiteja			
*Cc*4	Armour			
*Cc*5	Tomato			

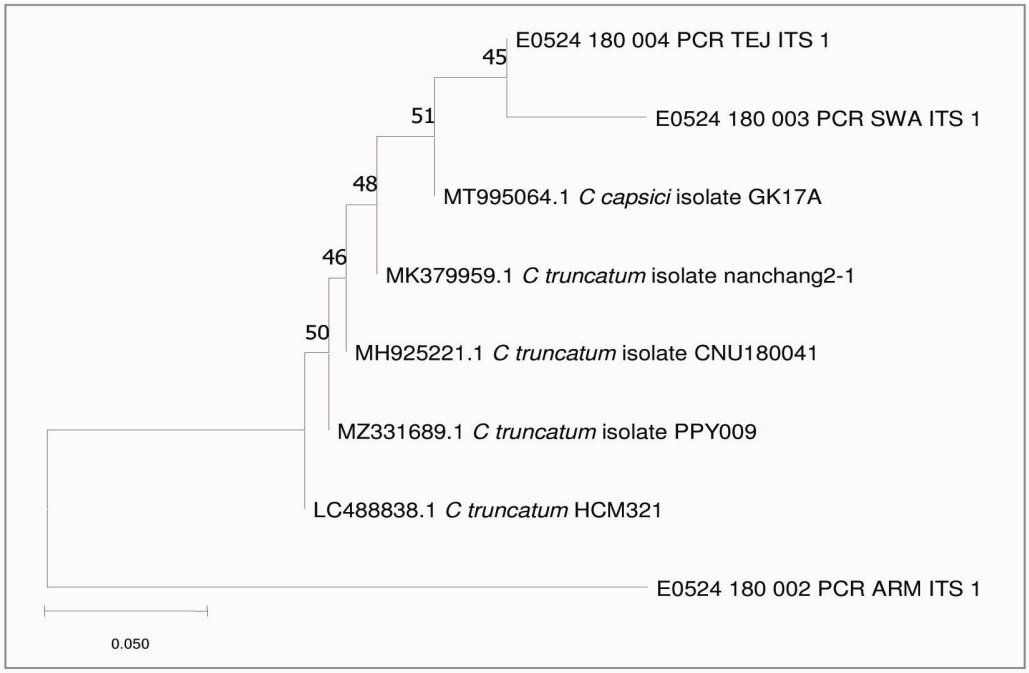
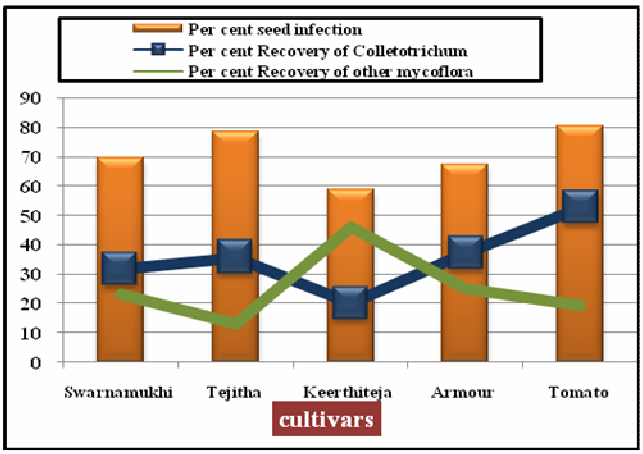
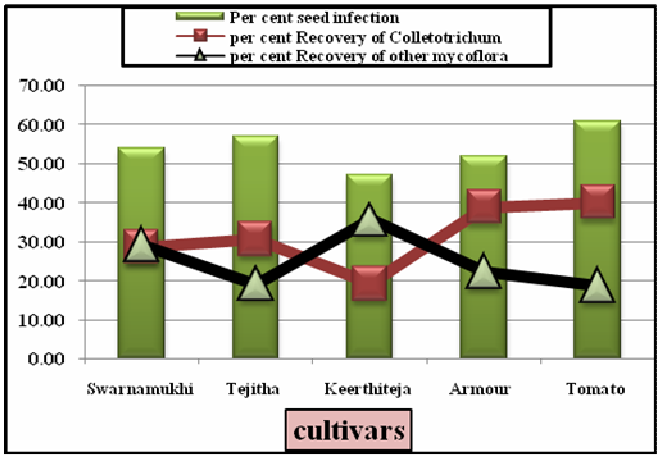


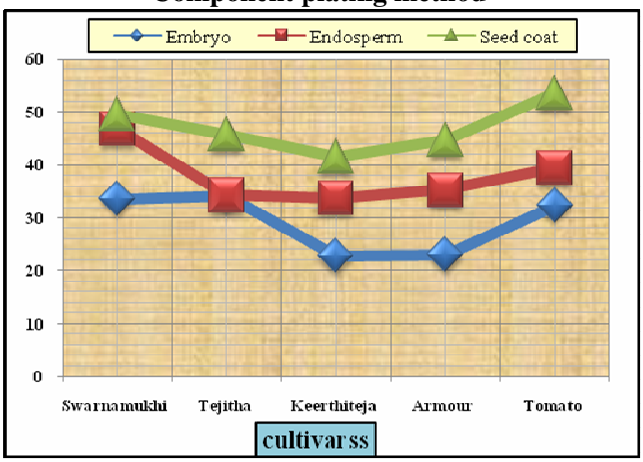
Fig. 1 : Phylogram generated from ITS sequence data for different Colletotrichum isolates Using MEGA11.



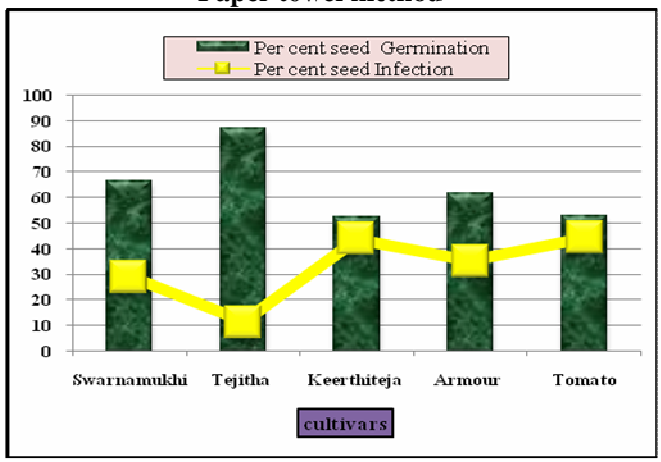
Component plating method



Paper towel method



Standard blotter method



Agar plate method

Fig. 2 : Detection of anthracnose affected chilli seed samples using standard seed health methods

Acknowledgement

The authors are thankful to Seed Research and Technology Centre, Department of Seed Science and Technology, PJTSAU, Rajendranagar, for providing the facilities and financial assistance for execution and completion of research work. I felt privileged to thank everyone who contributed directly or indirectly for successful completion of the present research study.

Funding

For execution and conduction of research work, the author(s) have received research grants and funding from Indian Institute of Seed Science, Mau, Professor Jayashankar Telangana State Agricultural University, Rajendranagar and Government of Telangana State.

Ethical approval

The current study was the authors' original research project, which has not been presented, published, or submitted to any publications. The findings of the work do have undesirable effects on the environment, animals, and people.

Conflict of interest

All the authors have declared and confirmed that they have no conflict of interest.

References

Afutu, E. (2012). Seed-borne fungi of chilli (*Capsicum frutescens*) and studies on the seed transmission of *Colletotrichum dematium* in the coastal savannah zone of central region of Ghana. M.Sc. (Ag) Thesis. Univ. Cape Coast.

Agarwal, V.K. and Sinclair, J.B. (1997). Principles of Seed Pathology (2nd Edition), CRC Press, Boca Raton, USA. 539.

Ahmed, S.S. (1982). Studies on seed borne aspects of anthracnose of chillies caused by *Colletotrichum capsici* (Sydow.) Butler and Btsby. M Sc. (Agn.) Thesis, Untv Agric. Set., Bangalore.

Anggrahini, D.S., Wibowo, A. and Subandiyah, S. (2020). Morphological and molecular identification of *Colletotrichum* spp. associated with chili anthracnose disease in Yogyakarta Region. *Journal Perlindungan Tanaman Indonesia*, 24(2): 161-174.

ANGRU chilli outlook 2020, https://angrau.ac.in/downloads/ AMIC/ OutlookReports/ 2022/

Anuradha, Sharma, A., Sood, S., Badiyal, A. and Sood, T. (2023). Fruit rot of Capsicum spp.: implications and management strategies. *The Journal of Horticultural Science and Biotechnology*, 98(6), 715-731.

Chandini, A., Kumar, J.H., Devi, G.U., Kumar, K.R. and Pushpavalli, S.N.C.V.L. (2022). Survey and collection of isolates of *Colletotrichum capsici* from different chilli growing areas of erstwhile Khammam district. *The Pharma Innovation Journal.* 11(7), 2393-2399.

Chigoziri, E. and Ekefan, E.J. (2013). Seed borne fungi of Chilli Pepper (*Capsicum frutescens*) from pepper producing areas of Benue State, Nigeria. *Agriculture and Biology Journal of North America*, 4(4), 370-374.

Dhiman, S. (2015). Role of *Colletotrichum* sp. in capsicum seed health and their management. M.Sc Thesis, Chaudhary Srawan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,* 39: 783-791.

Garg, R., Loganathan, M., Saha, S. and Roy, B.K. (2014). "Chilli Anthracnose: a review of causal organism, resistance source and mapping of gene," in Microbial Diversity and Biotechnology in Food Security, *Springer*. 589–610.

Guldekar, D.D., Potdukhe, S.R. and Gaikawad, M. (2009). Morphological Variation in *Colletotrichum capsici* Causing Anthracnose of Chilli. *Journal of Plant Disease Sciences,* 4(2): 204-207.

Handiso, S. and Alemu, T. (2015). Morpho-pathological Variability of Chili Anthracnose (*Colletotrichum capsici* (Syd)) Bisby and Butler) in Southern Nations Nationalities Peoples and Oromiya Region, Ethiopia. *International journal of life sciences*, 6(3): 80-90.

Ramdial, H. and Rampersad, S.N. (2015). Characterization of *Colletotrichum* spp. causing anthracnose of bell pepper (*Capsicum annuum* L.) in Trinidad. *Phytoparasitica*, 43(1): 37-49.

PJTSAU, chilli outlook, 2022.

Jogi, M.G., Padule, D.N. and Kamdi, S.R. (2010). Detection of seed mycoflora of chilli and its impact on seed germination and seedling vigour. *International J. of Pl. Sci.* (Muzaffarnagar), 5(2), 502-504.

Kanchana, U.C., Taylor, P.W.J. and Mongkolporn, O. (2004). Development of a bioassay to study anthracnose infection of *Capsicum* Chinese Jacq. fruit caused by *Colletotrichum capsici*. *Thailand Journal of Agricultural Sciences*, 37: 293-297.

Kiran, R., Akhtar, J., Kumar, P. and Shekhar, M. (2020). Anthracnose of Chilli: Status. Diagnosis and Management, 10.5772/intechopen.87455. https://doi.org/ 10.5772/intechopen.93614.

Kumar, K.S., Balabaskar, P., Kumar, S., Kumar, T.S. and Muthukumar, A. (2019). Studies on the cultural and pathogenic variability among isolates of *Colletotrichum capsici* (Syd.) Butler and Bisby causing anthracnose fruit rot incidence of chilli. 19(2): 3295-3299.

Kumar, K., Singh, J. and Khare, A. (2004). Detection, location transmission and management of seed borne *Colletotrichum dematium* causing dieback and anthracnose in chilli. *Farm Science Jounral*. 13(2): 152-153.

Lakshmesha, K., Lakshmidevi, K., Aradhya, N. and Mallikarjuna, S. (2005). Changes inpectinase and cellulase activity of *Colletotrichum capsici* mutants and their effect on anthracnose disease on *Capsicum* fruit. *Archives of Phyto pathological Plant Protection.* 38, 267–279.

Birla, M., Singh, R.K. and Barade, N. (2020). Validation of detection techniques and management of seed borne diseases of Chilli (*Capsicum annum*). *Journal of Pharmacognosy and Phytochemistry*, 9(6): 168-171.

Mongkolporn, O. and Taylor, P.W.J. (2018). Chili anthracnose: Colletotrichum taxonomy and pathogenicity. *Plant Pathology*, 67(6), 1255–1263.

Murray, M. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA, *Nucleic Acids Research*. 8: 4321-4326.

ISTA (2021). International rules for seed testing. International Seed Testing Association, Zurich, Switzerland.

Pakdeevaraporn, P., Wasee, S., Taylor, P.W.J. and Mongkolporn, O. (2005). Inheritance of resistance to anthracnose caused by *Colletotrichum capsici* in *Capsicum*. *Plant Breeding*. 124(2), 206–208.

Poonpolgul, S. and Kumphai, S. (2007). Chilli Pepper Anthracnose in Thailand. Country Report. In: Oh, D.G., Kim, K.T. (Eds.), Abstracts of the First International Symposium on Chilli Anthracnose. National Horticultural Research Institute, Rural Development of Administration, Republic of Korea :23.

Raj, T.S., Christopher, D.J. and Rani, S.U. (2006). Management of fruit rot of chilli with different plant products. *Indian Journal of Plant Protection*. 34(2): 274-275.

Ramachandran, N. and Rathnamma, K. (2006). "*Colletotrichum acutatum*—a new additionto the species of chilli anthracnose pathogen in India," in Paper presented at the Annual Meeting and Symposium of Indian Phytopathological Society, Central Plantation Crops Research Institute (Kasarago).

Ramesh, K.V., Jayalakshmi Devi, R.S., Gopal, K., Lakshmi, B.K.M., Krishna, T.M. and Reddy, B.R. (2018). Survey for the Incidence of Chilli Anthracnoseand Fruit Rot Disease in Major Chilli Growing Areas of Telangana, India. *International Journal of Current Microbiology and Applied Sciences,* 7(3): 3795-3803.

Ranathunge, N.P., Mongkolporn, O., Ford, R. and Taylor, P.W.J. (2012). *Colletotrichum truncatum* pathosystem on Capsicum spp: infection, colonization and defence mechanisms. *Australasian Plant Pathology*, *41*: 463-473.

Rao, S.R., Nagaswathi, K. and Rao, J.P. (2020). Extension study on fruit rot disease of chilli, in Khammam district of Telangana. *International Journal of Chemical Studies*, 8(3), 2182-2185.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution,* 4: 406-425.

Sahitya, L.U., Deepthi, S.R., Kasim, P.D., Suneetha, P. and Krishna, M.S. (2014). Anthracnose, a Prevalent Disease in *Capsicum*. Department of Biotechnology, KL University, India. Acharya N.G. Ranga University, Hyderabad. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 5(3): 1583-1604.

Sangchote, S. and Juangbhanich, P. (1984). Seed Transmission of *Colletotrichum capsici* on Pepper (*Capsicum* sp.). *Kasetsart Journal: Natural Science*. 18(1): 7-13.

Saxena, A., Raghuwanshi, R. Gupta, V.K. and Singh, H.B. (2016). Chilli Anthracnose: The Epidemiology and Management. *Frontiers Microbiology*, 7: 1527.

Singh, K., Vishanavat, K. and Rashmi, T. (2009). Detection, transmission and management of seed-borne inoculation of anthracnose (*Colletotrichum capsici*) in chilli. *Seed Research*, 37(1/2): 143-146.

Sruthy, M. and Kansara, S.S. (2020). Seed mycoflora associated with different varieties of chilli. *Pest Management in Horticultural Ecosystems*, 26(1): 152-155.

Tamura, K., Nei, M. and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

Tamura, K., Stecher, G. and Kumar, S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution* https://doi.org/10.1093/molbev/msab120

Tesfaye, A. and Kapoor, I.J. (2007). In Vivo Evaluation of Trichoderma Species against Botrytis Corm Rot/ Blight of Gladiolus. *Ethiopian Journal of Biological Sciences*, 6(2), 165-171.

Than, P.P., Jeewon, R., Hyde, K.D., Pongsupasamit, S., Mongkolporn, O. and Taylor, P.W.J. (2008). Characterization and pathogenicity of *Colletotrichum* species associated with anthracnose disease on chilli (*Capsicum* spp.) in Thailand. *Plant Pathology*, 57, 562–572.

Vinaya, H., Rao, M.S.L., Yashoda, H. and Mohankumar, D. (2009). Status of seed borne incidence of anthracnose of chilli in northern Karnataka and evaluation of seed health methods for the detection of *Colletotrichum capsici*. *Karnataka Journal of Agriculture Science*, 22(4), 807-809.

Welideniya, W.A., Rienzie, K.D.R.C., Wickramaarachchi, W.A.R.T., Aruggoda, A.G.B. (2019). Characterization of fungal pathogens causing anthracnose in capsicum pepper (*Capsicum annuum* L.) and their seed borne nature. *Ceylon J. Sci*.; 48(3), 261- 269.