Cultural, morphological and pathogenic variability in the *Colletotrichum capsici* isolates, inciting anthracnose and fruit rot of chilli (*Capsicum annuum* L.) in different agro-climatic zones of Kerala

Abstract

Chilli is a popular vegetable and spice crop, grown throughout the world. The global production and productivity is affected by many biotic as well as abiotic factors. Among them, anthracnose and fruit rot of chilli caused by *Colletotrichum capsici* results in significant yield loss in the chilli cultivating regions. A survey was conducted in the five agro-climatic zones (ACZs) of Kerala *viz*., Northern zone, Central zone, Special problem zone, High range zone and Southern zone to assess the incidence and severity of anthracnose disease. Maximum anthracnose incidence of 90 per cent and severity of 52.60 per cent were noticed at RARS, Pilicode (Northern zone). Characteristic leaf spot, fruit rot and die-back symptoms were observed in the survey locations. Nine *C. capsici* isolates obtained from different ACZs showed significant variation in cultural and morphological characters. Pathogen colonies showed different shades of white, off-white to grey turning brown or black with regular or irregular margins and concentric rings of black acervuli in the PDA medium. The diameter of growth of *C. capsici* isolates ranged from 7.20 to 8.60 cm at 7 days after incubation. All the *C. capsici* isolates initiated hyaline mycelium having a width of 1.73 - 2.36 µm and produced numerous sickle shaped conidia with a size range of 19.42 - 20.46 µm × 2.16 - 3.09 µm. Black coloured, circular or ellipsoidal acervuli (122.14 - 189.08 µm dia.) had 20 – 46 setae of length 74.13 to 107.30 µm. Also, brown or black appressoria of size 8.64 - 12.64 µm × 5.54 - 7.84 µm were formed in slide culture. Pathogenicity was established in the *C. capsici* isolates and Cc3 from CoA, Vellanikkara was obtained as the most virulent isolate producing a lesion size of 1.13 cm and PDI of 45.33 in the artificially inoculated chilli fruits (var. Vellayani Athulya) at 5 days after inoculation. Thus, all the nine *C. capsici* isolates exhibited variability in their cultural, morphological and pathogenic characteristics. Variability in phytopathogenic fungi helps in identifying novel and sustainable control methods to tackle the diseases incited by them.

Keywords: *Capsicum annuum*, Chilli anthracnose, *Colletotrichum capsici*, variability, pathogenicity, disease incidence, disease severity

Introduction

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Chilli is an important commercial spice crop of Solanaceae family, widely cultivated in the tropical and sub-tropical regions of the world. Fruits are the economic products, known for their pungency and fiery flavour. These form a popular, principal ingredient in the Indian gastronomy. The red ripe fruits are found to be rich in vitamins (A, C and E), riboflavin, thiamine, fibre, proteins and minerals. They are also high in capsaicin and β-carotene levels (Kumar and Kerketta, 2018).

India is not only the largest producer but also the major consumer and exporter of chillies in the world, contributing to 40 per cent of the total global chilli production and 25 per cent of international exports (Mehta, 2017). But chilli production is affected by a number of factors *viz*., climate change, lack of high yielding cultivars, poor quality seeds, susceptibility to pests and diseases. About 20-40 per cent yield loss is reported due to weeds, pathogens and pests (Oerke, 2006). Among them, anthracnose disease results in crop losses of 10-54 per cent in India (Saxena *et al*., 2016).

Colletotrichum capsici is the most common pathogen responsible for chilli anthracnose and fruit rot. The fungus infects almost all the above-ground parts of the plant. The various symptoms associated with the disease include damping off or seedling blight, leaf spots, die-back of branches, anthracnose and rotting of fruits. The typical symptoms on infected fruits are characterized by sunken, necrotic, circular to angular lesions with concentric rings of black acervuli. On severe infections, pink or orange conidial masses occur on the lesions (Oo and Oh, 2016). In India, anthracnose disease is reported to cause yield losses of 10 - 54 per cent (Lakshmesha *et al*., 2005). However, pre- and postharvest losses of more than 80 per cent have been found to be affecting the marketable yield of chilli plants (Thind and Jhooty, 1985; Than *et al*., 2008).

Pathogen identification is important in preventing the consequences due to development of new pathogen variants, which may result in severe crop losses. Also, variability studies in the pathogen are essential in understanding the prevailing disease situations and the possible control measures. Hence, in the present study, we investigated the cultural, morphological and pathogen variability within the *C. capsici* isolates obtained from the various chilli growing areas in different agro-climatic zones of Kerala.

Materials and methods

Survey and collection of infected fruit samples

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Comment [LR5R4]: 2.0 MATERIALS AND METHODS

Comment [LR6]: 2.1 SURVEY AND COLLECTION OF INFECTED FRUIT SAMPLES

A preliminary survey was conducted in the five agro-climatic zones (ACZs) of Kerala during 2018-2019. The survey locations included Northern Zone (College of Agriculture (CoA), Padannakad and Regional Agricultural Research Station (RARS), Pilicode of Kasargod district), Central Zone (CoA, Vellanikara, Thrissur and RARS, Pattambi, Palakkad), Special Problem Zone (ORARS, Kayamkulam, Alappuzha and RARS, Kumarakom, Kottayam), Southern Zone (CoA, Vellayani, Thiruvananthapuram and Farmer's field, Kottarakara, Kollam) and High Range Zone (Farmer's field, Idukki and RARS, Ambalavayal, Wayanad). Chilli growing areas were identified and 100 plants were selected in each location. Disease incidence as well as severity were assessed by counting the diseased and healthy fruits and assessing the extent of the infection in fruits.

Isolation, purification and maintenance of the pathogen

Comment [LR7]: 2.2 ISOLATION, PURIFICATION AND MAINTENANCE OF THE PATHOGEN

Anthracnose infected chilli fruit samples were collected from the surveyed regions and brought to laboratory for the isolation of the pathogen. Small bits of lesions, comprising of diseased and healthy portions were sterilized using 0.1 per cent sodium hypochlorite solution for 1 min and washed three times in sterile distilled water. The bits were blot dried and inoculated on to sterilized plates containing Potato Dextrose Agar (PDA) medium under sterile conditions. Later, the plates were incubated at room temperature (28 ± 2 ^{o}C) for 3 days. The emerging hyphal tip was inoculated onto prepared PDA slants. Further, the culture was sub-cultured at regular intervals to maintain the pathogen virulence. The procedure was repeated for all the nine isolates of *C. capsici* obtained from the survey locations.

Cultural characterization of *C. capsici* isolates

Comment [LR8]: 2.3 CULTURAL CHARACTERIZATION OF *C. capsici* ISOLATES

Sterile PDA plates were inoculated with 5 mm mycelial discs taken from actively growing 7-day old cultures of *C. capsici* isolates. The inoculated plates were incubated at room temperature (28 ± 2 ^{o}C) for 7 days. Three replications were maintained for each isolate. Mycelial growth rate of the different *C. capsici* isolates was measured after incubation for 7 days. The experiment was carried out in three replications of each isolate. Further, cultural observations on the appearance, colour and margin of mycelial growth of the isolates were also recorded.

Morphological characterization of *C. capsici* isolates

Comment [LR9]: 2.4 MORPHOLOGICAL CHARACTERIZATION OF *C. capsici* ISOLATES

Morphological characterization of the different *C. capsici* isolates was studied by slide culture technique. A glass slide together with cover slips was mounted on two

supporting glass rods in a petriplate containing sterile filter paper. The slide culture unit was then sterilized in an autoclave. Further procedures were carried out in a laminar air flow chamber under sterile conditions. 1 per cent agar medium was prepared and solidified in sterile Petri plates. Square-shaped agar blocks were cut out from these plates and placed at the ends of the sterilized glass slides. The pathogen was smeared on the four corners of each agar block and a cover slip was inserted. Humidity in the slide culture unit was ensured by moistening the filter paper with sterile double distilled water. These units were incubated for 48 h at room temperature (28 ± 2 ^{o}C). Further, the coverslips were transferred to a sterile glass slide and stained with lactophenol blue solution. These slides were microscopically observed and the morphological observations on the mycelial width, shape and size of conidia, diameter of acervuli, number and length of setae, and length and width of appressoria of the *C. capsici* isolates were recorded.

Preparation of spore suspension of the *C. capsici* isolates

Conidial suspension was prepared using 7 day-old *C. capsici* culture. Initially, the culture plates were flooded with sterilized double distilled water and the surface was slowly scraped with sterile fungal loop. The resulting spore suspension was collected and filtered in two layers of sterilized muslin cloth. Further, the concentration of final conidial suspension was adjusted to 1 × 10^{6} conidia ml^{-1} using a haemocytometer.

Pathogenicity of *C. capsici* isolates

The pathogen isolates were tested for pathogenicity by means of detached leaf and fruit method. Leaves, tender, mature and ripe chilli fruits of Vellayani Athulya (susceptible variety) were used for the experiment. Leaves and tender fruits were collected from 60-day old chilli plants whereas, mature and ripe fruits were picked from 90-day old plants. The detached leaves as well as fruits were surface sterilized by wiping with 70 per cent alcohol. Small incisions were induced, using a sterile needle and inoculated with spore suspensions (1 × 10^{6} conidia ml^{-1}) of the *C. capsici* isolates. The inoculated leaves and fruits were incubated at room temperature (28 ± 2 ^{o}C) in sterile plastic boxes lined with moist cotton. The days taken for symptom development and lesion size produced were observed regularly at 3, 5, 7, 10 and 15 days after inoculation. Disease severity was also scored for the different *C. capsici* isolates, based on the standard score chart (0 = healthy; 1 = 1-5 %; 2 = 5-25 %; 3 = 25-50 % and 4 = 50-100 % of fruit area infected) given by Vishwakarma and Sitaramaiah (1986). The

Comment [LR10]: Petri plate

Comment [LR11]: lactophenol cotton blue stain

Comment [LR12]: 2.5 PREPARATION OF SPORE SUSPENSION OF THE *C. capsici* ISOLATES

Comment [LR13]: 2.6 PATHOGENICITY OF *C. capsici* ISOLATES

pathogen was also re-isolated into PDA medium and compared with the original pathogen culture to confirm the Koch's postulates.

Analysis of data

Comment [LR14]: 2.7 ANALYSIS OF DATA

The experiments were analysed statistically by completely randomized design (CRD) using GRAPES software developed by Kerala Agricultural University. Further, the data was subjected to analysis of variance (ANOVA) tests and compared with Duncan's multiple range tests (DMRT) at 5 per cent level ($p < 0.05$).

Results

Comment [LR15]: 3.0 RESULTS

Northern zone recorded the maximum disease incidence and severity

A survey was carried out in the five ACZs of Kerala namely Northern zone, High range zone, Central zone, Special problem zone and Southern zone during 2018-2019. The incidence of chilli anthracnose varied from 20 to 90 per cent in the different ACZS. Highest disease incidence (DI) was recorded in the Northern zone, with 90 per cent at RARS, Pilicode, Kasaragod and 85 per cent at CoA, Padannakkad, Kasaragod in Anugraha variety grown in the area. This was followed by 83 per cent in RARS, Ambalavayal, Wayanad of High range zone (var. Anugraha); 74 per cent in RARS, Pattambi, Palakkad of Central zone (var. Jwalamukhi); 68 per cent in CoA, Vellayani, Thiruvananthapuram of Southern zone (var. Vellayani Athulya) and 55 per cent in CoA, Vellanikkara, Thrissur of Central zone (var. Anugraha). Lowest DI of 20 per cent was noticed at farmer's field, Kottarakkara, Kollam of Southern zone (local cultivar). Also, ORARS, Kayamkulam, Alappuzha and RARS, Kumarakom, Kottayam belonging to Special problem zone and Farmer's field, Idukki of High range zone recorded lower DI values of 32 per cent (local variety), 43 per cent (var. Ujwala) and 36 per cent (local variety) respectively.

Disease severity of chilli anthracnose in the various surveyed locations belonging to the five ACZs varied significantly between 23.63 and 52.60 per cent. Maximum PDI of 52.60 was observed at RARS, Pilicode, followed by 47.90 at RARS, Ambalavayal, 43.20 at CoA, Padannakkad and 40.33 at RARS, Pattambi. ORARS, Kayamkulam recorded the lowest anthracnose disease severity of 23.63 per cent. Relatively lower PDI values of 36.38, 29.88, 28.25, 27.25 and 24.13 were observed CoA, Vellayani; Farmer's field, Idukki; RARS, Kumarakom; Farmer's field, Kottarakkara; and CoA, Vellanikkara (Table 1).

Characteristic anthracnose symptoms were observed on the infected chilli plants of surveyed locations

The pathogen infected almost all the aboveground parts of chilli plants. Typical anthracnose symptoms on leaves were noticed as small, water-soaked necrotic lesions, surrounded by yellow halo (Figure 1A). Fruit rot symptoms were characterized by water-soaked, circular or elongated, yellowish brown lesions on the fruit surface. Black acervuli were found scattered or in concentric fashion on the lesions (Figure 1B). Anthracnose infection on the twigs or branches appeared as brown lesions which advanced from the tip downwards. Severe infection caused defoliation and drying up of affected branches and twigs (Figure 1C).

C. capsici isolates significantly varied in their cultural characteristics on the PDA medium

Nine *C. capsici* isolates obtained from the surveyed locations of the five ACZs were plated on PDA plates. Whitish to grey coloured mycelium was initiated; and on microscopic examination, hyaline and septate mycelium produced aseptate, hyaline, unbranched and short conidiophores. The conidia appeared hyaline, one-celled without any septation, sickle-shaped with an oil globule in the centre. Nine pathogen isolates (Cc1 - Cc9) were identified as *C. capsici* while, the isolate from RARS, Ambalavayal (Cc10) was identified as *Colletotrichum gloeosporioides*. Further, the pure cultures of *C. capsici* isolates were maintained at lab condition followed by regular sub-culturing at bimonthly intervals.

The *C. capsici* obtained from the different ACZs varied in their cultural characters. The isolates produced sparse mycelium on the PDA medium and concentric rings of black acervuli were observed in the culture. All the *C. capsici* isolates exhibited variation in the colony colour. The colonies varied from white to off-white turning grey, dark brown and black in colour, both on the front as well as rear sides of the culture plates.

The upper side of the culture plates inoculated with the isolates Cc1 of Northern zone, Cc6 of Special problem zone and Cc8 of Southern zone had white mycelium which later turned grey, while the reverse sides exhibited varying degrees of different colours *viz*., white turning grey, yellowish brown and white turning brown. Cc2 isolate appeared off-white on the front side and yellowish brown in colour on the rear side. In contrast, the isolates, Cc3 and Cc5 produced grey to dark grey mycelium on the PDA medium and their reverse sides exhibited colours of white turning brown and dark brown to black respectively. Cc4 isolate initiated creamy white mycelium on the front side of culture plate and brown to black colour on the reverse side. Cc7 showed greyish white mycelium on the upper side of culture plate

while, the lower side exhibited white to greyish white mycelium. However, grey to brown colonies were produced by Cc9 on the upper side compared to dark brown to black on the reverse side (Figure 2; Table 2).

The colony margins were observed as either regular or irregular. Seven *C. capsici* isolates *viz*., Cc2, Cc3, Cc4, Cc5, Cc6, Cc7 and Cc8 displayed regular margins whereas, the isolates Cc1 and Cc9 exhibited irregular margins on the PDA medium.

The *C. capsici* isolates showed significant variation in the growth rate on inoculation in the PDA medium. The isolate Cc4 grew faster in the medium, taking only 7 days to completely cover the Petri plate. This was followed by 8 days for isolates Cc3 and Cc6; 10 days for isolates Cc1 and Cc9 and 11 days for isolates Cc2, Cc5, Cc7 and Cc8. About 7 days after incubation, highest growth of 8.6 cm was observed for the isolate Cc4 followed by 8.5 cm in Cc6 and 8.2 cm in Cc3. In contrast, minimum growth of 7.2 cm was observed for the isolate Cc2 (Table 2). These plates produced orange to pink coloured spores in culture, on continued storage for about 20 to 25 days.

Morphological variability depicted remarkable variation in the *C. capsici* isolates

Microscopic observations of mycelia, conidia, setae, acervuli and appressoria of nine *C. capsici* isolates were recorded on the PDA medium. Width of mycelia in *C. capsici* isolates varied between 1.73 µm and 2.39 µm. Highest mycelial width was observed in isolate Cc5 of Special problem zone (2.39 µm), while the minimum was recorded in Cc2 of Northern zone (1.73 µm). All the isolates produced single-celled, sickle shaped conidia having a central oil globule. Conidial length varied between 19.42 µm and 20.46 µm whereas, the width of conidia ranged from 2.16 µm to 3.09 µm. Maximum length of conidia was observed in Cc4 isolate of Central zone (22.68 µm) while, conidial length was minimum for Cc5 of Special problem zone (19.42 µm). In contrast, Cc8 isolate from Southern zone produced conidia having maximum width of 3.09 µm and lowest conidial width was seen in the isolate Cc1 of Northern zone (2.16 µm) (Table 3).

The *C. capsici* isolates gave rise to black, round or elliptical acervuli having diameter ranging from 122.14 µm to 189.08 µm. The isolate Cc3 of Central zone produced acervuli with highest diameter of 189.08 µm whereas minimum acervular diameter of 122.14 µm was recorded in Cc8 isolated from Southern zone.

A number of long, dark brown to black, needle-like, elongated and septate setae were observed with the acervuli of *C. capsici* isolates, varying from 15 to 26. The number of setae

reached a maximum of 30-46 in the acervuli of isolate Cc3 of central zone compared to lowest number of setae in Cc1 isolate of Northern zone (15-36). Setae length in the nine *C. capsici* isolates varied between 74.13 µm and 107.30 µm. Maximum length of 107.30 µm was recorded in the setae produced by the isolate Cc6 (Special problem zone) and minimum of 74.13 µm in Cc9 of High range zone.

Penetration and attachment of the *C. capsici* isolates was made possible by the production of dark brown, round or elliptical appressoria. The appressorial size varied from 8.64 - 12.64 µm × 5.54 - 7.84 µm. Appressorial length and width was highest in the isolate Cc3 of Central zone (12.64 µm; 7.84 µm). Minimum length (8.64 µm) and width (5.54 µm) was measured for the appressoria initiated by the isolates Cc6 and Cc5 respectively, belonging to the Special problem zone (Table 4).

Cc3 isolate was the most virulent among the *C. capsici* isolates

Pathogenicity of *C. capsici* isolates were tested for their pathogenicity by pin pricking the chilli fruits, followed by inoculation. All the *C. capsici* isolates were pathogenic to chilli and produced anthracnose symptoms on the artificially inoculated fruits of chilli (var. Vellayani Athulya). The days taken for initiating anthracnose symptoms was reduced to 1 day in the *C. capsici* isolates, Cc1 and Cc2 from Northern zone, Cc3 from Central zone and Cc6 from Special problem zone. While, the other *C. capsici* isolates produced the symptoms in 2 to 3 days after inoculation. However, a maximum of 3 days for symptom appearance was recorded in the isolates Cc4 from Central zone, Cc7 and Cc8 from Southern zone.

The isolate Cc3 produced a highest lesion size of 1.13 cm, followed by Cc6 (0.91 cm) and Cc2 (0.73 cm). In contrast, minimum lesion size was recorded in chilli fruits inoculated with Cc8 (0.17 cm). The other isolates Cc1, Cc4, Cc5, Cc7 and Cc9 also formed lesions of 0.50, 0.33, 0.23, 0.28 and 0.37 cm respectively on the 5^{th} day after inoculation.

Maximum disease severity of 45.33 per cent was recorded at 5 days after artificial inoculation of chilli fruits with Cc3, followed by Cc6 (41.67 %) and Cc2 (33.33 %). Lowest disease severity of 16.67 per cent was observed in the fruits, on inoculation with Cc7 and 20.33 per cent due to Cc8. PDI of 25 was recorded in all the other *C. capsici* isolates (Cc1, Cc4, Cc5 and Cc9) (Table 5).

Discussion

The survey performed in the five ACZs of Kerala during 2018-2019 revealed the prevalence of *C. capsici* as the major causal agent of chilli anthracnose disease. Further, the disease incidence as well as severity varied significantly in the surveyed areas of various

districts. The anthracnose and fruit rot incidence ranged between 20 to 90 per cent while the severity as PDI values were observed between 23.63 and 52.60. The difference in incidence and severity of chilli anthracnose existing in the surveyed locations may be accredited to the cultivated variety, unpredictable climatic conditions, virulence of the strains and management strategies involved. Yadav *et al*. (2017) assessed anthracnose incidence in the four districts of Jaipur which ranged between 51.75 and 66.70 per cent. Anjana (2018) reported highest anthracnose incidence and PDI of 80 per cent and 54 respectively at RARS, Ambalavayal. Mishra *et al*. (2018) carried out an extensive survey in the 36 locations of Uttar Pradesh, where maximum anthracnose severity was recorded at Jaunpur (54.91 %). Harshitha *et al*. (2022) conducted a roving survey in the chilli cultivating districts of Karnataka during 2020-2021. Maximum PDI of 36.95 was observed in the Dharwad district compared to lowest in the Belagavi district (32.93).

Minute, circular to angular or irregular lesions were observed on the infected leaves which coalesced to give rise to blighted areas, surrounded by yellow halo. Severe infections displayed the presence of black acervuli in the centre of lesions. Characteristic fruit rot symptoms with concentric rings of acervuli and dieback symptoms on infected twigs were also recorded in the survey locations. Saxena *et al*. (2016) reported the presence of dark, greyish brown lesions on the anthracnose-infected stems and leaves, having scattered or concentric rings of acervuli. Javed *et al*. (2017) found the appearance of sunken, circular or round spots, having dark brown to black margins on the infected ripe chilli fruits. Ragul *et al*. (2021) observed circular, black coloured, necrotic lesions on unripe fruits. Under severe conditions, black acervuli were found on the lesions, causing fruit decay. Infected branches displayed die-back symptoms.

Significant variations in terms of cultural and morphological characters were observed among the nine *C. capsici* isolates. The fungal colonies produced on the PDA medium showed different shades of white, off-white to grey turning brown or black. The isolates of *C. capsici* initiated sparse mycelial growth having regular or irregular margins. Also, black acervuli appeared in the form of concentric rings in the pathogen culture. About 7 to 11 days were taken by the isolates to completely cover the PDA plates. The diameter of growth produced by the nine *C. capsici* isolates also varied between 7.20 and 8.60 cm at 7 days after incubation. Further incubation for 20-25 days resulted in the appearance of orange coloured spore masses.

Morphological observations also showed remarkable differences among the *C. capsici* isolates causing chilli anthracnose. Mycelial width varied significantly and measured between

1.73 and 2.36 µm. The pathogen culture produced abundant sickle-shaped conidia, with an oil globule in the centre. Conidial length varied between 19.42 µm and 20.46 µm while, the width of conidia ranged from 2.16 µm to 3.09 µm. Numerous black, circular or elliptical acervuli appeared in the culture plates and the diameter ranged between 122.14 µm and 189.08 µm. Mature acervuli contained many elongated and slender setae (20 – 46 in number), with length measuring between 74.13 µm and 107.30 µm. Brown coloured appressoria were produced by the isolates varying in size between 8.64 - 12.64 µm × 5.54 - 7.84 µm. Similar results have been reported by several researchers.

Masoodi *et al.* (2013) observed whitish grey mycelium of *C. capsici* isolates, producing condia with size 19.70 - 33.60 µm × 2.23 - 4.86 µm. White to grey, sparse to cottony mycelium with regular or irregular margins were found in the *C. capsici* cultures. Conidial length ranged from 13.50 µm to 21.20 µm while width varied between 3.20 µm to 4.80 µm. Black, circular acervuli measured 130 - 162.60 µm in diameter. The length of setae varied between 145.40 µm and 179.10 µm (Machenahalli, 2014). Similarly, Rajamanickam and Sethuraman (2015) reported that the ten isolates of *C. capsici* (Tamil Nadu) initiated fluffy to cottony white aerial mycelium having irregular to regular margins in the PDA medium and highest mycelial growth diameter of 8.15 cm was noticed at 7 days after inoculation. All the isolates produced falcate conidia having a centrally placed oil globule and acervuli varying in size (18 - 23 µm × 3.43 - 3.97 µm) and mature acervuli comprised of several setae (12 – 32). Srideepthi *et al*. (2017) recorded fluffy or cottony to felty mycelium produced by *C. capsici* isolates of Andhra Pradesh with a growth rate of 3.8 to 9.8 mm per day on PDA. Conidial size appeared to be measured between 18.10 - 22.36 µm in length and 2.84 - 4.05 µm in width. The pathogen cultures were observed as greyish to white in colour, having fluffy texture and regular margins. Hyaline, sickle shaped conidia of size ranging between 18 - 27 µm in length and 2.10 - 4.10 µm in width was produced in culture. The setae were dark coloured, septate measuring 110 - 272 µm × 4 - 6 µm in size (Prajapati *et al*., 2020). Sonakar *et al*. (2020) noticed maximum radial growth in all the nine isolates of *C. capsici* of Eastern Uttar Pradesh, grown in PDA medium at 9 days after inoculation. These isolates produced cottony or fluffy mycelium with either regular or irregular margins. Conidia were falcate shaped, with a size ranging between 18.10 - 27.10 µm × 1.6 - 2.3 µm. Mature acervuli contained many setae with varying length of 87.20 - 151.40 µm and width of 3.30 - 5.30 µm.

The differences in morphological as well as the cultural characteristics within the pathogen species may be due to the prevailing temperature, nutritional source and pH of

medium. Udhayakumar and Rani (2010) showed that the significant changes observed in colony colour as well as growth rate of *C. capsici* can be accorded to the nutritional factors present in the media. Significant increase in the conidial size as well as spore volume of the postharvest pathogens, *Botrytis allii* and *Pencillium hirsutum* were reported by Tian and Bertolini (1996). This was attributed to the changes in temperatures ranging from 20 to -2^{o}C. In contrast, spore size was noticed minimum at higher temperature and an enhancement in conidial size and spore volume was reported with decreasing temperature of -2^{o}C. Similarly, lower incubation temperatures marked an enhancement in the conidial size of entomopathogenic fungi, *Metarrhizium brunneum* (Seib *et al*., 2023). Whereas, Maitlo *et al*. (2017) reported the presence of large sized conidia in the chickpea wilt pathogen, *Fusarium oxysporum* f. sp. *ciceris* on exposure to higher temperatures of 30^{o}C, with additions of carbon and nitrogen, at pH of 6 - 7 in the culture medium. Also, recombinations and mutations form remarkable factors in the development of fungal variability. Feurty and Stukenbrock (2018) reported the involvement of random matings as well as mutations as a source of genetic variability in phytopathogenic fungi.

Comment [LR16]: Feurtey

The isolates of *C. capsici* were inoculated on the detached chilli fruits (var. Vellayani Athulya) by spraying spore suspension. Highest lesion size of 1.13 cm and thus, the maximum disease severity was recorded in the chilli fruits inoculated with the isolate Cc3 of Central zone. The days taken to initiate anthracnose symptoms was also considerably reduced for Cc3, taking only 24 h after artificial inoculation for symptom appearance. Minimum lesion size of 0.17 cm was observed for the isolate Cc8 obtained from Southern zone (Farmer's field, Kottarakkara). However, Cc7 isolate recorded lowest disease severity of 16.67 per cent. Sangdee *et al*. (2011) categorized the ten isolates of *C. capsici* (Thailand) into three groups *viz*., mildly virulent, moderately virulent and severely virulent, based on the disease scores incited in the inoculated fruits. Christopher *et al*. (2013) reported that among the twenty *C. capsici* isolates of Tamil Nadu, Cc1 incited maximum intensities of chilli fruit rot (69.90 %) and leaf rot (63.20 %) infections in artificially inoculated chilli plants. Tanwar *et al*. (2015) recorded that the *C. capsici* isolate, UDR Cc-01 to be the most virulent among the four isolates, causing a maximum PDI of 61.20 on the chilli variety, Pusa Jwala. Mishra (2023) observed the pathogenic variability of ten *C. capsici* isolates obtained from Rajasthan. The highly virulent isolate was recorded as UDP Cc1 with a severity of 47.17 per cent while, the least virulent was Raj Cc1 with a PDI of 26.42.

Conclusion

Survey conducted in the five ACZs of Kerala revealed that RARS, Pilicode of Northern zone (Kasargod district) is more prone to anthracnose infection with 52.60 PDI. Nine *C. capsici* isolates were obtained from the survey locations and their cultural, morphological as well as pathogenic differences were studied. Among the *C. capsici* isolates, Cc3 of CoA, Vellanikkara (Central zone) was obtained as the most virulent isolate. These variability studies comprehend towards the selection of better management options and also reduce the risk of creation of new pathogen races. The integration of different management measures can help in mitigating severe crop losses.

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Table 1. Incidence and severity of anthracnose and fruit rot of chilli at different survey locations in the five agro-climatic zones (ACZs) of Kerala

Zones	Locations	Variety	*DI (%)	**PDI (%)	Isolates
Northern	CoA, Padannakad (Kasargod)	Anugraha	85	43.20	Cc1

Comment [LR17]: It should be written in full form (not abbreviated)

Comment [LR18R17]: Make corrections in all abbreviated CoA

Zone	RARS, Pilicode (Kasargod)	Anugraha	90	52.60	Cc2
Central Zone	CoA, Vellanikara (Thrissur)	Anugraha	55	24.13	Cc3
	RARS, Pattambi (Palakkad)	Jwalamukhi	74	40.33	Cc4
Special Problem Zone	ORARS, Kayamkulam (Alappuzha)	Local variety	32	23.63	Cc5
	RARS, Kumarakom (Kottayam)	Ujwala	43	28.25	Cc6
Southern Zone	CoA, Vellayani (Thiruvananthapuram)	Vellayani Athulya	68	36.38	Cc7
	Farmer's field, Kottarakkara (Kollam)	Local variety	20	27.25	Cc8
High range Zone	Farmer's field, Idukki	Local variety	36	29.88	Cc9
	RARS, Ambalavayal (Wayanad)	Anugraha	83	47.90	Cc10

***** DI – Disease incidence; ** PDI – Percentage Disease Index

The chilli cultivating areas of various districts representing the five ACZs of Kerala were chosen for the survey. About 100 chilli plants were sampled in each location for assessing the disease incidence and severity. The chilli variety cultivated in each location during survey was also considered.

Figure 1. Different symptoms observed on the anthracnose infected chilli plants of surveyed locations

(A) The photograph shows minute, water-soaked, dark brown circular or irregular necrotic lesions on the anthracnose infected chilli leaves

(B) Typical fruit rot symptoms depicting greyish brown lesions on the fruit surface, enlarging to cover the entire fruit resulting in drying up and mummification. Severe infection shows concentric rings of acervuli on lesions.

(C) Anthracnose infection on twigs produce small necrotic spots which progress downwards leading to die-back of branches, with acervular structures on the lesions.

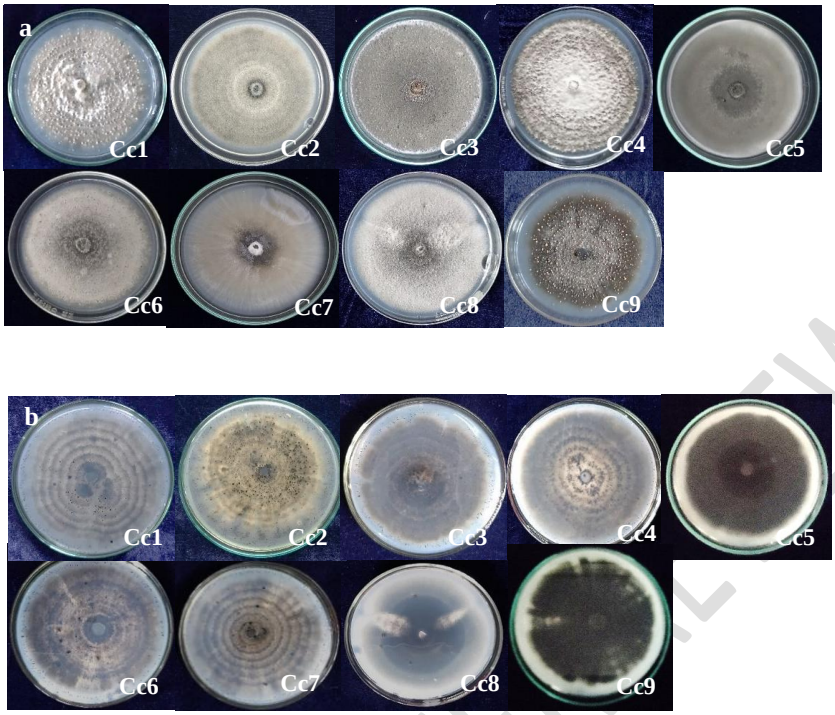

Figure 2. Colony characters of the nine *C. capsici* isolates on PDA medium at 7 days after incubation. depicting the upper as well as reverse side of the culture plates

(a) Photographs show the upper side of the culture plates inoculated with the nine *C. capsici* isolates numbered from Cc1 to Cc9

(b) Pictures represent the rear view of the culture plates inoculated with the nine *C. capsici* isolates numbered from Cc1 to Cc9

Table 2. Cultural characteristics of the different *C. capsici* isolates on PDA medium

Isolates	Appearance	Colour of mycelia		Margin	DTCP	Radial growth of pathogen mycelium (cm)
		Upper side	Reverse side			
Cc1	Sparse mycelial growth with concentric rings of acervuli	White turning grey	White turning brown	Irregular	10	7.8 ± 0.24^{c}
Cc2		Off-white	Yellowish brown	Regular	11	7.2 ± 0.22^{e}
Cc3		Grey to dark grey	White turning brown	Regular	8	8.2 ± 0.26^{b}
Cc4		Creamy white	Brown to black	Irregular	7	8.6 ± 0.17^{a}
Cc5		Grey to dark grey	Dark brown to black	Regular	11	7.3 ± 0.08^{de}
Cc6		White turning grey	Yellowish brown	Regular	8	8.5 ± 0.13^{ab}
Cc7		Greyish white	White turning brown	Regular	11	7.4 ± 0.15^{de}
Cc8		White turning grey	White turning black	Regular	11	7.4 ± 0.22^{d}
Cc9		Grey to brown	Dark brown to black	Irregular	10	7.6 ± 0.21^{cd}
SE(m) ±						0.037
CD(0.05)						0.281

DTCP – Days taken to completely cover Petri dish

The nine *C. capsici* isolates were inoculated on the sterilized PDA plates and incubated for 7 days at room temperature (28 ± 2 ^{o}C). The colour of pathogen colonies on the upper as well as reverse side of culture plates, margin of growth and diameter of mycelial growth were recorded for each isolate. The data of radial growth is based on nine independent experiments with three replications in each treatment per experiment and errors represent SEs.

Table 3. Morphological characteristics of different *C. capsici* isolates

Isolates	Width of Mycelia (µm)	Conidia			Diameter of acervuli (µm)
		Shape	Length (µm)	Width (µm)	
Cc1	1.83 ± 0.37^{cd}	Sickle shaped single celled conidia with a central oil globule	21.46 ± 1.24^{b}	2.16 ± 0.42^{e}	146.26 ± 3.14^{e}
Cc2	1.78 ± 0.39^{d}		21.20 ± 1.02^{b}	2.30 ± 0.49^{de}	142.19 ± 2.08^{f}
Cc3	1.86 ± 0.11^{cd}		20.46 ± 3.57^{bcd}	3.00 ± 0.38^{a}	189.08 ± 2.58^{a}
Cc4	2.03 ± 0.13^{bcd}		22.68 ± 1.38^{a}	2.32 ± 0.51^{cde}	173.02 ± 2.57^{b}
Cc5	2.39 ± 0.24^{a}		19.42 ± 1.87^{d}	2.52 ± 0.34^{bc}	143.02 ± 1.63^{f}
Cc6	2.05 ± 0.24^{bcd}		20.52 ± 0.85^{bc}	2.40 ± 0.20^{bcd}	153.31 ± 4.09^{d}
Cc7	2.12 ± 0.11^{abc}		21.83 ± 0.59^{bc}	3.02 ± 0.42^{a}	128.89 ± 1.70^{g}
Cc8	2.21 ± 0.23^{ab}		19.90 ± 3.68^{cd}	3.09 ± 0.46^{a}	122.14 ± 1.47^{h}
Cc9	2.25 ± 0.15^{ab}		20.57 ± 2.09^{bc}	2.57 ± 0.43^{b}	167.97 ± 1.42^{c}
SE(m) ±	0.06		4.41	0.17	5.98
CD (0.05)	0.31		1.06	0.21	2.18

Slide culture technique was carried out to study the morphological characters of the nine *C. capsici* isolates. 1 per cent agar medium was prepared and square blocks were cut out of the solidified medium. Slide culture unit was set up and agar blocks were placed on the sterilized glass slides. Four corners of the blocks were inoculated with the *C. capsici* isolates. The units were inoculated at room temperature for 48 hours and were microscopically observed. The mycelial width, conidial length and acervular diameter were the mean of ten observations while conidial width was the mean of thirty observations. The errors represent SEs.

Table 4. Setae and appressorial characteristics of different *C. capsici* isolates

Isolates	Setae		Appressoria	
	Number	Length (µm)*	Length (µm)	Width (µm)
Cc1	15-36	80.92 ± 2.33^{d}	11.13 ± 0.46^{c}	5.63 ± 0.62^{ef}
Cc2	20-32	78.72 ± 3.31^{d}	11.48 ± 0.69^{bc}	5.71 ± 0.81^{def}
Cc3	30-46	106.37 ± 5.08^{a}	12.64 ± 0.87^{a}	7.84 ± 0.57^{a}
Cc4	32-45	101.98 ± 2.78^{b}	9.05 ± 0.90^{e}	6.48 ± 0.41^{bc}
Cc5	25-37	94.22 ± 3.30^{c}	11.95 ± 0.58^{abc}	5.54 ± 0.64^{f}
Cc6	38-43	107.30 ± 2.91^{a}	8.64 ± 1.62^{e}	6.02 ± 0.21^{cde}
Cc7	24-40	96.62 ± 1.85^{c}	11.21 ± 0.32^{c}	6.10 ± 0.67^{cd}
Cc8	20-30	94.60 ± 1.18^{c}	12.33 ± 0.91^{ab}	6.13 ± 0.26^{cd}
Cc9	27-40	74.13 ± 0.86^{e}	9.96 ± 1.63^{d}	6.78 ± 0.19^{b}
SE(m) ±		8.31	0.98	0.28
CD (0.05)		2.57	0.88	0.47

Square shaped blocks were cut out from the solidified agar medium (1 %). Slide culture unit was set up and agar blocks were placed on the sterilized glass slides. Four corners of the blocks were inoculated with the *C. capsici* isolates. The units were inoculated at room temperature for 48 hours and were microscopically observed. The setal length and appressorial parameters were the mean of ten observations and the errors represent SEs.

Table 5. Pathogenicity of *C. capsici* isolates on mature chilli fruits of variety Vellayani Athulya

Isolates	Days taken for symptom appearance	Lesion size on fruits (cm) at 5th day after inoculation	PDI (%)
Cc1	1	0.50 ± 0.20^{cd}	25
Cc2	1	0.73 ± 0.05^{bc}	33.33
Cc3	1	1.13 ± 0.20^{a}	45.33
Cc4	3	0.33 ± 0.15^{de}	25
Cc5	2	0.23 ± 0.06^{e}	25
Cc6	1	0.91 ± 0.20^{b}	41.67
Cc7	3	0.28 ± 0.17^{de}	16.67
Cc8	3	0.17 ± 0.06^{e}	20.33
Cc9	2	0.37 ± 0.15^{de}	25
SE(m) ±		0.023	
CD (0.05)		0.260	

The nine isolates of *C. capsici* were inoculated on the detached fruits of chilli variety, Vellayani Athulya. Pin-pricking was done and the fruits were inoculated with the spore suspension of *C. capsici* isolates (1×10^6 spores ml^{-1}). The artificially inoculated fruits were incubated at room temperature for 5 days. The days taken for symptom development, lesion size produced and disease severity were recorded at 5 days after incubation. The data of radial growth is based on nine independent experiments with three replications in each treatment per experiment and errors represent SEs.