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Anthracnose of Chilli: Status, Diagnosis, and Management

Raj Kiran, Jameel Akhtar, Pardeep Kumar and Meena Shekhar

Abstract

Chilli (*Capsicum annuum* L.) is one of the most economically important vegetable crops in the world. Among different biotic constraints, anthracnose disease is the major limiting factor affecting yield and production of chilli crop. Different symptoms associated with disease are fruit rot, leaf spots, dieback on stem, seedling blight, or damping off. Many species of genus *Colletotrichum* are found associated with the disease worldwide. In India, primarily three important species, namely, *Colletotrichum truncatum*, *C. acutatum*, and *C. gleosporoides*, are responsible for the chilli anthracnose. Accurate identification of pathogen is needed for choosing the proper management strategy for controlling this disease. Both conventional and molecular methods are adapted along with different management strategies, recommended for this disease namely cultural, chemical, and other eco-friendly methods.

Keywords: *Colletotrichum*, biocontrol agents, diagnosis, molecular methods

1. Introduction

The genus *Capsicum* includes many cultivated species, of which *Capsicum annuum* L. is one of the most widely cultivated one; besides this, other domesticated species are *C. baccatum*, *C. chinensis*, *C. frutescens*, and *C. pubescens* [1]. *C. annuum* comprises of both sweet (bell pepper) and pungent (chilli) fruits of numerous shapes and sizes. It is a good source of Vitamin A and C, potassium, and folic acid [2]. Fresh green chilli has more vitamin C than a citrus fruit, whereas red chilli has more vitamin A than in carrots [3, 4]. Besides its wide use as vegetable, spice, and condiments, it is also used in medicines and beverages. Capsaicinoid and caretenoids are the active ingredients of the chilli; the capsaicinoids are nonvolatile alkaloids that make chilli pungent [5], and caretenoids have nutritional value that also provides color to the chilli fruit [6]. In tropical and subtropical countries, chilli is considered the most important constituent of different cuisines. As the native home of chillies are considered to be tropical America, where it is still found growing in the wild state [7]. Its introduction to India is credited to voyage of Columbus who brought seeds from Spain, introducing it to Europe, which subsequently spread to Africa and Asia [8].

India is the world's largest producer of dried chillies and in 2018 India produced 1.8 million tons, out of 4.1 million tons produced worldwide [9]. There are two important commercial qualities that makes Indian chilli world famous are color and

pungency levels. Chilli crop is attacked with different pests and pathogens in field and during post-harvest, contamination with mycotoxins are major constraints in chilli production. Worldwide, *Capsicum* is vulnerable to various pests, weeds, fungal, bacterial, and viral pathogens; among the fungal diseases, anthracnose/die-back/fruit-rot of chillies is an important disease causing serious losses in field, transit, transport, and storage [10, 11].

2. Anthracnose disease losses

The word anthracnose derived from Greek language meaning ‘coal’ it is the common name of plant disease with very dark, sunken lesions and containing fungal spores [12]. Typical symptoms (**Figure 1**) of anthracnose on chilli fruit include dark spots, sunken necrotic tissue with concentric rings of acervuli. Besides fruit rot, it also causes leaf spots, dieback on stem, seedling blight, or damping off. This disease not only affects the quality of fruit by appearance of anthracnose lesion but also reduces dry weight of fruit, and quantity of capsaicin and oleoresin [13, 14].

Losses are caused by this disease worldwide; it is reported that in Vietnam it causes 20–80% yield loss [15], 10% yield loss in Korea [16], 50% yield loss in Malaysia [17] and as high as 80% yield loss (during severe epidemics) in Thailand [18]. In India, a calculated loss of 10–54% has been reported in yield due to this disease [19, 20], and this disease is reported throughout India but it found to be more common and aggressive form in Assam, Bihar, Andhra Pradesh and Uttar Pradesh [10]. The anthracnose pathogen has been intercepted in seed and it has been reported that there is occurrence of pathogen in seed samples, upto 5% infection index indicates its wide spread occurrence in India [21].



Figure 1.
(a) Healthy chilli plant, (b) chilli plant affected with anthracnose disease, and (c) chilli fruits showing anthracnose symptom.

3. Causal organisms

This disease is caused by the species of genus *Colletotrichum*, which belongs to Ascomycetes. Worldwide, different species of *Colletotrichum* are reported to cause chilli anthracnose disease (**Table 1**), In India, among different species known to cause this disease, there are primarily three important species *Colletotrichum capsici* Syd. Butler and Bisby (Synonym *C. truncatum*), *C. acutatum* and *C. gloeosporioides* have been reported to be associated with the disease, however *C. truncatum* causing major damage at the ripe fruit stage of the plant [35, 52–55].

Country	Pathogen	References
Australia	<i>C. acutatum</i> , <i>C. atramentarium</i> , <i>C. dematium</i> , <i>C. gloeosporioides</i> var. <i>minor</i> and <i>C. gloeosporioides</i> var. <i>gloeosporioides</i> and <i>C. brisbanense</i>	[22, 23]
Brazil	<i>C. boninense</i>	[24]
India	<i>C. capsici</i> / <i>C. dematium</i> / <i>C. truncatum</i> , <i>C. gloeosporioides</i> , <i>C. graminicola</i> , <i>C. acutatum</i> , <i>C. piperatum</i> , <i>C. atramentarium</i> , <i>C. fructicola</i> and <i>C. siamense</i> , <i>C. cliviae</i> , <i>C. coccodes</i> and <i>C. karstii</i>	[20, 25–35]
Indonesia	<i>C. gloeosporioides</i> , <i>C. truncatum</i> and <i>C. acutatum</i>	[36]
South Korea	<i>C. acutatum</i> , <i>C. gloeosporioides</i> , <i>C. coccodes</i> and <i>C. dematium</i>	[37]
Mexico	<i>C. truncatum</i>	[23]
Malaysia	<i>C. truncatum</i>	[17, 38]
New Zealand	<i>C. coccodes</i> , <i>C. kartsii</i> , <i>C. novae-zelandiae</i> and <i>C. nigrum</i>	[39, 40]
Papua New Guinea	<i>C. truncatum</i> and <i>C. gloeosporioides</i>	[41]
Philippines	<i>C. gloeosporioides</i> , <i>C. truncatum</i> and <i>C. scovillei</i>	[42, 43]
Sri Lanka	<i>C. truncatum</i>	[44, 45]
Taiwan	<i>C. acutatum</i> , <i>C. truncatum</i> and <i>C. gloeosporioides</i>	[46]
Thailand	<i>C. acutatum</i> , <i>C. truncatum</i> and <i>C. gloeosporioides</i>	[47, 48]
USA	<i>C. gloeosporioides</i> , <i>C. acutatum</i> , <i>C. truncatum</i> and <i>C. coccodes</i>	[49, 50]
UK	<i>C. acutatum</i> and <i>Glomerella cingulata</i>	[51]
Vietnam	<i>C. acutatum</i> , <i>C. truncatum</i> , <i>C. gloeosporioides</i> and <i>C. nigrum</i>	[15]
Zimbabwe	<i>C. nymphaeae</i>	[23]

Table 1.
 Different *Colletotrichum* species associated with the disease anthracnose of chilli in different countries.

4. Diagnosis

Identification of *Colletotrichum* species based on morphological characteristics (size and shape of conidia; presence of setae) and colony characteristics is generally used by several workers [56–59]; it is widely used in seed health testing labs for detection of *C. capsici* in germplasm for pest free conservation of chilli seeds [21]. As the pathogen is seed-borne, there is threat of introduction of this pathogen along with import of germplasm (including Chilli) from different countries; therefore, while importing from any other country, there is a need to examine the samples very critically including sensitive molecular diagnostic tools to prevent entry of this pathogen associated with germplasm [60]. Moreover, for the accurate identification of the pathogen at species level molecular methods are widely adapted. Loop-mediated isothermal amplification (LAMP) assay was used for the accurate and sensitive detection of *C. capsici* LAMP primers (β -tubulin gene sequences based) were designed and it was reported that it could detect as little as 10 fg/ μ l of *C. capsici* pathogen in comparison with only upto 1 ng/ μ l of *C. capsici* detection using polymerase chain reaction [61]. A sequence characterized amplified region (SCAR) marker was developed for specific and sensitive detection of *C. capsici* in chilli seeds and fruits. This markers did the amplification of an expected 250-bp fragment from genomic DNA and these markers were very much sensitive as it was reported that the marker could detect purified *C. capsici* DNA template up to 1 pg and DNA from *C. capsici* infected

chilli fruits up to 25 ng [59]. As these two markers are very sensitive, these may be very useful in detection of the pathogen in imported germplasm in plant quarantine laboratories.

COL1/COL2 primers were used for amplification of the specific internal transcribed spacer region of tested *Colletotrichum* species (*C. acutatum*, *C. truncatum* and *C. gloeosporioides*) with a specific band of 460 base pairs. *C. gloeosporioides* was detected at a low level of 1000 conidia on chilli leaf and fruit by this primer [62]. Another, primer set based on the sequences of the ribosomal internal transcribed spacer (ITS1 and ITS2) regions of *C. truncatum* was designed and standardized for the detection of *C. truncatum* in infected plant tissues using PCR assay. The sensitivity was 10 pg of genomic DNA from the pathogen [63]. Machenahalli et al. [64] detected pathogens from different parts of plant like seeds, fruits, infected twig/stem by PCR-based method by using specific primers. *C. truncatum* was amplified by species specific primer (C.cap-f and C.cap-r) as single band at 450 bp. *C. gloeosporioides* was amplified by species specific primers CgInt at 450 and *C. acutatum* by CaInt at 490 bp respectively. The accurate identification is very important for choosing the correct management strategy for this disease.

5. Management strategies

For the management of anthracnose disease of chilli, different strategies are adapted. These are use of cultural practices, chemical control, eco-friendly measures like use of biocontrol agents, plant extracts and use of resistant cultivars. Generally, use of different strategies in combination has been recommended for managing the disease [65]. The summarized information is given from across the world for the management of this disease.

5.1 Cultural practices

Several cultural practices have been reported to manage chilli anthracnose due to the special etiology of the pathogen. These precautionary measures are implemented to reduce the rate of infection and minimize the inoculum pressure even before fruits are mature and harvested. Than et al. [47] and Ali et al. [66] in their review reported that different cultural practices like disease free seeds, weeding, crop rotation, proper drainage, removal of crop residue are being followed for the chilli cultivation. It was suggested that disease free chilli seeds should be planted and elimination of weeds should be done in chilli field and rotation of chilli crop with other crops which are not alternative hosts to *Colletotrichum* spp. after every 2–3 years is very effective for controlling this disease. Good drainage systems on the field to channel out waste water during irrigation regimes, on-farm fruit disinfection such as fruit washing at packing houses and finally removal of plant debris which may serve as source of inoculum are some other clean crop and sanitation practices [47]. If there was history of disease in a particular field, then other crops should be rotated in isolation from other solanaceous plant for at least alternate years [50]. Deep plow is recommended to completely cover diseases plants or removing infected plant debris from the field at the end of growing season [67]. Early planting of chilli or planting cultivars that bear fruit within a short ripening period to allow the fruit to escape fungal infection is also recommended. Other alternative sanitation practices such as weeding, removal of infected or wounded fruits should be carried out regularly to prevent the pathogens from using such wounds as sites of infection.

5.2 Chemical control

Different strategies for managing the disease are recommended and chemical control is found most effective and practical method [68]. As time required for controlling the disease with chemical method is much lesser as compared to the time required for the development of resistant cultivar. Use of protective fungicide like manganese ethylene bisdithiocarbamate (Maneb) is widely recommended for managing this disease. Other dithiocarbamate fungicides like Mancozeb (0.2%), ziram (0.1%), copper oxychloride fungicide (Blitox 50), and Bordeaux mixture (0.5 or 1%) of a copper sulphate fungicide were found effective in managing this disease. Seed dressing with benzimidazole fungicides (Benlate, delsene M) and strobilurin fungicide (azoxystrobin) are recommended [69] and soaking of chilli seeds for 12 h in 0.2% Thiram, a dithiocarbamate fungicide was also found effective for better control of the disease [70].

Among different systemic fungicides recommended Bavistin (carbendazin 50%WP) 0.1%, Plantvax (oxycarboxin) and vitavax (carboxin) were found effective as use of Bavistin resulted in 80.84% disease reduction [71] and Plantvax and Vitavax were reported to reduce the disease by checking the spore germination of *C. truncatum* [72]. Additionally other systemic fungicides from triazole group propiconazole [73], difenoconazole, benzimidazole fungicide (Benomyl) [74] have been used in both pre and post-harvest management of chilli anthracnose, as propiconazole, exhibited the highest level of inhibition of in vitro mycelial growth, biomass production, sporulation and spore germination at concentrations as low as 0.1 mg/ml. Other workers also reported that Tilt (propiconazole) is highly effective in controlling *Colletotrichum* spp. [75, 76] concentration of Tilt at 150 ppm was found effective in inhibiting the pathogen as it caused 50% inhibition (ED 50) of *C. acutatum* growth in culture media [77]. It is to be noted that Benomyl and its associated fungicides Carbendazim and thiophanate methyl (both of which registered) has raised major health concerns and these are proved unacceptable and dangerous [78]. Different strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint) and pyraclostrobin (Cabrio) have also been recommended for effective management of the disease [47, 79].

Moreover, dependence on only single chemical resulted in the emergence of resistant strains of *C. truncatum* isolates from chilli fruit against different chemicals benomyl, which were cross-resistant to thiophanate methyl and carbendazim [80], resistance of *C. truncatum* to benomyl and strobilurin-fungicides (azoxystrobin and kresoxim-methyl) is also reported [81–83]. Under such circumstances, combined application of Bioagents with chemicals are recommended, *Pseudomonas fluorescens* along with half of the recommended dose of azoxystrobin fungicide has been found effective and viable option to control fruit rot [79]. As use of chemicals are not eco-friendly and it leaves chemical residue in chilli fruits, which hinders the export, and there are numerous reports describing negative effects of using chemicals on farmer's health in developing countries [36]. To overcome the undesirable effects of chemical usage alternate methods such as use of bioagents, plant extracts or use of chemicals in combination with these are recommended to control the infection.

5.3 Biological control

Trichoderma species is the fungal antagonist which is widely applied to control *Colletotrichum* species in chilli [84, 85]. It is also believed that *Trichoderma* species are able to effectively compete for surface area, thereby reducing pathogen infection success [86–88]. Chloroform extracts of nonvolatile antibiotics (NVAC) of *T. viride*

added to the culture media inoculated with *C. truncatum*, showed reduction in biomass and synthesis of RNA, DNA and protein [89]. It has been reported that antifungal metabolites (100 mg/L) secreted from *Trichoderma harzianum* Rifai strain number T-156co5 significantly controlled *C. truncatum* isolated from *C. annuum* [90]. *In vitro* studies indicated that *T. viride* and *P. fluorescens* are very effective in inhibiting mycelial growth of the pathogen [91]. It is suggested that the use of *T. viride* and *P. fluorescens* individually or in combination known to significantly lower the anthracnose disease incidence and should be used as an alternative to chemical control [92].

Other bioagents like *Bacillus subtilis* and *Candida oleophila* (a yeast species) have been tested for efficacy against *C. acutatum* [93]. *Pichia guilliermondii* Wick strain R13 is another yeast species which is reported to reduce the disease incidence on *C. truncatum* infected chilli fruit as low as 6.5%. It has also been proposed that this fungal strain with other yeasts suppressed *Colletotrichum* spp. through multiple modes of action (nutrient competition, competition for space between antagonist and the pathogen, toxin production, induction of plant resistance and hydrolytic enzyme production) [94–96]. Intanoo and Chamswarng [97] reported that DGg13 and BB133 were antagonistic bacterial strains found very effective in controlling *C. truncatum*. *Pseudomonas aeruginosa* FP6 also found effective against *C. acutatum* [98].

Rhizosphere and rhizoplane fungal isolates (*Chaetomium globosum*, *T. harzianum* and *F. oxysporum*) from perennial grasses has been reported to decreased disease incidence and severity in seedlings and mature plants, and promoted plant growth and increased yield in the greenhouse and field [99]. In an experiment crude extracts from *Chaetomium cupreum* CC, *C. globosum* CG, *T. harzianum* PC01, *T. hamatum* PC02, *Penicillium chrysogenum* KMITL44 and antibiotic substances Rotiorinol, Chaetoglobosin-C and Trichotoxin A50 was used against *C. gloeosporioides* isolate WMF01 (the most virulent on all tested varieties of grape). The results revealed that application of all bioproducts significantly reduced the disease incidence on leaves, twigs and fruits of grape in all varieties as compared to the chemical control [100]. *Cordyceps sobolifera* an entomopathogenic fungi have also been reported for use as a biocontrol agent against *C. gloeosporioides* [101, 102].

5.4 Plant extracts

Antimicrobial plant secondary metabolites compounds are one of the best options to controlling plant diseases. In chilli, several workers have shown the efficacy of plant extracts against *Colletotrichum* spp. [103–108]. Among the plant extracts, *Allium sativum* (10%) and *Azadirachta indica* (10%) demonstrated the highest inhibition of mycelial growth of *C. gloeosporioides* [91]. *A. indica*, *Datura stramonium*, *Ocimum sanctum*, *Polyalthia longifolia* and *Vinca rosea* were used against *C. truncatum*. Among the five fermented leaf extracts tested against *C. truncatum*, *A. indica* extract at 20% concentration highly inhibited the growth of *C. truncatum* *in vitro* condition. And *in vivo* the application of fermented leaf extract of *A. indica* alone reduced the fruit rot incidence (@3%) and increased plant height, number of fruits and yield significantly [109]. In an experiment the botanicals or plant extracts from *Catharanthus roseus*, *Coleus aromaticus*, *Manilka razapota* and *A. indica* used against fungi, it was concluded that these botanicals confer antifungal effects on the radial mycelial growth of *C. truncatum* [107]. The organic pesticides were prepared from the extract of neem leaves, soursop leaves, lemongrass extract, tuba root extract, and kenikir/*Cosmos caudate* extract [110]. The result indicates that neem leaves are the most effective organic pesticides to control the chilli pepper disease especially in Indonesia.

Nine plants extracts viz., *Lawsonia inermis*, *A. indica*, *Bougainvillea spectabilis*, *Withania somnifera*, *Ocimum tenuiflorum*, *Aegle marmelos* L., *Justicia adhatoda* and *Calotropis gigantean* were tested under *in vitro* condition through poisoned food technique against chilli fruit rot pathogen *Colletotrichum* sp., among them *W. somnifera* (10%) was found to highly inhibit the mycelial growth of the anthracnose pathogen up to 84.88% [111]. Further, Singh and Khirbat [112] reported the efficacy of aqueous extract of three wild plants viz., *Albizza lebbeck*, *Acacia arabica* and *Clerodendrum infortunatum* to control chilli fruit rot. Alves et al. [113] reported the efficacy of 1% aqueous or 20% ethanol plant extracts to control bell pepper anthracnose caused by *C. acutatum*. In this study, 6% aqueous garlic, mallow and ginger extracts reduced disease severity by more than 97%. Even though recent research suggests the use of these plant extracts as bio-fungicides, but still more studies on their efficacy in the controlling of chilli anthracnose need to be performed under field conditions.

5.5 Resistant cultivars

As use of resistant or tolerant cultivar is the most cost-effective management strategy. Due to the lack of resistance in the *C. annuum* gene pool, no commercial resistant varieties have been developed in *C. annuum* [114]. The introgression of the resistance gene from *C. baccatum* to *C. annuum* is difficult. There are some studies on introgression of anthracnose resistance into *C. annuum* to develop a new variety [115, 116]. Five lines of *C. annuum* from AVRDC, Taiwan, namely AVPP1102-B, AVPP0513, AVPP0719, AVPP0207 and AVPP1004-B, as the promising lines with good fruit yield and tolerance to anthracnose [117]. Two chilli varieties, Lembang-1 and Tanjung-2, have been reported as moderately resistant from IVEGRI, Indonesia, [118].

In India, some anthracnose-resistant lines listed are LLS, PBC932 (VI047018), Breck-2, PBC80 (VI046804), Breck-1, Jaun, and PBC81 (VI046805) [119]. Other nine resistant varieties (BS-35, BS-20, BS-28, Punjab Lal, Bhut Jolokia, Taiwan-2, IC-383072, Pant C-1 and Lankamura Collection) were identified which could be employed for developing successful resistant cultivars through breeding programs [120]. The information on the resistance varieties against *Colletotrichum* spp. may also be utilized for studying the inheritance of the resistance and also to locate and study the quantitative trait loci (QTLs) maps for resistance [121]. Further studies need to be undertaken to investigate the importance of these distinct genes in the management of chilli anthracnose. Nevertheless, the genetic mechanism associated with chilli resistance to anthracnose is still poorly understood mainly due to lack of information on the defense signaling modules governing the resistance mechanism.

6. Conclusion

Anthracnose of chilli is main constraint for its production in the India as well as worldwide. Detection this pathogen in the seed by the morphological features and with the developed molecular markers are very important especially in quarantine laboratories. The accurate detection of pathogen also helps in choosing the best management strategy for the control of this disease. Involvement of many *Colletotrichum* species in the disease and absence of resistance gene in *C. annuum* makes breeding for resistance is more challenging. Moreover, injudicious use of chemicals for the control of this disease leaves residue in the chilli fruit poses threat to the export. Combining the use of resistance cultivars with other disease

control measures would enhance the efficiency in integrated management of chilli anthracnose. Moreover, more research is required to find better alternative methods to control chilli anthracnose by involving vigorous evaluation and identification of resistant cultivars of chilli against this disease.

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