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REVIEW ARTICLE

Recent advances on *Colletotrichum capsici* causing anthracnose of chilli: A review

Kartik Sawant[1], Devendra Kumar[1]*, Archana TS[1], Sanket Sudhakar Chavan[1], Ajay Chavan[1], Sumanth Kumar Reddy[1], Chandhrapati Akhilesh[1], Vipul Kumar[1], Rafakat Hussain[2] & Siddhesh Kolhe[1]

[1]Department of Plant Pathology, School of Agriculture, Lovely Professional University, Punjab, 144411, India
[2]Department of Plant Science, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China

*Email: Devendra.26885@lpu.co.in

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Abstract

Chilli is one of the most significant spice crops cultivated with numerous culinary, medicinal, and industrial applications. *Colletotrichum* has a wide host range, causing anthracnose disease in various crops, whereas in chilli, anthracnose caused by *Colletotrichum capsici* is the major constraint in chilli production. Under severe environmental conditions, the symptoms appear all over the plant, including the fruit, which leads to complete crop damage. The particular disease has a huge economic impact on yield reduction and also debars the export criteria due to the lower quality of the chilli crop. Management of the disease is difficult under field conditions, and the majority of the management strategies are highly dependent on the use of chemicals. Solely relying on chemicals is harmful to the environment and human health, so an integrated disease management approach can help in managing the disease at the initial stage. Detection and identification of pathogens play a major role in pathogen management. Early identification and detection of the pathogen based on conventional and molecular methods are very important to reduce yield loss under field conditions. The current review consists of a detailed understanding of the pathogen, its identification, detection and diagnosis, as well as the most recent research updates.

Keywords

Anthracnose; *Colletotrichum capsici*; detection; management

Introduction

The chilli (*Capsicum annuum* L.), sometimes referred to as "red pepper," is a significant fruit that is valued as a spice in India, Pakistan, and other nations (1). The chilli pepper is rich in protein, fibres, vitamins (A, C, and E), capsidiol, capsaicinoids, potassium, folic acid, and capsaicin. It is also used in drinks and medications (2). Capsaicin has the ability to stop platelet aggregation in addition to having immunomodulatory, antioxidant, anticancer, antibacterial, and analgesic characteristics. India is now the world's greatest producer, user, and exporter of dried peppers (3). The major states in India that cultivate chillies are Uttar Pradesh, Tamil Nadu, Andhra Pradesh, Maharashtra, Karnataka, West Bengal, Rajasthan, and Orissa, which together account for more than 80% of the country's land area (4). According to recent research, the estimated production in India of green chilli around 0.00679 million tonnes andsee dried chilli 1.389 million tonnes, respectively, over an area of 0.797 million hectares (5,6).

The worldwide output of chilli fruit must be maintained due to its therapeutic properties and widespread use. In the past several years, a number of biotic and abiotic diseases have attacked, resulting in a decrease in fruit output. The main culprits for these losses are thought to be fungi, including anthracnose, Fusarium wilt, damping-off, dry root, stem rot, and collar rot (7). Anthracnose of chilli, due to *Colletotrichum truncatum* alone, resulted in a 50% yield loss globally (8).

The seeds and air are responsible for the spreading of anthracnose-causing agents, and primarily affect foliage and fruits. Additionally, it influences fruit, seed, and plant development as well as quality. However, fruit rot (anthracnose) disease brought on by the damaging fungus *C. capsici* alone has been linked to a yield loss of 8-60 percent in India (10). Anthracnose has been managed through integrated disease management strategies that include cultural, physical, biological, and chemical control techniques. Fungicide use has achieved the greatest decrease; nevertheless, this is unsustainable for agriculture, environmentally unfriendly and promotes disease resistance (3). The simplest, least expensive and most efficient method of preventing anthracnose is the adoption of resistant cultivars. However, while biocontrol agents (BCAs) are economical, environmentally benign, and a practical method for disease control, it is crucial to utilise them as resistant cultivars have not yet emerged (11). Fungicides and biological agents may be used as anthracnose disease control strategies. Utilizing fungicides to halt the disease has various negative effects, resulting in the emergence of resistance in the pathogen, lingering toxicity, and pollution at a high cost to the environment (12). In order to improve crop health and productivity, it has become vital to employ environmentally friendly practises (13,14). In comparison to biocontrol agents, chemically induced resistance may offer a more effective method of plant protection. Chemicals that cause resistance in plants are referred to as phytoalexin inducers or elicitors in different plant species (15). Chemicals like salicylic acid, acetyl salicylic acid, nicotinic acid, and jasmonic acid have shown induced resistance in various crops. Application of silicon to soils deficient in silicon is found to be promising for controlling various diseases, including anthracnose disease. Silicon (Si) has been utilized to manage fungal infections with promising results in recent years, and silicon buildup has been found to be one of the key elements responsible for greater resistance against many agricultural plant pathogens (85). Various *Colletotrichum* spp. reported globally are given in Table 1.

Table 1. Geographic distribution of Colletotrichum species on Chilli

S. No	Country	Colletotrichum species	References
1	India	*Colletotrichum capsici, Colletotrichum cocodes, Colletotrichum fruticola* and *Colletotrichum siamense*	[16-19]
2	Australia	*Colletotrichum siamense, Colletotrichum simmondsii, Colletotrichum queenslandicum, Colletotrichum truncatum* and *Colletotrichum cairnsense*	[19]
3	Indonesia	*Colletotrichum acutatum, Colletotrichum capsici, Colletotrichum gloeosporioides*	[20]
4	South Korea	*Colletotrichum acutatum, Colletotrichum gloeosporioides, Colletotrichum coccodes, Colletotrichum dematium*	[21]
5	Myanmar	*Gloeosporium poperatum E.* and E., *Colletotrichum. nigrum E. and Hals, Colletotrichum capsici*	[22,23]
6	New Zealand	*Colletotrichum coccodes*	[24]
7	Papua New Guinea	*Colletotrichum capsici, Colletotrichum gloeosporioides*	[25]
8	Taiwan	*Colletotrichum acutatum, Colletotrichum capsici, Colletotrichum gloeosporioides*	[26]
9	Thailand	*Colletotrichum acutatum, Colletotrichum capsici, Colletotrichum gloeosporioides*	[27]
10	Vietnam	*Colletotrichum acutatum, Colletotrichum capsici, Colletotrichum gloeosporioides, Colletotrichum nigrum*	[28]

Commercial importance of chilli

The red colour in chilli is due to carotenoid pigments, namely capsanthin and capsorubin, which are referred together as oleoresin (29). To give processed meat and drinks a stunning colour, concentrated oleoresin is added. As a result, it is regarded as the finest alternative to synthetic colour used in the food and cosmetic sectors (30). Enhancing fruit pungency and colour not only improves marketability but also assures that chilli fruits are easily accepted by consumers and food processing businesses (31). Chilli pepper contains anti-cancer and anti-inflammatory characteristics, and it is used to treat rheumatism, bronchitis, chest colds, and stiff joints with cough and headache, arthritis, cardiac arrhythmias, and stomach ulcers. (32). Chilli pepper leaves include capsaicin, which has enzymatic synthesis and anti-obesity characteristics, boosts the immune system, and lowers blood pressure (33). Capsaicin causes cancer cell death in mice and even helps the pancreatic cells to produce insulin again in type 2 diabetes patients. (34). Furthermore, capsaicin suppresses the production of prostate-specific antigen, limits the ability of the most powerful form of testosterone, dihydrotestosterone, to activate PSA, and directly inhibits PSA transcription, resulting in a drop in PSA levels (35).

Symptoms and Identification

Anthracnose can cause considerable pre- and post-harvest injury, as well as symptoms that appear all over the plant, like leaves, fruits, and stems, before harvest (Figure 1). The phenomenon of quiescence, in which symptoms of post-harvest disease do not usually appear until the fruit is ripe. Appressoria are discs of adhesive that adhere to and penetrate the surface of plants until fruit physiological changes take place.

The appressoria that grow on immature fruits are dormant until the fruit undergoes genetic transformation.

Fig. 1. Infection and symptoms from different stages: **A** = symptoms appear on chili plants causing dark spots on leaves, **B** = brown sunken visible on chilli fruit, gradually changes into black colour, **C** = at the higher stage, the fruits and leaves to turn black, and chilli is not able for use.

Cell distraction is due to epidermal cytoplasm, which became condensed and the size of vacuoles increased and the cell demolition extended to the further subepidermal cells, which resemble being injured by the pathogen enzymes. The pathogen penetrates and colonizes within the plant tissue through inter and intracellular mycelium growth at later stages of infection. This kind of mechanism is found in necrotrophic fungal pathogens during infection.

Globular and angular depressed lesions on fruits have typical signs in the form of acervuli within the concentric rings. Acervuli frequently develop orange conidial masses, resulting in a moist and slimy surface. Under extreme disease strain, lesions may merge (8). Since water is necessary for spore germination and penetration into hosts, *Colletotrichum capsici* spore dispersal is strongly dependent on water splashes onto host plants or wind-driven rain. Due to its necrotrophic lifestyle, C. capsici secretes several cell wall-degrading enzymes into the space in between the host cells after penetrating the surface of the host. *C. capsici* spends most of its life in the conidial stage and overwinters as conidia or sclerotia. Acervuli, a subepidermal fruiting body, generate hook-shaped conidia, which make up *C. capsici*, the asexual stage. The colony's appearance was reported to be white to grey with a dark green core and thick, filamentous mycelium when cultivated on plates (28).

Morphological characterization

In 2020, research was done for the identification of the *C. capsici* pathogen. Under a stereo-binocular microscope (40X), mycelium was dense, filamentous, and septate, while acervuli were dark brown, spherical, and elongated. Setae were dark brown to black in colour, with a long needle-like structure that was inflated at the base and narrowed at the end, measuring 110-272 m in length and 4-6 m in width, with 2-5 septa per setae. Profuse thick-walled conidia that were hyaline, uninucleate, falcate (sickle-shaped), somewhat tapering or rounded towards the end, and measured 18-27 m-1.8-4.1 m in size (mean 21.642.85 m). At 550X, acervuli measured 39.84 m in diameter, as seen via an electron microscopic image. Setae are long, needle-like structures that arise from ruptured acervuli. Setaes were inflated at the base and tapered towards the tip, with a length of 107.74 m. The test fungus was grown on autoclaved, molten, and cooled medium that was poured aseptically onto sterilized petri plates and allowed for solidification of the media before each plate was centrally inoculated with a 5 mm culture disc of pathogen cut from the margins of a 7 to 10 day old culture using a sterilized cork borer under aseptic conditions and incubated at 282 oC in a BOD incubator. Pathogens were discovered using stereo binocular and electron microscopes to analyse morphological characteristics such as acervuli, the presence and absence of setae septation, conidia form and size, and the presence of oil globules (86).

Culture characters

C. capsici may be isolated using standard culture medium, such as Potato Dextrose Agar. Acervuli can be seen in mature lesions originating from leaves or fruits. Conidia are hyaline, unicellular, or cylindrical, measuring 7 to 14 mm and 2.5 to 3.5mm, and mycelia are branching and hyaline. The colony is white with orange conidial masses on the reverse side, 2.5 to 3.5 mm.

Molecular characterization

Morphological features of pathogens changed by environmental conditions are one of the significant causes of anthracnose of chilli because two species, *C. gloeosporioides* and *C. acutatum*, cannot be easily distinguished based on characteristics such as cultural and morphological. Therefore, to solve this issue, *Colletotrichum*'s taxonomic complexity has been characterised and examined using DNA sequence studies. The most trustworthy framework for categorising *Colletotrichum* is based on data from DNA studies since DNA is not affected directly due to environmental influences (41). In order to ascertain the evolutionary relationships among the Colletotrichum species, sequence analysis of the internal transcribed spacer (ITS) sections, which are situated between the 18S and 5.8S and the 5.8S and 28S genes, has proven particularly effective (42-44). Analysis of protein-coding genes, such as the incomplete tubulin gene and introns from two other genes, helped us better understand the evolutionary relationships between the many species of *C. acutatum* (synthetase of glyceraldehyde-3-phosphate dehydrogenase and glutamine) (42,45). Although ITS sequences cannot distinguish *C. gloeosporioides* complex from other species of *Colletotrichum*; some single and combination genes, like glutamine and glyceraldehydes-3-phosphate dehydrogenase (GAPDH), can (46). A1 to A4 subgroups of *C. acutatum* isolates were identified phylogenetically based on partial-tubulin 2 sequences (exons 3-6) (42). A more thorough and trustworthy method for investigating *Colletotrichum* species is an integrated strategy that uses both morphological characterization and molecular diagnostic methods (41) (Table 2).

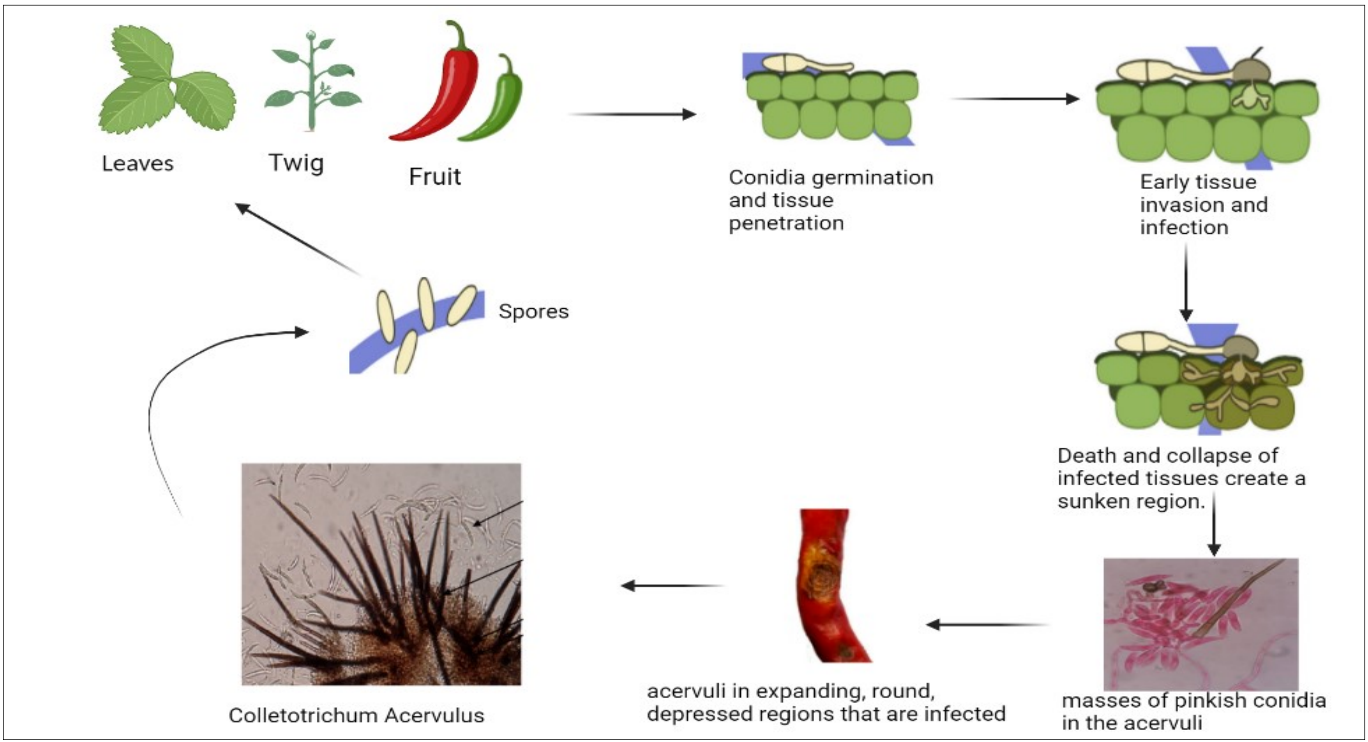

Fig. 2. Disease cycle of anthracnose disease of chilli (*Capsicum annum* L.) caused by *Colletotrichum* spp.

Pathogenic variability

Any offspring that displays a trait that differs from the traits present in the ancestral individuals is referred to as a variation (47). The gene-for-gene paradigm frequently governs the plant-pathogen interactions in numerous pathosystems (48). It is known that some pathogen populations are pathogenically varied, and this variation appears to be caused by the ongoing emergence of novel pathogenic variants (49). A genotype that is partially resistant would lead to decreased infection rates, which would eventually lead to a reduction in the quantity of inoculum present in the field and a reduction in the likelihood of epidemics. *C. acutatum*, a particularly virulent species (8), was tested against chilli genotypes in a number of studies (50,51), and it was discovered that the “PBE 80” genetic resource pool provided by *Capsicum baccatum* for resistance to chilli anthracnose. A different *C. baccatum* genotype called "PBC81" has shown great sensitivity to several isolates of *C. acutatum*. In contrast to *C. baccatum*, the cultivar *Capsicum annuum* has been shown to be susceptible in various investigations (52, 53). Additionally, 'PBC932' has been noted as a resistant variant of *Capsicum chinense* against *C. capsici* (54). However, the sole species produced worldwide, *C. annuum*, has not yet shown any significant resistance (53). The complete life cycle of the pathogen is given in figure 2.

Infection strategies of Colletotrichum

Colletotrichum gloeosporioides and *C. acutatum* are the two species of *Colletotrichum* that are most frequently mentioned in connection with fruit rots in the literature. Previously, it was thought that the cause of anthracnose fruit rot or ripe rot on blueberries was *C. gloeosporioides*. Recent research has discovered that *C. acutatum* is more often related to this condition (55,56). *C. acutatum* has a diverse host range, and only a few extensive studies on its host tissue penetration and colonisation have been conducted (57-60). These findings suggest that the *C. acutatum* infection approach is determined by the host being colonised. Thus, *C. acutatum* functions as a subcuticular, intramural necrotroph on strawberry stolons and

Table 2. Morphological feature of Colletotrichum species. (39).

Species	Colony colour	LC	WC	SC	LA	WA	SA	Sclerotia	Setae
Colletotrichum acutatum	White, pinkish, gray and orange	8.5 to 10	4.5 to 6	Fusiform, medianly constricted	8.5 to 10	4.5 to 6	Irregular and clavate	Existing	Absent
Colletotrichum capsici	White, gray and dark green	18 to 23	3.5 to 4	Falcate, fusiform apices acute	9 to 14	6.5 to 11.5	Circular and clavate	Abundant	Absent
Colletotrichum coccodes	White mycelia	16 to 23	3 to 4	Fusiform medianly constricted	11 to 16.5	6 to 9.5	Long clavate and irregular	Existing	Abundant
Colletotrichum dematium	White to grey or dark brown	19.5 to 24	2 to 2.5	Falcate, fusiform apices acute	8 to 11.5	6.5 to 8	Circular and clavate	Abundant	Abundant
Colletotrichum gloeosporioides	Varied	9 to 24	3 to 4.5	Straight, obtuse at apex	6 to 20	4 to 12	Irregular and clavate	Diverse	Diverse

Note: **CL**=Length of conidia, Width of conidia, **SC**= shape of conidia, **LA**=Length of appressoria, **WA**= width of appressoria, **SA**=shape of appressoria

leaves, with no visible biotrophic phase, but in almond, the fungus appears to use both infection tactics depending on the tissue being colonised (59,60). The pathogen is expected to thrive on citrus as appressoria or biotrophically as quiescent infections on leaves throughout most of the year, but has a brief necrotrophic phase on blossoms (61,58).

Disease management

Since no effective control techniques have been developed so far, managing chilli anthracnose has been a hot topic for agriculturalists and farmers. The necessity for creating a sustainable strategy for preventing the disease's spread has been further exacerbated by the decline in chilli output and the decline in fruit quality. There isn't a single management strategy that can effectively control the disease. Generally speaking, it has been recommended to treat the disease by combining a number of management strategies, including chemical, biological, and physical control, as well as inherent resistance (47). The four main categories under which the management strategies for preventing *Colletotrichum* spp. from dispersing and forming a disease can be stated are: use of cultural practises, use of chemical control, use of resistant varieties, and lastly, use of biological control.

Use of Cultural Practices

In addition to being soil transmitted, the fungi are also seed, wind and water borne; therefore, strategies to restrict its spread should concentrate on three key aspects of disease and pathogen-free yield of crops in the field: appropriate water drainage, exclusion of any infested plant parts from the field, and non-host crop rotation. Water splashes can quickly transfer the pathogen's conidia from diseased to healthy plant sections. Additionally, favourable moisture percent supports the pathogen's effective colonization (3). Therefore, the land has to be properly irrigated and drained to stop the spread of the disease. Additionally, the plants should be spaced properly apart in order to prevent a thick canopy, which allows for the creation of moisture (27). Utilizing transplants grown from disease-free chilli seedlings is another crucial approach. It is important to keep transplanting and propagative material away from weeds and other host plants, such as solanaceous plants (62). It has also been claimed that plastic mulch and rice straw may be used to effectively manage the disease (63). Building resistance against the pathogen in the host is believed to be the most essential and long-term method of disease management. This technique not only removes disease losses, but also the mechanical and chemical costs of disease management (48). However, there has been little success in producing resistant chilli variations in the species *Capsicum annum* L., which is the sole species farmed worldwide (54). Due to the presence of several pathogen species in the *Colletotrichum chilli* pathosystem, the arduous task of resistance breeding is extraordinarily challenging.

Fungicides used in chemical form

The most effective approach considered chemical control to manage the spread of the pathogen in the absence of any precise control strategy. This approach of disease control, especially for anthracnose disease, is becoming more common due to the longer period necessary for growing the resistant variety and the fact that using fungicides is not an extensive, permanent solution (64). However, the hazardous remnants of the chemicals still present in the fruits make it difficult to export the anticipated chilli goods to other nations, which have an impact on the national economy. Additionally, reliance on a single chemical component causes pathogenic isolates to acquire resistance, which makes treating the condition more challenging (27). Traditionally, manganese ethylene-bis-dithiocarbonate acid (nabam) has been advised as a fungicide to manage the illness. Chemical fungicides based on benzimidazole, triazole, dithiocarbonates, and cooper compounds are typically advised for controlling anthracnose disease (65). Its control has also included the use of more recent substances such as strobilurin-based fungicides (such as azoxystrobin and pyraclostrobin). However, there are few instances of this type of fungicide being used to suppress chilli anthracnose in extensive field experiments (66,67).

By using chemical fungicides promptly within the crucial window that is favourable for the illness's emergence, the disease can be effectively controlled. Fungicides should typically be given to young, growing tissues like fruits, leaves, and flowers to prevent the disease from entering the plant system (64). But it is impossible to ignore the countless reports detailing the detrimental impacts of fungicide usage on farmers' health, financial situation, and toxin-filled environmental degradation, particularly in poor nations (20). The fungicides suppress the pathogen to keep it in an inactive state; it depends on the mode of action of the particular compound. As a result, farmers in a certain location should choose fungicides carefully and in accordance with the local environmental circumstances. The rotation of more than two distinct classes of fungicides and compounds is strongly advised to improve the likelihood of improved disease protection in the fields. (68). Chemicals used to target species of *Colletotrichum: C. capsici* was controlled with difenoconazole (69). *C. gloeosporioides* was controlled through Bavistin (70).

Management through botanicals and biological control

Recently, research into the potential for using medicinal plants' powerful antifungal and antibacterial characteristics to manage disease has increased (71,72). Their usage for regulating phytopathogens has become increasingly popular due to their simple disintegration, lack of residual action, and non-phytotoxic qualities (3). Several investigations on the management of *Colletotrichum* species on chilli have also been undertaken using unprocessed plant extracts (73). In order to regain the environment's lost balance, a sustainable biocontrol strategy for disease management is now recognized. Applying biocontrol agents is a specific technique that has not achieved much attraction for the management of the chilli anthracnose pathogen (74).

Biocontrol agent (Trichoderma against Colletotrichum spp.)

Trichoderma was well researched against ascomycete fungi. It is recognized as a saprophytic fungal pathogen with strong adaptation potential, as evidenced by its capacity to colonise wood, bark, agricultural wastes, and other surfaces, in addition to its omnipresence in a range of soil types (76). Its biocontrol ability against a wide range of key phytopathogens, including *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Alternaria*, *Colletotrichum*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotinia*, and *Verticillium* (75,78). The processes involved include mycoparasitism, antibiosis, competition for resources and space, and the potential to build systemic resistance in plants against pathogens (79-81). The application of *Trichoderma* species has also been linked to an increase in plant growth and a large rise in biomass (82,83). Recently, the fungus' capability to promote biotic tolerance in plants by strengthening the mechanical strength of the plant system against phytopathogenic infection has been examined (84).

With its quick colonization capacity and mycoparasitic character, which results in coiling and parallel development of the pathogen in the *Colletotrichum* plant pathosystem, its potential has been revealed (77). This trait has also been related to the production of extracellular enzymes like glucanases and chitinases, which destroy the pathogenic mycelia, limit its proliferation and colonization in the host tissue (79).

Current status future directions

Though the illness's epidemic character has been researched for centuries, many areas remain undiscovered in terms of host-pathogen interaction, disease propagation, and effective management options. There is an urgent need to establish an effective integrated management approach that takes into account the many environmental conditions and pathogenic resistances that drive pathogen colonisation in host tissues. An understanding of the virus's lifecycle would provide significant information for establishing targets for producing resistant chilli cultivars against the infection. Modifications to traditionally advised cultural practises tailored to a specific agroclimatic zone will also aid in disease management. More research is needed to have a thorough understanding of the pathogen's numerous mechanisms of infection and the pathogenic diversity related to areas with post-harvest and pre-harvest crop loss. A comprehensive understanding of the major parts of a disease triangle would allow for improved disease control while keeping track of the quality and quantity of crops produced, therefore contributing efficiently to the country's economy.

Conclusion

In summary, the significance of chili as a versatile spice crop with various applications has been highlighted. The presence of Colletotrichum capsici, causing anthracnose disease, poses a major challenge to chili production, resulting in extensive crop damage and economic losses. Over-reliance on chemical treatments is not sustainable due to their negative environmental and health impacts. Therefore, there is a critical need for integrated disease management strategies to combat this pathogen effectively. The early detection and identification of the pathogen through conventional and molecular methods play a vital role in minimizing yield losses under field conditions. This review provides a comprehensive understanding of the pathogen, its detection and identification techniques, as well as recent research advancements. By offering valuable insights, this review contributes to the development of sustainable management practices for anthracnose in chili crops, ensuring improved productivity and quality.

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Authors contributions

The authors of this review paper, Sanket Sudhakar Chavan, Ajay Chavan, Sumanth Kumar Reddy, Chandhrapati Akhilesh, and Siddhesh Kolhe, have made significant contributions to the study. Sanket Sudhakar Chavan contributed to the study's conceptualization and design, while Ajay Chavan provided valuable perspectives during the literature review. Sumanth Kumar Reddy played a crucial role in analysis, enhancing scientific rigor. Chandhrapati Akhilesh offered insights into practical implications, and Siddhesh Kolhe actively participated in writing and revising the manuscript. Collectively, their contributions have shaped the content and conclusions of the paper, enriching its overall quality.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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