



# **Etiology, Symptomatology and Management of Black Spot of *Aloe vera* (*Aloe barbadensis* Miller) through Botanicals and Biocontrol Agents – A Brief Review**

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Author MK collected the literature and wrote the first draft of the manuscript. Author MK will work on the management of black spot of *Aloe vera* during her PhD programme at Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar. Author PKJ is major advisor of author MK and he designed the study and protocols. Both authors read and approved the final manuscript.*

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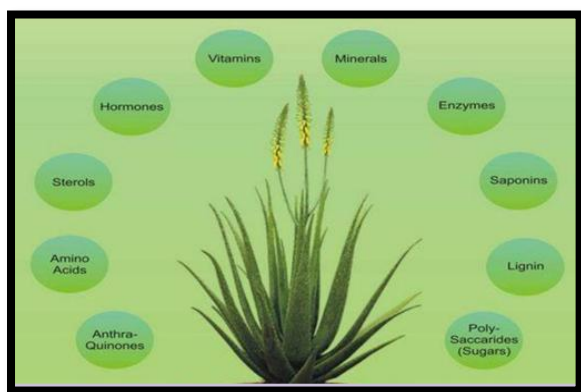
## **ABSTRACT**

*Aloe vera* is an important medicinal plant of Asphodelaceae (Liliaceae) family. It is widely used owing to its wound healing, anti-inflammatory, immunity, antidiabetic, antioxidant, laxative, antibacterial, antifungal and antiviral properties. Similar to other crop plants, *Aloe vera* is infected by several plant pathogens that deteriorate its yield and quality. Among various fungal diseases, black spot disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is the most serious disease which not only reduces the crop yield and market value but also the antioxidant properties and other medicinal efficacy of the plant. *C. gloeosporioides* is commonly controlled by chemical fungicides but due to the limitations of applying chemical control methods on medicinal plants, there is a need to look for some safe alternatives. The objective of this paper is to review the previous work on etiology, symptomatology and management of *C. gloeosporioides* (Penz.) Penz. & Sacc. through botanicals (natural plant extracts), biocontrol agents and some safe and ecofriendly chemicals like Azoxystrobin.

**Keywords:** *Aloe vera*; black spot; *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.; biocontrol agents; botanicals.

## 1. INTRODUCTION

*Aloe vera* (*Aloe barbadensis* Mill. = *A. vera* L.) is an important medicinal plant. The name *Aloe vera* has been derived from the Arabic word “Alloeh” which means “shining bitter substances” while “vera” in Latin means “true” [1]. According to Adodo [2], over 325 species of the genus *Aloe* have been identified and some of the common varieties of *Aloe vera* are: *Aloe barbadensis* Miller, *A. saponaria*, *A. chinensis*, *A. variegata*, *A. forex*, *A. latifolia* and *A. curacao*. However *Aloe barbadensis* is more popular all over the world because it propagates faster than any other known species and has more therapeutic value and hence referred as ‘True Aloe’.



**Figure. 1. Constituents of *Aloe vera* plant**

The aloe plant is an important and historical medicinal plant, often known as “Doctor in pot” [3]. It is known as “miracle plant” because it contains more than 200 constituents beneficial to humans. No other herbs in nature contain so many different elements beneficial to human. It is also known as “nature’s tonic” as it is extremely rich source of polysaccharides that store energy and sustain energy levels to keep one active throughout the day. Phytochemical studies of *Aloe vera* gel have revealed the presence of more than 200 active substances including vitamins, minerals, enzymes, sugars, anthraquinones, lignin, saponins, sterols, amino acids and salicylic acid [4]. Owing to sky rocketing prices and known side effects of allopathic medicines, medicinal plants and ayurvedic medicines are becoming popular. Recently due to the commercialization and high demand of natural products, aloe has become the ingredients of choice in several skin and health products.

For the maintenance of good health, many herbal drugs and drinks have been formulated from *Aloe vera* plants. Many of the health benefits associated with this plant have been attributed to the polysaccharides contained in the gel of aloe leaves. *Aloe vera* gel has been used as a natural remedy to sooth the pains of burns, rashes, insect bites and other skin irritations [5,6]; to cure ringworm and eczema [7], for treatment of sore and wounds, skin diseases, reduce blood sugar and diabetes, arthritic swelling, constipation, piles, liver and kidney problems, asthma, ulcer, jaundice and fungal infection [8,9,10]; and has a modulating effect on human immunodeficiency virus-HIV [2]. Further, the anti-inflammatory [11,12,13] and antioxidant [14,15] potentials of *Aloe vera* have also been established. In the pharmaceutical industry, it is for the manufacturing of topical products such as ointments and gel preparations, as well as in making tablets and capsules.

*Aloe* is a very hardy perennial plant best suited for cultivation in arid and semi-arid regions. It is cultivated in Andhra Pradesh, Rajasthan, Gujarat, Madhya Pradesh, Maharashtra, Chhattisgarh, Karnataka, Tamil Nadu, Bihar, West Bengal, and Jharkhand in India. It is native to Africa & Mediterranean regions. Africa, China, Unites States, Australia, Mexico, tropical regions of Latin American countries and coastal region of south India are the major producers of *Aloe vera* in the world.

Due to increasing demand of *Aloe vera* in pharmaceutical industry, emphasis is being given to bring this crop in cultivation at large scale. This crop has been identified as potential crop of Bihar by National Medicinal Plant Board (NMPB) due to favorable climatic condition of this state. Government of Bihar has also placed this crop as a priority crop in Bihar Krishi Road Map.

## 2. BLACK SPOT DISEASE OF *Aloe vera*

Like others, *Aloe* plants are also attacked by a number of fungal and bacterial pathogens which cause numerous diseases. Besides bacteria, fungal pathogens are more likely to be associated with *Aloe vera* both in natural and artificial conditions and cause diseases like leaf spots, collar and root rot which affects the growth, development and therapeutic potential of the plant [16]. *Aloe vera* plant is prone to these

diseases due to lack of knowledge on scientific cultivation techniques and favorable abiotic factors, which favour the pathogen growth. As the Aloe plants are generally used in medicine and cosmetics [5,6], the contamination with fungal pathogen can have a large impact on human health. Some fungal pathogens produce mycotoxins in their infected hosts and substrates on which they grow [17] and these mycotoxins are harmful for human and animal health.

Aloe plants are commonly affected by leaf spot diseases caused by different pathogens and the diseases have been reported from India and other countries caused by *Fusarium* spp. [18]; *Fusarium phyllophilum* [19]; *Alternaria alternata* (Fr.) Keissler [20]; *Alternaria alternata* [21]; *Alternaria tenuissima* [22]; *Colletotrichum gloeosporioides* [23]; *Phoma betae* [24]; *Curvularia lunata* and *C. ovoidea* [25]; *Fusarium oxysporium* [26]; *Nigrospora oryzae* [27]; *Sphaeropsis sapinea* [28] and *Phomopsis* sp. [29].

Black spot disease of *Aloe vera* is caused by the pathogen *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. Occurrence of this disease on Aloe leaves caused by *C. gloeosporioides* was earlier reported by Cedeño et al. [30] in Venezuela and Avasthi et al. [23] in India. This disease has also been reported from Bihar (Annual Report AICRP-MAPB, 2017-18). During visit of medicinal plant garden of Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, incidence of this disease has been frequently observed.

### 3. PATHOGEN

*Colletotrichum* is one of the most important phytopathogens worldwide causing an economically important disease anthracnose/black leaf spot disease in a wide range of hosts with characteristic symptoms. *Glomerella cingulata* is the sexual stage while the asexual stage is known as *C. gloeosporioides*. *G. cingulata* occurs on a broad range of host species producing acervuli within the host tissue during asexual phase of its life cycle.

The mycelium of the fungus consists of numerous, sparsely septate hyphae which are initially hyaline but later become slightly dark colour. The characteristic feature of the fungi is that their conidia are born on asexual fruiting body called acervuli, which is small, flask shaped

structures with cushion-like masses of conidiophores. Brown colour, long hair or spine like structure called setae are also emerges from acervuli. The conidia are hyaline, one-celled and ovoid to oblong. *Colletotrichum* species naturally produce micro-sclerotia to allow dormancy in the soil during the winter or when subjected to stressful conditions and these micro-sclerotia can survive for many years. *Colletotrichum* species produce a series of specialized infection structures such as germ tubes, appressoria, intracellular hyphae and secondary necrotrophic hyphae [31].

### 4. HISTORY

The generic name *Colletotrichum* was introduced by Corda (1831) for *C. lineola*, a species associated with a member of plants in the family *Apiaceae*, in the Czech Republic. The pathogenic *C. gloeosporioides* was proposed for the first time as *Vermicularia gloeosporioides* by Penzig in 1882. From India it was first reported by Butler in 1918 on coffee leaves [32]. After that, McRae (1924) reported it as the causal pathogen of mango anthracnose.

Detailed scientific studies on *C. gloeosporioides* in India progressed throughout last century and are still in continuation. Traditionally the identification and characterization of *Colletotrichum* spp. relied on differences in morphological features such as colony color, size, and shape of conidia and appressoria, optimal temperature for growth, growth rate, presence or absence of setae. Now molecular techniques provide alternative methods for taxonomic studies and are important tools in solving the problem of species delimitation [33]. There are various species under genus *Colletotrichum* but only *C. graminicola* and *C. higginsianum* genomes were completely sequenced. *C. gloeosporioides* genome is under study but various genes have been identified which involve in pathogenesis and host defense mechanism.

### 5. MODE OF INFECTION

Studies have shown that the ubiquitous species is common in the tropics either as a primary disease-causing organism (parasite), isolated from deteriorated plant parts (saprophyte) or live inside plant tissue asymptotically as endophytic fungi [34,35,36]. *C. gloeosporioides* follows the hemibiotrophic mode of infection where, biotrophic and necrotrophic phases

sequentially occur. During the infection, initially the pathogen establishes an interaction with host by producing a melanized appressorium and then penetrates the host cuticle. After penetration, infection vesicles and primary hyphae are formed. This stage of infection is called biotrophic phase. Later, necrotrophic secondary hyphae develop and spread causing the death of host cells [37].

## 6. SYMPTOMS

The initial symptom of the disease is the appearance of small round to oval, dark green water-soaked lesions which later become circular spots with tan to light brown center. As the spots mature the center of the lesion becomes reddish brown to brown color. With progress in disease the lesions join together to form big necrotic area. The disease causes loss of mucilaginous gel and death of the leaves. Disease is favored by warm and wet weather, spreads easily during wet weather by water splash [23].

## 7. DISEASE CYCLE AND EPIDEMIOLOGY

The fungus prefers warm and humid environment for spreading the disease uniformly and effectively [38]. The fungus primarily invades the injured or weakened tissues of plants, produces several specialized structures during infection process viz. conidia, acervuli, setae and appressoria. *C. gloeosporioides* colonizes and injures plant tissues and forms number of acervuli and conidia. The pathogen survives or overwinters on the infected plant debris. When it finds favorable environmental conditions, it produces acervuli in lesions or infected plant tissues containing spores/conidia. These conidia spread over relatively short distances by rain splash or overhead irrigation and infect other healthy plant tissues. Infection ends up in development of black, sunken, rapidly expanding lesions on leaves. The whole infection process, including the formation of conidia, acervuli, setae and appressoria, and infection results into necrosis of infected tissues.

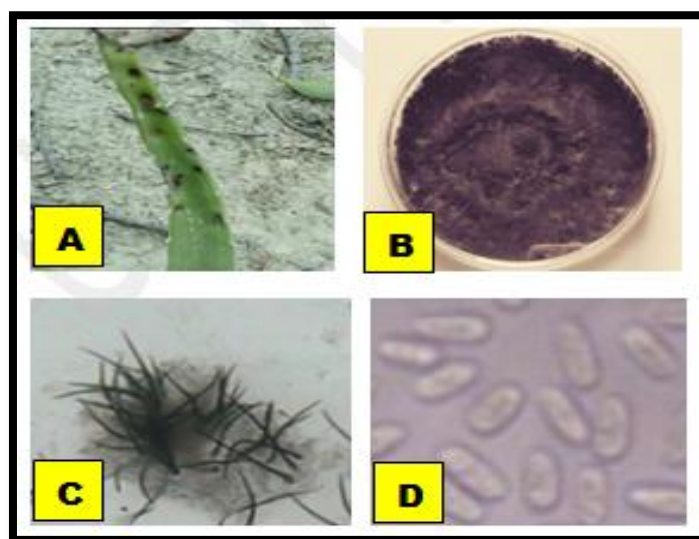


Figure. 2. A) Black spot symptoms on *Aloe vera* leaf, B) 30 days old culture of *C. gloeosporioides*, C) Setae (hair or spine like), D) Conidia



Figure. 3. Symptoms of black spots on *Aloe vera* plant

## 8. DISEASE MANAGEMENT

Limited study has been undertaken on disease of *Aloe vera* and their management. Since the use of toxic fungicides for management diseases in *Aloe vera* is not advocated, the most favourable option is to use effective botanicals and biocontrol agents to suppress the disease. In the present paper an attempt has been made to compile available reports of earlier studies by different investigators on use of botanicals and biocontrol agents against *C. gloeosporioides* (Penz.) Penz. & Sacc. causing infection on different horticultural crops including *Aloe vera* and other medicinal plants.

## 9. BIOCONTROL AGENTS

Biocontrol potential of *Trichoderma viride*, *Beauveria bassiana*, plant growth promoting rhizobacteria (PGPR) and some other microorganisms along with different phytoextracts against *C. gloeosporioides* were evaluated in past by various researchers. Deshmukh et al. [39] evaluated several bioagents such as *Trichoderma harzianum*, *T. viride*, *T. longibrachyatum*, *Gliocladium virens*, *Chaetomium globosum*, *Pseudomonas fluorescens*, *Aspergillus niger*, and *Bacillus subtilis* to monitor their antagonistic effects against *C. gloeosporioides* (Penz.) Penz. & Sacc. causing anthracnose in Indian bean (*Lablab purpureus* L). They reported that among all the 8 bioagents three bioagents significantly inhibited the growth of pathogen mycelia, *T. viride* by (60.69, 58.57, 63.83), *T. harzianum* by (58.12, 52.27, 58.87) and *A. niger* by (55.56, 50.46, 57.44) per cent. Ghosh and Chakraborty [40] reported that five isolates of *Trichoderma viride* and two isolates of *Beauveria bassiana* were antagonistic to *C. gloeosporioides* causing anthracnose of Sargandha (*Rauvolfia serpentina*). Antagonistic effect of yeast (*Meyerozyma caribbica*) against *C. gloeosporioides* in mango was evaluated. *M. caribbica* showed significant inhibition of anthracnose (86.7%). The result showed that the combination of the different mechanisms of the yeast increases their ability to control *C. gloeosporioides* [41]. The mechanisms of action and effectiveness of the antagonistic yeast (*Cryptococcus laurentii*) [(Kuff.) C. E. Skinner] strain L5D against *C. gloeosporioides* (Penz.) Penz. & Sacc. causing anthracnose in mango was examined. They observed a high antagonistic potential *in vivo* with significant inhibition of anthracnose (75.88%) [42]. Antagonistic activity of two species of yeasts

(*Candida intermedia* and *Wickerhamomyces anomalus*) against *C. gloeosporioides* (Penz.) & Sacc. causing anthracnose in Avocado was studied. The results showed that *W. anomalus* caused a significant reduction of incidence and severity of anthracnose [43]. An investigation was carried out to find the effect of volatile and non-volatile compounds produced by native *Trichoderma* spp. on *C. gloeosporioides* causing anthracnose on *Aloe vera*. The results showed that the volatile compounds produced by all the *Trichoderma* isolates showed inhibition of 24 to 47% of *C. gloeosporioides*. However, non-volatile compounds or culture filtrate at 7.5% and 15% showed 15-29% and 26-39% respectively [44].

## 10. BOTANICALS AS PESTICIDES

The application of plant extracts as biopesticides acts as a vital component for the management and control of plant diseases. It is a novel, safe and easy application against plant pathogens. Besides natural origin botanicals are valued for their biodegradability and low environmental persistence. It is now well documented that phytoextracts induce resistance in plants against many pathogenic fungi [45]. Many workers have reported antifungal activities of different plant species and stressed the importance of plant as a possible source of natural fungicides. Different plant species namely, *Allium sativum* bulb, *Zingiber officinale*, *Mangifera indica*, *Curcuma longa* and *Lantana* leaves, *Cannabis sativus*, *Azadirachta indica*, *Tagetes erecta* and *Annona squamosa*, had been screened for their antifungal activity against *C. gloeosporioides* [46-50]. Ogbemor et al. [51] evaluated the antifungal effects of extracts from five selected plants against the mycelial growth as well as conidial germination of *C. gloeosporioides* causing leaf spot of rubber plant and reported that among the all selected plants, *Ocimum basilicum* showed the highest effectiveness followed by *Allium sativum*. They reported that treatment with 100% *O. basilicum* resulted in disease index of 31.7% significantly lower than the control 65% disease index at 5% level of probability. Similarly, antifungal effects of some plant extracts such as tobacco leaf, Keora seed, giant Indian milky weed, garlic and ginger at different concentration (30%, 40%, 50%, 60% and 70%) was evaluated by Mukherjee et al. [52] on growth and development of causal organism of mango anthracnose (*Colletotrichum gloeosporioides*) and observed highest growth inhibition by garlic extracts at 70% concentration. Garlic extracts at 50% and 60% concentration were also effective

than other treatment. Prapassorn et al. [53] reported that the inhibition of mycelium growth and spore germination of *C. gloeosporioides* (Penz.) Penz. & Sacc. was achieved by application of crude methanol extract of *Piper sarmentosum* leaves and crude chloroform extract of *Mentha cordifolia* leaves.

Essential oils have been shown to exert several biological activities including antibacterial and antifungal actions. Barrera-Necha et al. [54] evaluated efficacy of nine essential oils *in vitro* on conidial germination and mycelia growth inhibition of *C. gloeosporioides* isolated from papaya (*Carica papaya* L.). They observed strong antifungal activity on conidial germination with *Cinnamomum zeylanicum* and *Syzygium aromaticum* at 50, 100, 150, 200 and 250  $\mu\text{g mL}^{-1}$ . They also found lowest infection percentage on papaya fruit during storage at 28°C and 14°C when treated essential oils of *S. aromaticum* at 50  $\mu\text{g mL}^{-1}$ . So, *S. aromaticum* oils may be a possibility to control *C. gloeosporioides* on papaya fruits. Anaruma et al. [55] studied the activity of 28 essential oils from medicinal plants against *C. gloeosporioides* (Penz.) Penz. & Sacc. causing anthracnose disease in yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa*), as well as evaluated their effects in the control of post-harvest decay. They reported that the disease index of the samples treated with the essential oil obtained from *Cymbopogon citratus* did not differ ( $P \leq 0.05$ ) from that of the samples treated with fungicide.

## 11. ECOFRIENDLY FUNGICIDES

Azoxystrobin is a novel fungicide, discovered during research on *Oudemansiella mucida* and *Strobilurus tenacellus*, which are small white or brown coloured mushrooms commonly found in European forests. Azoxystrobin is a systemic, broad-spectrum fungicide highly effective at very low doses and considered safe for environment, so may be used on medicinal plants. This fungicide was first introduced in 1998. Azoxystrobin has been evaluated for its effectiveness both *in vivo* and *in vitro* and was found to completely inhibit mycelia growth. Azoxystrobin at 1, 2 and 4 ml/l suppressed the development of both panicle and leaf anthracnose and observed total control of mango anthracnose. Azoxystrobin treated mango tree produced more fruits as compared to control and showed no phytotoxicity [56]. Chitosan loaded nanoemulsions was evaluated as a natural product to control anthracnose in dragon fruits caused by *C. gloeosporioides* (Penz.) Penz. &

Sacc. Chitosan was used as nanoemulsions of different droplet sizes (200, 400, 600, 800 and 1000 nm) at 1.0% concentration and evaluated *in vitro* for controlling conidia growth, dry weight of mycelia and sporulation of the pathogen. They observed that chitosan at 1.0% concentration at 600 nm droplet size given best results in terms of inhibiting conidial germination, reducing dry weight of mycelia as well as sporulation [57].

## 12. INTEGRATED DISEASE MANAGEMENT

To reduce the harmful effect of chemical pesticides on human and environment, integrated plant disease control strategies are developed by combining compatible bioagents, botanicals and chemical agents. Different effective botanicals, fungicides and biocontrol agents were evaluated to manage anthracnose in papaya caused by *C. gloeosporioides*. They found extracts of *Lantana camara* was superior botanical agent than the turmeric extract against the test pathogen. Among 10 tested fungicides, carbendazim found most effective and showed maximum mycelia inhibition of *C. gloeosporioides* at all concentration and among the biocontrol agents, *T. virens* found superior of all the tested biocontrol agents [58]. An *in vitro* and *in vivo* investigation was carried out to elucidate the efficacy of PGPR, bioagents and botanicals in the management of anthracnose of pomegranate. A significant difference in antifungal activity of bioagents was observed. *Trichoderma viride* (79.1%) was found to be more effective in inhibiting the mycelia growth. Bacterial bioagents, *Pseudomonas fluorescens* shown maximum mycelia growth inhibition (54.8%). Among plant extracts maximum 93.4% and 88.1% inhibition of mycelia growth was observed with eucalyptus extract followed by garlic extract at 10% concentration. The best performing bioagents and botanicals under laboratory condition were tested in field condition for management of anthracnose of pomegranate. The result shown minimum disease index in treatments with *T. viride* (18.9%) with highest yield (7290 kg/ha) in field condition [59]. An *in vitro* study was conducted for controlling *C. gloeosporioides* (Penz.) Penz. & Sacc. causing inflorescence die back in arecanut by combining two compatible bio agents, *Trichoderma virens* and *T. viride* and two fungicides viz. Blitox 50 W and Mixol 72. The treatments shown significant inhibitory effect on the growth of pathogen and also reduced colony diameter. Highest percent inhibition was observed when 0.05% of Mixol 72

was used with *T. virens* (87.61%). They reported that the combination of bioagents with fungicides provided higher disease suppression than achieved with fungicides and bioagents when used alone [60]. *In vitro* efficacy of biocontrol agents and fungicides were tested against *C. lindemuthianum* (Sacc. and Magn.) causing anthracnose in French bean. They observed that carbendazim alone and in combination (carbendazim + *Pseudomonas fluorescens*) showed complete inhibition of *C. lindemuthianum* at 100 ppm, while among three biocontrol agents, *Trichoderma harzianum* shown maximum growth inhibition of pathogen [61].

### 13. FUTURE STRATEGIES

Popularity and demand of Aloe vera is increasing day by day among the people. It is cultivated commercially throughout the world due to its distinctive importance in medicinal and cosmetics industry. In India farmers can sell their products direct to the herbal manufacturing companies as it is demanded by herbal pharma and cosmetic industries like Patanjali, Himalaya, Dabur and Baidhnath etc. Its cultivation is also being popularized by National Medicinal Plant Board (NMPB) and other government agencies in the country and hence the area under *Aloe vera* is gradually expanding and consequently the incidence of black spot disease may also increase due to inoculum build up. Therefore, there is an urgent need to develop ecofriendly management strategies using botanicals and biocontrol agents. In view of importance of the disease the first author has taken up the following aspects during her PhD programme at Dr. Rajendra Prasad Central Agricultural University (DRPCA), Pusa, Samastipur in order to workout effective and ecofriendly management strategies against black spot of *Aloe vera*.

1. To identify potential native biocontrol agents in the rhizosphere and phylloplane of *Aloe vera* under agroclimatic condition of North Bihar for management of *Colletotrichum gloeosporioides*, causing black spots of *Aloe vera*.
2. To study the compatibility of various effective botanicals and ecofriendly molecules with the potential bio control agents.
3. To study the integrated disease management (IDM) strategies for the management of black spots of *Aloe vera* using potential native biocontrol agents, compatible and effective molecules and botanicals.

### 14. CONCLUSION

*Aloe vera* is an important medicinal plant. It is highly demanded in Indian as well as in International market. Black spot is a potential problem for growers throughout the country. So, management of this disease is very important as it reduces the quality, yield and market price of *Aloe vera*. The aloe plants are generally used in medicine and cosmetic industries, contamination with pathogen will have very harmful effects on human health. Some fungal pathogen produces mycotoxins in their infected hosts and on substrates on which they grow. Application of various fungicides to combat plant diseases are quick and easy methods, but this approach is associated with several demerits such as pathogen resistance, pathogen resurgence, effects on non-target organisms, and ecological and human health hazards and also on pharmacological important constituents of *Aloe vera*. There are also chances of pesticides residues in plant materials treated with chemicals. So, these and many other factors leads to develop some better alternatives which will be safe and ecofriendly in management of *C. gloeosporioides*. Exploitation of several biocontrol agents and plant extracts for controlling *C. gloeosporioides* may be less expensive, safer to the users as well as environment and could also serve as a better alternative to synthetic fungicides. So, the integrated approach which combines compatible biocontrol agents, plant products and ecofriendly chemicals can be exploited in future for the management black spot of *Aloe vera*.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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