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STUDIES OF BLACK SPOT DISEASE AND ITS MANAGEMENT IN *ALOE VERA* (*ALOE BARBADENSIS* MILLER)

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ABSTRACT

The present investigation was carried out to study the effectiveness of the native biocontrol agents, botanical and eco-friendly fungicides for the management of black spot in *Aloe vera* (*Aloe barbadensis* Miller) incited by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. In course of study, 15 fungal isolates, obtained from the phylloplane and rhizosphere of *Aloe vera* were evaluated for their effect on plant health under pot condition. The beneficial mycoflora which promoted plant growth in pot condition, were also found effective in suppressing the pathogen *in vitro*. Among them *Trichoderma harzianum* exhibited maximum pathogen growth inhibition (76.19%) followed by *T. viride* (73.59%). Out of nine botanicals, neem leaf extract was found superior in checking the pathogen growth by 74.82% followed by tulsi (59.78%) at 10% concentration. Among two fungicides tested, carbendazim was most effective and inhibited the pathogen growth by 93.52% followed by azoxystrobin (93.07%) at 250 ppm, but azoxystrobin was found more compatible with the bio-control agent. During integrated management trial, the most effective treatment was foliar spray with azoxystrobin @ 250 ppm accompanied with soil drenching of *T. harzianum* 5% resulted in 82.63% reduction in PDI. The foliar spray of azoxystrobin @ 250 ppm accompanied with soil drenching of *T. harzianum* @ 5 % and further foliar spray with neem extract @ 10% also showed promising disease reduction over control (81.40%).

***Key words*:** *Aloe vera*, *Colletotrichum gloeosporioides*, Phylloplane, Rhizosphere, Botanicals, fungicides.

Introduction

Aloe vera is an important medicinal plant of Asphodelaceae (Liliaceae) family originated in Africa & Mediterranean regions, also known as Ghrit Kumari, Kunvarpathu and Indian Aloe. It can be used in three forms *viz.*, aloe gel, aloe latex and the whole leaf extract. The colorless mucilaginous gel obtained from its leaves has been widely utilized for pharmacological and cosmetic applications (Sánchez *et al.*, 2020). It is a succulent, drought tolerant and short-stemmed plant, growing to 60-100 cm tall, spreading offsets. The leaves are thick, fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower leaf surfaces. The margin of the leaf is serrated and has small white teeth (Kumar and Yadav, 2014; Lakshmi & Rajalakshmi, 2011). The flowers are produced in summer on a spike up to 90 cm tall, each flower being pendulous with a yellow tubular corolla 2-3 cm long (Raksha *et al.*, 2014). The plant is widely distributed in hot and arid regions, including North Africa, the Middle East, the southern Mediterranean region, and the Canary Islands. Among approximately 420 species of *Aloe*, *Aloe barbadensis* Miller is considered one of the most important species, containing around 75 nutrients and over 200 bioactive compounds (Kumar *et al.*, 2024). It possesses several functional properties, including antimicrobial and antioxidant activities, making it effective in wound healing, diabetes management, immunomodulation, anti-aging treatments, and various skin-related disorders. The leaves, which are primarily used for medicinal purpose, consist of three

distinct parts: latex, gel and rind, each containing diverse phytochemicals (Matei *et al*., 2025).

Like other plant it is also affected by several diseases like leaf spot, base rot, rust, black spot, black spot, soft rot etc. Among these diseases, black spot caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc is considered very serious as it reduces the quality and quantity of Aloe gel and thereby reduces its market value (Chandel *et al*., 2025). The symptoms of black spot appear as a small round to oval, water-soaked area about 1-2 mm in diameter. These areas increase into circular spots with tan to light brown centre bordered by water soaked tissue. As these spots expand, the centre of the lesion become reddish brown to brown color. The acervuli on infected leaves produced black coloured spore mass under high humid condition. In the advance stage of infection, spots appear on both the surfaces of leaf, affected area loose the mucilaginous gel and ultimately leads to the death of infected leaves (Avasthi *et al*., 2011). Hot and humid weather favors the pathogen development. It is more serious during rainy season as its conidia are dispersed easily by rain splash and cause severe disease (Kamle & Kumar, 2016).

As the plants are generally used as medicine and cosmetics (Heggers, 1996; Olusegun, 2000), the contamination with fungal pathogens may cause adverse effect on human health. Some fungal pathogens and non-pathogens produce mycotoxins in their infected hosts and substrates on which they grow (Husaini *et al*., 2009) and these mycotoxins are hazardous to human and animal health (WHO, 1979). Among various options for the management of plant diseases, use of bio-control agents (preferably native bio-control agents), is considered more appropriate, eco-friendly and sustainable particularly in case of medicinal and aromatic plants due to limitation in use of chemicals pesticides on these crops.

Plant health is largely influenced by the presence of various micro-organism in the rhizosphere and phylloplane. Rhizosphere is regarded as the zone of microbial proliferation in and around roots, harbors a large microbial population than the non-rhizospheric soil owing to the release of large amounts of organic carbon by the plant roots (Broeckling *et al*., 2008). Most rhizosphere fungi act as saprophytic organisms and could develop mutualistic associations with host roots as well. Likewise, the phylloplane is a complex terrestrial habitat of a numerous microorganisms including bacteria, filamentous fungi and yeasts that are growing on the surface of leaves (Al Nuaimy & Hawar, 2024). Considering the huge losses to quantity and quality of Aloe gel due to black spot, the present study was conducted to identify the resident bio-control agent against the pathogen and to work out the eco-friendly management strategy against the disease by using the resident bio-control agents, effective botanicals and safe fungicides.

Materials and Methods

Isolation, purification and identification of pathogen

For isolation of the pathogen, the diseased leaves were collected and brought to laboratory, thoroughly washed in running tap water and then small bits (2-3 mm) were cut from diseased portions along with adjoining healthy tissues with the help of sterile razor blade then surface sterilized by dipping in 0.1% mercuric chloride for 30-60 seconds followed by three washings in sterilized distilled water. The disinfested leaf bits were blotted between sterile Whatman No.1 filter papers and aseptically plated in PDA plates (3 pieces per plate). These inoculated plates were incubated in Biological Oxygen Demand (BOD) incubator at 25 ± 2°C for the growth of the pathogen. After 2- 3 days, fungal growth appeared as white to grayish white on PDA plates. The mycelia growing from the tissues were transferred onto fresh PDA medium amended with 1.0 mg/ml streptomycin sulfate and sub-cultured repeatedly until pure cultures of the isolates were obtained. The identification of the fungus was done on the basis of the morphological (under microscopes) and cultural characters.

The pathogenicity of the isolated fungus on *Aloe vera* plant was established by following the standard methodology. The pathogen was cultured on PDA medium for 8-10 days at 28±2°C in BOD incubator. Conidial suspension of pathogen was prepared and sprayed with conidial suspension of pathogen on artificially pricked upper surface of healthy *Aloe vera* leaves by sterilized needle in greenhouse conditions. Leaves sprayed with sterile distilled water served as control. Plants were covered with polythene bag to create sufficient humidity. After 48 hours, polythene bag was removed. Observations were taken after five days onwards. After 10 days of inoculation, disease symptoms appeared on the leaves. The same fungus was re-isolated from the inoculated plants which confirmed the pathogenicity of the fungus. These cultures were kept for further experiments.

Isolation, purification and identification of mycoflora from phylloplane and rhizosphere of *Aloe vera*

Isolation and identification of phylloplane mycoflora:

The phylloplane fungi were isolated from leaves by leaf washing technique, involved leaf washing under running tap water, cut into small pieces (2-3 cm) then

placed in one-liter conical flask containing 500 ml of distilled water and flask was vigorously shaken. From this suspension serial dilution was made up to 10^{-5} dilution. Thereafter, 1 ml of each dilution was transferred into Petri plates containing Rose Bengal Agar Base medium and kept in BOD for incubation at 28±2°C for seven days. After one-week, individual fungal colonies were picked from the edge of the growth and transferred onto fresh PDA plates amended with 1.0 mg/ml streptomycin sulfate. Pure culture obtained after repeatedly sub-culturing were subsequently maintained on PDA slants for further studies. The fungal isolates were identified on the basis of their morphological and cultural characters as per standard mycological manuals (Alexopoulus *et al*., 2002; Barnett and Hunter 1999). The published literature was also used to identify the fungi (Choudhary *et al.*, 2000).

Isolation, purification and identification of rhizosphere mycoflora:

Rhizosphere mycoflora were isolated by serial dilution method (Sinclair and Dhingra, 1985). Five healthy plants were uprooted, and then soil adhering to the roots was separated and allowed for shade drying. 10 g of rhizosphere soil was added in 90 ml of sterilized distilled water and dilution up to 10^{-5} was prepared, one ml of each dilution was plated on Petri dishes containing Rose Bengal Agar Base medium and incubated for seven days at 28±2°C in BOD incubator. Three replications were maintained for each dilution. The emerging fungal colonies were picked individually and transferred to PDA slants and purified by hyphal tip method. The identity of the fungal isolates was established by observing their morphological and cultural characters. Each fungal isolate, was mounted on the sterile slides, then stained with lacto-phenol/cotton blue and examined in 40X light microscope. The observations were made on characters like spore shape and spore type, colony color and growth rate using standard manual (Alexopoulus *et al*., 2002; Barnett and Hunter, 1998). Finally, the published literature was also used to identify the fungi (Choudhary *et al.,* 2000).

Effect of phylloplane and rhizosphere mycoflora on plant under pot condition:

Pylloplane and rhizosphere mycoflora were evaluated for their effects on plant under pot condition in greenhouse. Top garden soil (0-20 cm depth) collected from field was used in pots for growing young *Aloe vera* plants and inoculation of phylloplane and rhizosphere fungal isolates to assess their effects on plant health. These inoculated plants were maintained in greenhouse under prevailing condition of temperature (28±2°C) and light. Plants were regularly observed and data on number of plantlets (suckers per plant), plant height, fresh and dry weight (shade drying) of leaves and roots was recorded after 120 day of planting. On the basis of their effects on plants they were categorized into beneficial and pathogenic ones (Kumari and Jha, 2021).

Evaluation of antagonistic activity of beneficial mycoflora from phylloplane and rhizosphere against the target pathogen:

Each isolate of beneficial mycoflora from phylloplane and rhizosphere, were tested for their antagonistic activity against the pathogen using the dual culture method as described by Sinclair and Dhingra (1985). A mycelial disc (5 mm) cut from 7-day old actively growing culture of each of the isolated mycoflora was placed at 1 cm distance from the edge of the Petri plates. Similarly, a mycelial plug obtained from 7- days old pathogen culture was placed at the opposite side, 1 cm away from the edge of Petri plate and the control plate was prepared only with the pathogen mycelial disc at the centre of plate. All the plates were then incubated for seven days at 28±2°C. The percentage inhibition was calculated by using the following formula suggested by Ramesh *et al*., (1995):

$$I = \frac{R1 - R2}{R1} \times 100$$

Where,

I = Percent inhibition of pathogen growth by antagonists,

R1 = Radial growth of the pathogen (mm) in control plate,

R2 = Radial growth of the pathogen (mm) in the treatment plate (dual culture).

Evaluation of various botanical against the target pathogen under *in vitro* condition

Preparation of Botanical extract:

Botanical extract was prepared from fresh leaves of Brahmi (*Bacopa monnieri*), Mandookparni **(***Centella asiatica*), Neem (*Azadirachta indica*), Sarpgandha (*Rauvolfia serpentine*), Ashwagandha *(Withania somnifera*), Pipli (*Piper longum*), Tulsi (*Ocimum sanctum*), Bael (*Aegle marmelos*) and Oak (*Calotropis gigantea*) collected from Herbal Garden, RPCAU, Pusa, Samastipur. Leaf extracts was prepared by cold water extraction method described by Shekhawat and Prasad (1971). 100 g leaves of each plant was washed separately with distilled water. Washed leaves were chopped into small pieces with sterilized sharp knife. Each sample was separately grounded and homogenized in mechanical grinder by adding 100 ml sterilized distilled water 1:1 (w/

v). The collected extract was first strained through two layer of muslin cloth so as to remove the fibrous and suspended materials and again filtered through Whatman No. 1 filter paper. The clear leaf extract obtained was considered as the stock solution of 100% concentration.

***In vitro* evaluation of botanical:**

Nine botanical were evaluated for their efficacy against the target pathogen by poisoned food technique (Nene and Thapliyal, 1993). In this technique, from the stock solution of each botanical extract 5, 10, 15 and 20% concentration was prepared. The PDA amended with botanical extract was poured separately into sterilized Petri plates @ 20 ml per plate under aseptic conditions and after solidification, each plate was inoculated by placing 5 mm diameter mycelial disc from seven day-old culture of pathogen at the centre of each Petri plate. Suitable control was maintained where the culture disc is grown under the same conditions on PDA without botanical extract. Three replications were maintained for each plant extract. These inoculated plates were incubated at 28±2°C for seven days. The colony diameter of the test pathogen was measured and compared with check was taken as measure of inhibitory effect of plant extract and inhibition over control (I) was calculated by following the formula of Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent inhibition over control

C = Mean maximum radial growth of colony in control plate (mm)

T = Mean maximum radial growth of colony in treatment plate (mm)

***In vitro* evaluation of fungicides against the target pathogen:**

Two fungicides (Carbendazim and Azoxystrobin) were tested against *C. gloeosporioides* by employing poisoned food technique. Stock solution of the selected fungicides was made by adding requisite amount of molecule in sterilized water. Appropriate quantity of the stock solution of molecule was added to the PDA medium (luke warm) so as to get desired concentration (250, 500 and 750 ppm) of each molecule in conical flask and poured into sterilized Petri plates at luke warm condition. Simultaneously, a control was also maintained by growing test fungus on PDA medium only. Three replications were kept for each treatment. Each plate was inoculated with a 5 mm mycelial disc of the pathogen taken from 7-day-old culture at the centre and the inoculated plates were incubated at 28±2°C. The colony diameter of the test pathogen grown on PDA was measured when the plates under control were fully covered with the mycelium of the test pathogen. The colony diameter, compared with check was taken as measure of inhibitory effect of tested fungicides and per cent inhibition of the test fungus was calculated by following the formula suggested by Vincent (1947):

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of mycelial growth over control

C = Mean maximum colony diameter in control (mm)

T = Mean maximum colony diameter in treatment (mm)

Compatibility of effective botanical and fungicides with efficient bio-control agent

Compatibility test of effective botanical and fungicides with efficient bio-control agent was done by using poisoned food technique as mentioned above. After screening, compatible combination of effective bio-control agents, botanical and fungicides were used for disease management under field condition.

Evaluation of different combination of bio-control agents, effective botanical and fungicides for the management of disease under field conditions

Rainy season is favorable for black spot disease as spores are rapidly spread by rain splash. So, disease incidence and severity were also observed comparatively high during this period. Considering this, field experiment was conducted during rainy season for management of this disease. Based on results of *in vitro* evaluation for antagonistic effect of bio-control agents and inhibitory of chemical and botanical against pathogen as well as compatibility test, two efficient bio-control agents, two botanical and both fungicides were evaluated in suitable combinations to control the disease development under field condition. The details of the treatments are presented as follows.

Design of Experiment

Field trial was designed to evaluate the effect of ten different treatment combinations under Randomized Block Design (RBD) using three replications. Each plot size was 2.5 m long with 0.5.m width. Plot to plot and block to block distance was maintained as 0.5 m. Plant to plant distance is 45 × 45 cm. In each plot 12 suckers were planted with 6 suckers in each row. Total 30 plots

were prepared in RBD and maintained during the study period. Three plots were kept as control plots. Recommended cultural operations were done as per requirement of crop. Continuous monitoring was done during the experimental duration to observe the development of disease symptoms in treated as well as control plot.

Preparation of botanical extract, suspension of biocontrol agents, molecule solution and suspension of pathogen

For field experiment, botanical extract was prepared by using the method of Shekhawat and Prasad (1971) discussed earlier (in section 2.5.1). The effective native biocontrol agents were multiplied in PDB for 15 days in BOD. The broth culture, thus obtained, was thoroughly mixed which was considered as 100% concentration; from this 5% concentration was prepared for field spray as well as soil drenching. Molecule solution was prepared by mixing thoroughly the requisite quantity of molecule with clean water on the scheduled day of spray. Pathogen suspension was made by growing the pathogen in PDB for 15 days in BOD. Suspension was prepared on the day of spraying following the same procedure as was performed in preparation of biocontrol suspension.

Spray schedule

Spraying was done in standing plant of the field. Culture of biocontrol agents were applied as soil drenching @ 5% suspension of both biocontrol agents just after planting. Two sprays of the selected treatment were done at 20 days' interval. First spray was done with effective concentration of botanicals and molecule as preventive spray. After 10 days, pathogen suspension was sprayed uniformly on each plant to develop disease symptoms. On appearance of disease symptoms, curative spray was made after 20 days of spraying pathogen inoculum with same treatment as used for preventive spray.

Observation on disease development:

Fields was visited regularly to observe the disease occurrence. The observation on disease development was recorded on 0-5 scale in each treatment on 5 plants randomly selected.

Based on the above observations, per cent disease index (PDI) was worked out by following the formula Wheeler (1970):

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Sum of individual disease ratings}}{\text{(No. of leaf observed} \times \text{Highest disease rating)}} \times 100$$

Further, percent disease reduction over control was calculated by using following formula:

$$\text{Per cent disease reduction over control (PDR)} = \frac{C - T}{T} \times 100$$

Where,

C = Per cent disease index in untreated/ control plot

T = Per cent disease index in treated plot

Statistical analysis

Experimental design used for the *in vitro* and *in vivo* studies were complete randomized design (CRD) and randomized block design (RBD), respectively. The data was analyzed statistically by OPSTAT Online Agriculture Data Analysis Tool. Analysis of variance (ANOVA) was used to find out the variation of result from experiment treatments at 5% level of significance. The percent data were converted to angular transformed values.

Results and Discussions

Symptoms

The black spot symptoms with sunken, tan to brown center were observed on leaves. Initially symptoms appeared as brown to black spots, which later coalesced. Later on the center of mature spots on leaves became sunken, dried and turned necrotic.

Isolation, purification and identification of the pathogen:

Infected leaves showing typical black spot symptoms were used for the isolation of the pathogen by agar plate method and the pure culture was obtained by hyphal tip method. The profuse growth of cottony fungal colony was observed as white to grayish white and sub-aerial to aerial mycelium. Colonies required 8-10 days for completely covering the PDA plates of 90 mm diameter when incubated at 28 ± 2°C. Later the colony was covered with orange slimy mass of conidia. The microscopic observation revealed the presence of hyaline, septate, highly branched mycelia with large number of more or less cylindrical, hyaline and single celled conidia with oil globules at the centre. Based on morphological as well as cultural characters, the pathogen was identified as *Colletotrichum gloeosporioides*. The observation in the present study pertaining to morphological and cultural characters of the fungus showed similarity with observations of earlier workers (Bhagya *et al*., 2011; Chowdhry and Varshney, 2000 and Photita *et al*., 2004).

Pathogenicity test

The pathogenicity test of the pathogen was conducted by inoculating pathogen inoculum on healthy *Aloe vera* plants under greenhouse. The pathogen was found to produce characteristic symptoms of black spot disease and re-isolation of the fungus from the inoculated plants showed similarity with the original isolate, hence Koch's postulate was confirmed. Earlier also *C. gloeosporioides* was found to produce characteristic disease symptom in

Table 1: Details of treatment.

Treatment	Management input	Common name/active ingredient	Concentrations
T_1	*T. harzianum* + Neem	Culture + leaf extract	5% +10%
T_2	*T. harzianum* + Tulsi	Culture + leaf extract	5% + 10%
T_3	*T. viride* + Neem	Culture + leaf extract	5% + 10%
T_4	*T. viride* + Tulsi	Culture + leaf extract	5% + 10%
T_5	Azoxystrobin + *T. harzianum*	23% SC + Culture	250 ppm + 5%
T_6	Carbendazim+ Neem	50% WP + Culture	250 ppm+ 10%
T_7	Azoxystrobin + *T. harzianum* + Neem	23% SC + Culture + leaf extract	250 ppm+ 5% + 10%
T_8	Azoxystrobin + *T. viride* + Neem	23% SC + Culture + leaf extract	250 ppm+ 5%+ 10%
T_9	Azoxystrobin + *T. viride* + Tulsi	23% SC + Culture + leaf extract	250 ppm + 5% + 10%
T_{10}	Control		

Aloe vera (Avasthi *et al.,* 2011).

Mycoflora associated with rhizosphere and phylloplane of *Aloe vera* plant

In course of study various mycoflora associated with *Aloe vera* were isolated from rhizosphere by serial dilution method and from phylloplane by leaf washing technique on Rose Bengal Agar Base Medium. Total 15 mycoflora was found associated with rhizosphere and phylloplane of *Aloe vera* and their identity was confirmed by their morphological and cultural characteristics. The fungal population was dominated by *Aspergillus* (3 species), *Trichoderma* (3 species) followed by *Penicillium* (2 species). Among 15 isolates of mycoflora, 11 isolates (*Aspergillus niger, A. flavus A. fumigatus, Trichoderma harzianum, T. viride, T. asperellum, Mucor* sp., *Cladosporium sphaerospermum, Fusarium solani, Penicillium* sp. and *Alternaria* sp.) were associated with both rhizosphere and phylloplane, 2 isolates (*Penicillium chrysogenum* and *Rhizopus stolonifer)* were obtained only from rhizosphere and 2 isolates (*Curvularia lunata* and *Colletotrichum gloeosporioides*) were present only in phylloplane. This shows that microclimate of phylloplane and rhizosphere of *Aloe vera* is favorable for these fungal species. The presence of diverse range of mycoflora in the rhizosphere and phylloplane, might be due to favorable microclimate and availability of nutrient required for survival and growth of mycoflora in these niches.

In the present investigation, mycoflora associated with phyloplane of *Aloe vera* represented the prevelence of *Aspergillus* and *Penicillium*, which is in line with the observation of Jalander and Gachande (2012). The present findings also showed agreements with the findings of Thakur (2017), reported various fungal species from the phylloplane of different medicinal plant and observed *Aspergillus niger, Trichoderma harzianum, Penicillium frequentans* as common fungal species from phylloplane of *Rauwolfia serpentina*. Earlier also presence of different fungi in rhizosphere and phylloplane of various herbs including *Aloe vera* have been reported. *Domsch and Gams* (1972) remarked that spores of these fungi might have come in contact with *Aloe vera* plant by air movement. Similarly, Dongo and Ayodele (1997) found *Cladosporium, Fusarium* and *Mucor* as common fungal airspore of Abraka.

Antagonistic activity of beneficial mycoflora from rhizosphere and phylloplane of *Aloe vera* against *C. gloeosporioides*

The beneficial isolates of rhizosphere and phylloplane mycoflora, which showed plant growth promotion in pot experiment, were evaluated for their antagonistic effect against the pathogen. In course of investigation, the native bio-control agents such as *T. harzianum, T. viride, T. asperellum, Aspergillus* spp. and *Penicillium* spp. obtained from the rhizosphere and phylloplane of *Aloe vera* plants, were found to successfully inhibit the pathogen growth in dual culture test, where *T. harzianum* showed the highest inhibitory effect on pathogen growth (76.19% inhibition) followed by *T. viride* and *Penicillium* sp. (65.80 %).

In the present study, the colonies of *Trichoderma* were found to grow faster than the pathogen and in all the cases, the colony of *C. gloeosporioides* stopped growing before direct contact was made, probably due to diffusible inhibitors that were secreted by the bio-

Table 2: Disease rating scale categories - *C. gloeosporioides* for black spot of *Aloe vera.*

Disease grade	Per cent area of infection on leaf	Description
0	0	No lesions on leaves
1	1-5	One small lesion
2	6-10	Few lesions on few leaves
3	11-20	Few (2-5 mm) lesions on many leaves
4	21-50	Many large lesions (>5 mm)
5	> 50	Mostly large lesions (15-20 mm)

Table 3: Antagonistic effect of beneficial mycoflora against *C. gloeosporioides.*

S. No.	Mycoflora	RG	PIRG
1.	*Aspergillus niger*	21.33	72.29
2.	*Aspergillus flavus*	22.00	71.43
3.	*A. fumigatus*	23.67	69.26
4.	*T. harzianum*	18.33	76.19
5.	*T. viride*	20.33	73.59
6.	*T. asperellum*	20.83	72.95
7.	*Penicillium* sp.	26.33	65.80
8.	*P. chrysogenum*	23.00	70.13
9.	Control	77.00	00.00
SE(m)±		0.99	1.28
SE(d)±		1.39	1.81
C.D. (at 5%)		2.95	3.83
C.V.(%)		6.08	3.49

Mean of three replications; RG: Radial growth (mm); PIRG: Per cent inhibition of radial growth

agents. The observations in the present study showed similarity with the observation made by Lee and Wu (1984). Earlier the antagonistic effect of various phylloplane and rhizosphere fungi (*Trichoderma* sp., *Aspergillus niger* and *Penicillium* sp.) against pathogenic ones have been reported by Adebanjo and Bankole (2002), Evueh and Ogbebor (2008), Fitsum *et al*., 2014, Pandey *et al.*, (1993), Shovan *et al*., (2008) and Thakur and Harsh (2014). Thus, the antagonistic potential of these native isolates of mycoflora may be exploited for development of effective management strategy against black spot of *Aloe vera*.

In the present investigation, *T. harzianum* and *T. viride* showed stronger inhibitory effect on the growth of *C. gloeosporioides* as compared with *T. asperellum*. This is due to production of different types of secondary metabolites by different species or isolates in the same species of *Trichoderma* with differential abilities to inhibit the growth of pathogenic fungi (Vinale *et al.*, 2008; Wu *et al.*, 2017). Vinale *et al.*, (2006) reported that the secondary metabolites of *T. harzianum* strain T22 and T39 showed antifungal activity against *Leptosphaera muculans*, *Phytophthora cinnamomi*, and *Botrytis cinerea* by reducing its growth. According to Dennis and Webster (1971), a number of antibiotics such as trichodermin, trichodermol, trichotoxin are known to be produced by *Trichoderma* species. These compounds might have been responsible for inhibition of *C. gloeosporioides* isolated from *Aloe vera* in the present study. The inhibitory effect on the growth of *C. gloeosporioides* may also be due to different enzymatic activities such as pectin lyase by *A. niger* and through antibiotic (toxin) production by *T. harzianum* (Howell, 2003). The present finding is in agreement with the findings of Nurbailis *et al*., (2019), who studied the antifungal activity of the culture filtrate of *T. harzianum, T. viride, T. koningii, Trichoderma* PP2, *Trichoderma* PP3 against *C. gloeosporioides* and observed that culture filtrate of *Trichoderma* spp., isolated from chili rhizosphere, inhibited the pathogen growth through antibiosis.

In vitro evaluation of different botanical (leaf-water extract) against the target pathogen

All the nine botanical evaluated at different

Table 4: Effect of botanicals at different concentration on colony growth of *Colletotrichum gloeosporioides.*

S. No.	Botanical	5%		10%		15%		20%	
		CG	PICG	CG	PICG	CG	PICG	CG	PICG
1.	Neem	25.00	72.22 (58.25)**	22.67	74.82 (59.93)	19.06	78.82 (62.65)	10.68	88.13 (69.91)
2.	Mandookparni	54.75	39.17 (38.78)	52.41	41.77 (40.30)	48.85	45.72 (42.59)	44.06	51.05 (45.64)
3.	Tulsi	39.31	56.33 (48.67)	36.20	59.78 (50.68)	31.69	64.79 (53.64)	25.26	71.93 (58.05)
4.	Sarpgandha	49.00	45.56 (42.49)	46.51	48.33 (44.08)	43.54	51.63 (45.97)	38.28	57.46 (49.33)
5.	Ashwagandha	54.13	39.86 (39.19)	50.17	44.26 (41.74)	47.20	47.55 (43.64)	42.12	53.21 (46.88)
6.	Pipli	54.49	39.46 (38.96)	52.18	42.02 (40.45)	50.15	44.27 (41.75)	47.75	46.95 (43.29)
7	Brahmi	52.48	41.69 (40.26)	48.47	46.14 (42.83)	39.36	56.27 (48.64)	37.16	58.71 (50.05)
8.	Bael	53.00	41.11 (39.92)	49.90	44.55 (41.91)	48.76	45.82 (42.64)	45.28	49.69 (44.86)
9.	Oak	55.49	38.35 (38.30)	53.34	40.73 (39.70)	51.94	42.29 (40.61)	47.36	47.37 (43.53)
10	Control	90	0.00	90	0.00	90	0.00	90	0.00
Factors				SE(m)±		SE(d)±		C.D. (at 5%)	
Factor (Botanical)				0.34		0.48		0.96	
Factor (Concentrations)				0.23		0.32		0.64	
Factor (Botanical × Concentration)				0.68		0.96		1.91	

CG: Colony growth*; PICG: Per cent inhibition of colony growth

*Mean of three replications; **Value given in parenthesis is angular transformed value; SE(m), SE(d), C.D. (at 5%) of per cent inhibition of colony growth at all the four concentrations of botanical extract.

concentrations resulted in more than 38 per cent inhibition in mycelial growth of the pathogen. Out of them, the neem leaf extract was found most effective followed by tulsi leaf extract at all concentration. At 10% concentration, neem extract shown maximum growth inhibition of the pathogen (74.82%) followed by tulsi (59.78%). The effectiveness of these botanical extracts in the present studies call for exploration of their potential for formulation of bio-pesticide in order to manage the black spot disease in *Aloe vera* and other medicinal plants.

Earlier several workers have reported antifungal activities of many plant species and underlined the importance of plants as a possible source of natural fungicides (Ogbebor *et al*., 2005; Philip and Sharma, 1997 and Tewari, 1995). Watve *et al*. (2009) evaluated leaf extract of neem, garlic, sadafuli, tulsi and glyricidia against growth of jatropha leaf spot pathogen (*C. gloeosporioides*) and they recorded maximum growth inhibition by leaf extract of neem (78.15%). Neem has been asserted to contain 18 active principles including the terpenoids azadirachtin, azadiradione, epoxy-azadiradione, nimbin, solannin, 6-diacethyl-nimbin amongst others. Azadirachtin is the most potent of the components and possesses strong antioxidant properties (Da Costal *et al*., 2010; Raheja and Thakore, 2002). Extract of *Ocimum basilium* arrested the ravages of the pathogens or their enzymes or both (Ugwuoke *et al*., 2008) and thereby inhibit rot causing fungi as *O. basilium* possess active principles. *Curcuma longa* and *A. indica* contain bioactive terpenoids which have been implicated for activities against a broad array of plant pathogenic fungi and bacteria (Gurjar *et al*., 2012). Thus, the findings of earlier studies are in conformity with the finding of the present study. The investigation on the mechanisms of disease suppression by plant products have suggested that the active principles present in plant extract may either act on the pathogen directly (Amadioha, 2000) or induce systemic resistance in host plants resulting in reduction of disease development (Kagale *et al*., 2004).

In vitro evaluation of fungicides against *C. gloeosporioides*:

In the present investigation, two fungicide-azoxystrobin and carbendazim was evaluated against *C. gloeosporioides* at different concentrations, markedly suppressed the growth of the pathogen at all the concentrations. Among them carbendazim was found more effective which allowed the pathogen growth to 5.83 mm only at 250 ppm and resulted in 93.52% growth inhibition, the inhibitory effect was further increased to 94.37% inhibition at 750 ppm concentration. Likewise, the azoxystrobin was also found highly effective against the pathogen at 250 ppm and resulted in 93.07 % growth inhibition of the pathogen whereas, at 750 ppm concentration resulted in 94.22% pathogen growth inhibition. Similarly, Patel (2009), reported that carbendazim and propiconazole showed 85% inhibition of *C. gloeosporioides*. The effectiveness of carbendazim against *C. gloeosporioides* and other species of the fungus under *in vitro* and *in vivo* condition has been reported by various workers (Jagtap *et al*., 2012; Prabhakar *et al*., 2008). Likewise, Sundarvadana *et al* (2006) and Ingle *et al*., (2014) also reported the effectiveness of azoxystrobin against *C. gloeosporioides* in mango and *C. truncatum* inciting black spot in soyabean respectively.

Table 5: Effect of fungicides against C. *gloeosporioides*.

S. No.	Fungicides	CC	RG	PIRG
1	Carbendazim	250	5.83	93.52
2	Carbendazim	500	5.40	94.00
3	Carbendazim	750	5.06	94.37
4	Azoxystrobin (Strobilurin)	250	6.23	93.07
5	Azoxystrobin (Strobilurin)	500	5.47	93.93
6	Azoxystrobin (Strobilurin)	750	5.20	94.22
7	Control	-	90.00	0.00
SE(m)±			0.06	0.06
SE(d)±			0.08	0.09
C.D. (at 5%)			0.18	0.20
C.V.(%)			0.58	0.14

CC: Concentrations (ppm); RG: Radial growth* (mm); PIRG: Per cent inhibition in radial growth (%)
*Mean of three replications.

Compatibility of potential native biocontrol agents with effective botanicals and fungicides

The compatibility of effective botanical extract and fungicides was tested with efficient native biocontrol agents in order to find out the most suitable combination for effective management of black spot in *Aloe vera* under field condition. Among fungicides, the radial growth of *T. viride* with azoxystrobin @ 750 ppm was recorded 55 mm whereas radial growth of *T. harzianum* was only 32 mm. Azoxystrobin at 750 ppm and carbendazim at all concentration were not compatible with *Trichoderma* spp., which recorded quite less growth with azoxystrobin (750 ppm) and complete growth inhibition with carbendazim. Among two biocontrol agents *T. viride* was more compatible than *T. harzianum*. Both bio-control agents (*T. harzianum* and *T. viride*) were highly compatible with leaf water extracts of neem and tulsi at all concentrations which allowed full growth of the bio-control agents, but in case of fungicides, the bio-control agents were highly compatible with azoxystrobin only. Similar observation about poor compatibility of

Table 6: Effect of efficient native biocontrol agents, botanicals and compatible fungicides in different combinations on plant height, number of leaves per plant, per cent disease index and per cent disease reduction over control under field conditions.

Treatment	Plant height (cm)*	No. of leaves per plant*	Per cent Disease Index (PDI)#	Per cent disease reduction (PDR) over control
T_1 - *T. harzianum* + Neem	32.02	9.61	11.00	78.85
T_2 - *T. harzianum* + Tulsi	31.10	9.04	13.83	73.40
T_3 - *T. viride* + Neem	29.03	9.20	12.26	76.43
T_4 - *T. viride* + Tulsi	26.99	7.78	15.83	69.55
T_5 - Azoxystrobin + T. *harzianum*	29.52	8.10	9.03	82.63
T_6 -Carbendazim + Neem	25.03	7.00	10.28	80.22
T_7 - Azoxystrobin + *T. harzianum* + Neem	33.12	10.00	9.67	81.40
T_8 - Azoxystrobin + *T. viride* + Neem	31.27	9.10	10.00	80.77
T_9 -Azoxystrobin + *T. viride* + Tulsi	30.43	8.07	11.86	77.18
T_{10} - Control	24.15	6.15	52.00	0.00
SE(m)±	0.92	0.37	0.39	0.72
SE(d)±	1.30	0.53	0.55	1.02
C.D. (at 5%)	2.75	1.12	1.16	2.15
C.V.(%)	5.43	7.69	4.31	1.78

*Mean of five plants; #Mean of three replications

carbendazim with *Trichoderma* spp was reported by earlier workers (Gowdar *et al*., 2006; Lal and Maharishi, 2007). Bheemaraya *et al*., (2012) reported compatibility of neem seed kernel extract, leaf extract of eucalyptus and pongamia with *Trichoderma* species. The present findings of compatibility test of effective bio-control agents with efficient botanical and fungicides also gained support from the above stated reports by various workers.

Integrated effect of native bio-control agents, effective botanical and compatible fungicides on management of black spot disease and plant growth parameters under field condition

In course of study on integrated management of black spot disease, the most effective treatment was foliar spray with azoxystrobin @ 250 ppm accompanied with soil drenching by *T. harzianum* @ 5% resulted in 82.63% reduction in PDI. Likewise the foliar spray of azoxystrobin @ 250 ppm accompanied with soil drenching of *T. harzianum* @ 5% and further foliar spray with neem extract @ 10% also showed promising disease control (81.40%). The treatment combination having *T. harzianum* as a component showed striking growth promoting effect on plants as evident from the observation in the present study that foliar spray with azoxystrobin @ 250 ppm accompanied with soil drenching of *T. harzianum* @ 5% and further foliar spray with neem extract @ 10% recorded maximum plant height (33.12 cm) with maximum number of leaves (10/plant). Likewise, soil drenching of *T. harzianum* @ 5% accompanied by foliar spray with neem @ 10% also recorded the plant height of 32.02 cm and number of leaves (9.61/plant). The effectiveness of the treatment combinations like azoxystrobin @ 250 ppm accompanied with soil drenching of *T. harzianum* @ 5% or other combination such as foliar spray of azoxystrobin @ 250 ppm accompanied with soil drenching of *T. harzianum* @ 5% and further foliar spray with neem extract @ 10% in controlling the black spot disease and promotion of plant growth parameters in *Aloe vera* plants under field condition in the present investigation call for large scale testing of these combination in farmers field.

Earlier also the combined applications of bio-control agents, chemical and botanical extract have been found effective in plant disease management. Nidhina and Hegde (2013) successfully controlled *C. gloeosporioides* (Penz.) Penz. & Sacc. causing inflorescence die back in arecanut by combining two compatible bioagents (*T. virens* and *T. viride*) and two fungicides (Blitox 50 W and Mixol 72). The highest percent inhibition was observed when Mixol 72 @ 0.05% was used with *T. virens*. They further reported that the combination of bio-agents with fungicides provided higher disease suppression than achieved with fungicides and bio-agents alone. Similar observations were made by Pandey *et al*., (2019) and Rao *et al*., (2020) who were successful in managing the disease by combination of bio-agent and chemical. The significant enhancement in plant growth parameter due to incorporation of *T. harzianum* in treatment combination is also supported from the observation of greenhouse experiment in the present investigation. Plant growth promoting abilities and biofertilizer potential of *Trichoderma* spp are well

documented by Chet *et al*., (1997), Harman and Bjorkman (1988) and Molla *et al*., (2012). The superiority of combined application of bio-agent and chemical/botanical extract in effective disease management in the present investigation and also in the reports of earlier workers might be due to synergistic interaction among various components of integrated disease management strategy. The combination of compatible fungicide- azoxystrobin, neem extract and *T. harzianum*, which has been found quite effective in disease management in the present investigation, may prove as an effective tool for protection of *Aloe vera* crops from the effect of *C. gloeosporioides*.

Conclusion

The present study showed the effectiveness of native isolates of bio-control agents, obtained from the rhizosphere and phylloplane of *Aloe vera*, botanical and safe molecule for management of black spot disease in *Aloe vera* caused by *C. gloeosporioides*. Among various isolates, *T. harzianum* showed the strongest inhibitory effect on the pathogen growth (76.19%) followed by *T. viride* (73.59%). Among botanicals, neem leaf extract, showed maximum pathogen growth inhibition (74.82%) followed by tulsi (59.78%) at 10% concentration. Among two fungicides - azoxystrobin and carbendazim evaluated at different concentrations resulted in more than 90% suppression of the pathogen growth at all the concentrations. The test bio-control agent was more compatible with the azoxystrobin and neem and tulsi extract. In course of field trial, the most effective treatment was foliar spray with azoxystrobin @ 250 ppm accompanied with soil drenching by *T. harzianum* @ 5% resulted in 82.63% reduction in PDI. Likewise the foliar spray of azoxystrobin (250 ppm) accompanied with soil drenching of *T. harzianum* (5%) and further foliar spray with neem extract (10%) also showed promising disease control (81.40 % reduction).

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