Mycoflora Associated with Phylloplane and Rhizosphere of *Aloe vera* and their Effect on Plant Growth Parameters

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Authors’ contributions

The work was carried out by author MK under the supervision of author PKJ. Statistical analysis and first draft of the manuscript was written by author MK. Author PKJ read the manuscript and prepared the final manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The present investigation was conducted to study the spectrum of mycoflora associated with phylloplane and rhizosphere of *Aloe vera* and to assess their effect on plant growth parameters and antagonistic activity against the *C. gloeosporioides* causing black spot disease in *Aloe vera*. During the study total 15 mycoflora belonging to ten genera were isolated from *Aloe vera* plant by leaf washing technique from phylloplane and serial dilution from rhizosphere soil. Among these, fungal species belonging to the genera of *Aspergillus*, *Trichoderma* and *Penicillium* was found to be more abundant. In course of study some of mycoflora associated with phylloplane and rhizosphere of *Aloe vera* were found to exert plant growth promoting effect and also exhibited strong antagonistic activity against *Colletotrichum gloeosporioides*.

Keywords: Phylloplane; rhizosphere; *Aloe vera*; mycoflora; antagonistic.

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1. INTRODUCTION

Aloe vera, also known as Ghrit Kumari, Kunvarpathu and Indian Aloe, is one of the most important medicinal plant belonging to Liliaceae family. It is a succulent, drought tolerant and very 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 [1].

Like other plant Aloe vera is also affected by several diseases like leaf spot, base rot, rust, black spot, soft rot etc. Black spot disease caused by Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. is very serious among them as it reduces the quality and quantity of Aloe gel and thereby reduces it market value. The symptoms of anthracnose began as a small round to oval, water-soaked area about 1-2 mm in diameter. These area increase into circular spots with tan to light brown centre bordered by water soaked tissue. As these spots expand, 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 appeared on both the surfaces of leaf, affected area lost the mucilaginous gel and leads the death of infected leaves [2]. 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 [3].

It is well established that plant health is largely influenced by the presence of various micro-organism in the rhizosphere and phylloplane. Rhizosphere, which 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 [4]. 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. Phylloplane mycoflora are the fungi that are growing on the surface of leaves. There are two groups of phylloplane fungi, residents and casuals. Residents can multiply on the surface of healthy leaves without noticeably affecting the host whereas, casuals land on the leaf surface but cannot grow [5].

In the present investigation, an attempt has been made to work out the spectrum of mycoflora in the rhizosphere and phylloplane of Aloe vera and to assess their effect on plant health in relation to growth parameters, development of disease symptoms on Aloe vera plant and antagonistic activity against the C. gloeosporioides.

2. MATERIALS AND METHODS

2.1 Collection of Samples

For isolation of mycoflora from phylloplane, samples of leaves showing disease symptoms as well as healthy leaves were randomly selected from different plants. For isolating rhizosphere mycoflora, plants were uprooted with intact soil adhering to the roots.

All samples were collected from Herbal garden of Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur. These collected samples were placed in paper bags and then, brought to the Plant Pathology laboratory. All samples were kept at 4°C until further analysis.

![Fig. 1. Aloe vera plant showing black spot symptoms](image-url)
2.1.1 Isolation of pathogen

Disease samples of Aloe vera leaves showing black spot symptoms were collected and used for isolation of the pathogen. Leaves were 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, surface-sterilized with 0.1% mercuric chloride solution for 30-60 seconds followed by three washings with sterilized distilled water, so as to make them free from any traces of mercuric chloride. The disinfected tissue pieces were blotted between sterile Whatman No. 1 filter papers and aseptically plated randomly in 9 cm diameter Petri-dishes containing Potato dextrose agar (PDA) medium (3 pieces per plate). These inoculated Petri plates were incubated in Biological Oxygen Demand (BOD) incubator at 28 ± 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 by comparing the morphological characters and reproductive structures under compound microscope.

Subsequently the pure cultures thus obtained were maintained on PDA slants. The slants were incubated at 28 ± 2°C in BOD incubator. These cultures were revived every month and maintained throughout the course of studies on PDA.

2.1.2 Pathogenicity test

To establish the pathogenicity of the isolated fungus (pathogen) on Aloe vera, the standard methodology was followed. The pathogen was cultured on Potato Dextrose Agar (PDA) medium for 8-10 days at 28±2°C in BOD incubator. Conidial suspension of pathogen was prepared. Healthy leaves of the Aloe vera plants that were grown in greenhouse conditions were artificially pricked on the abaxial surface by sterilized needle and sprayed with conidial suspension of pathogen. 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 five days onwards. After 10 days of inoculation, disease symptoms were appeared on the leaves, pathogen was re-isolated from infected plants and confirmed with parent cultures. These cultures were kept for further experiments.

2.2 Isolation and Identification of Phylloplane Mycoflora

The phylloplane fungi were isolated from five leaves collected from different Aloe vera plants. Leaves were taken as whole from each plant, washed under running tap water. The leaves were cut into small pieces (2-3 cm) and placed in one liter conical flask containing 500 ml of pre sterilized distilled water and flask was vigorously shaken. From this suspension of microorganisms serial dilution was made up to 10⁻⁵ dilution. Thereafter, one ml of each dilution was transferred into Petri plates containing Rose Bengal Agar Base medium. The inoculums was spread uniformly and kept undisturbed in Laminar Air flow after that it was kept in BOD for incubation at 28±2°C for seven days. The emergence of fungal colonies was monitored regularly. 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/l streptomycin sulfate. Subculturing was done repeatedly until pure cultures were obtained for each fungus, and they were subsequently maintained on PDA slants for further studies. The fungal isolates were identified on the basis of morphological and cultural characters as per standard mycological manuals [6,7].

2.3 Isolation and Identification of Rhizosphere Mycoflora

Rhizosphere mycoflora were isolated by serial dilution method [8]. Five plants were uprooted, and then soil adhering to the roots was separated from roots and allowed for shade drying. Stock solution was prepared by adding 10 g of rhizosphere soil in 90 ml of sterilized distilled water. From this stock solution up to 10⁻⁵ dilution 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. Each emerging fungal colonies were picked individually and transferred to PDA slants and purified by hyphal tip method. The pure cultures, thus obtained, were maintained in PDA slants and kept in refrigerator for further studies. The fungal isolates were identified based on morphological and cultural characters.

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The sample (fungus mycelium) of each fungal isolate, was mounted on the sterile slides, then stained with lactophenol/cotton blue and examined in 40X light microscope. The fungal cultures were identified on the basis of microscopic characters for spore shape and phenotypic characteristics for spore type, colony color, growth rate using standard manual [6,7].

2.4 Greenhouse Experiment

2.4.1 Effect of phylloplane mycoflora on plant health

Various mycoflora isolated from phylloplane were studied for their effect on plant health (pathogenic or beneficial). All the fungal isolates were multiplied in potato dextrose broth for 15 days in BOD. After 15 days of incubation, culture of each isolates was used as suspension for foliar spray. One kg soil was filled in each pot and then planting (one plant in each pot) was done at 5-10 cm depth. After one week, suspension of each fungus was sprayed using three pots for each fungus and three pots for control. Plants were regularly monitored for health condition and data on crop health and plant growth parameters like plant heights (after 120 days by using a scale), fresh weight of leaves and roots, dry root weight of leaves and roots were taken after 120 days of planting. On the basis of their effects on plants they were categorized as beneficial or pathogenic.

2.4.2 Effect of rhizosphere mycoflora on plant health

Each fungal isolates obtained from rhizosphere soil were separately multiplied on sand-maize media (9:1) for 25 days in BOD incubator. Plastic pots were prepared by mixing 2 kg soil and inoculum @ 50g/kg soil of different mycoflora. After 48 hours, young suckers obtained from healthy plants in Herbal garden of RPCAU, Pusa were planted and maintained in greenhouse under prevailing conditions of temperature (28±2°C) and light. Plants grown under similar condition without any mycoflora inoculation served as control. Plants were regularly monitored for health condition and data on crop health (disease) and plant growth parameters like plant heights, fresh weight of leaves and roots, dry weight of leaves and roots, number of plantlets or suckers was taken after 120 day of planting. On the basis of their effects on plants, they were categorized as beneficial or pathogenic.

2.4.3 Antagonistic activity of phylloplane and rhizosphere mycoflora against *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc

Each isolated phylloplane and rhizosphere mycoflora were tested for their antagonistic activity against the pathogen-*Colletotrichum gloeosporioides* using the dual culture method as described by Sinclair and Dhingra [4]. A 5 mm mycelial disc cut from an actively growing 7-days old culture of each of the isolated mycoflora was placed 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 of the same Petri plate, 1 cm away from the edge of Petri plate. The control plate was prepared only with the pathogen mycelial disc at the centre of plate. These plates were incubated at 28±2°C for seven days. The percentage inhibition was calculated by using the following formula suggested by Ramesh et al., [9].

\[ I = \frac{R_1 - R_2}{R_1} \times 100 \]

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.

3. RESULTS

3.1 Isolation of Pathogen and Pathogenicity Test

The isolated pathogen was identified as *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. which was found to incite black leaf spot disease in *Aloe vera* plant. Its pathogenicity to *Aloe vera* plant was also confirmed by employing Koch’s postulates.

3.2 Isolation and Identification of Phylloplane and Rhizosphere Mycoflora from *Aloe vera* Plant

In the present study, totally 15 mycoflora were isolated from phylloplane and rhizosphere of *Aloe vera* (Table-1). The fungal population was dominated by *Aspergillus* (3 species) *Trichoderma* (3 species) followed by *Penicillium*...
(2 species). Among 15, 12 mycoflora were associated with rhizosphere soil, 13 with phylloplane and 10 were common on both phylloplane and rhizosphere.

Phylloplane shown the presence of Aspergillus niger, A. flavus, A. fumigatus, Trichoderma harzianum, T. viride, T. asperellum, Fusarium solani, Mucor sp., Cladosporium sphaerospermum, Penicillium sp., Curvularia lunata, Alternaria sp. and Colletotrichum gloeosporioides. Whereas, rhizosphere soil shown the presence of Aspergillus niger, A. flavus, A. fumigatus, Trichoderma harzianum, T. viride, T. asperellum, Fusarium solani, Mucor sp., Cladosporium sphaerospermum, Rhizopus stolonifer, Penicillium sp., and Penicillium chrysogenum. It was interesting to note that Aspergillus niger, A. flavus A. fumigatus, Trichoderma harzianum, T. viride, T. asperellum, Mucor sp., Cladosporium sphaerospermum, Fusarium solani and Penicillium sp. were common in both phylloplane and rhizosphere soil of Aloe vera.

3.3 Effect of Rhizosphere Mycoflora on Plant Growth Promotion

Mycoflora obtained from rhizosphere soil of Aloe vera plant were studied under pot condition in green house to test their growth promotion potential on Aloe plant. The result of the study shown marked variation among the various rhizosphere mycoflora in relation to their effect on plant growth. Isolates of Trichoderma harzianum and T. viride were showed increased plant height, fresh shoot and root weight, dry shoot and root weight, number of plantlets (Table-2). The other isolates such as Fusarium, Alternaria, Curvularia and Cladosporium were not effective in promoting growth of Aloe vera, rather shown pathogenic effect and resulted in poor growth and development of Aloe plant compared to control.

The data presented in Table 2 showed that Trichoderma harzianum, T. viride, T. asperellum and two Penicillium spp. had promising effect on all growth parameters. The maximum plant height was obtained with T. harzianum (28.09 cm) followed by T. viride (26.33 cm), T. asperellum (26.11 cm), Penicillium chrysogenum (25.02 cm) and Penicillium sp. (23.32 cm). Least plant height was observed from Alternaria sp. (18.32 cm) followed by Fusarium solani (19.14 cm) Cladosporium sphaerospermum (19.72 cm) which have shown characteristic disease symptoms and poor plant growth.

3.4 Effect of Phylloplane Mycoflora on Plant Growth Promotion

Mycoflora isolated from phylloplane were also studied for their effect on Aloe vera plant by spraying inoculum on plant. Result presented in Table 3 showed that among various mycoflora, The isolates of Fusarium, Cladosporium, Curvularia and Alternaria showed negative effects after 15 days of inoculation with reduced plant height, fresh leaf and root weight, dry leaf and root weight. The other isolates such as Mucor sp., Aspergillus spp, Trichoderma spp, Penicillium spp. showed more or less growth promoting effect as evident from their effect on plant growth parameter.

**Table 1. Mycoflora isolated from phylloplane and rhizosphere of Aloe vera**

| S. No. | Mycoflora                     | Class          | Isolated from (phylloplane or rhizosphere) |
|-------|------------------------------|----------------|-------------------------------------------|
| 1.    | Aspergillus niger            | Ascomycetes    | Phylloplane and rhizosphere               |
| 2.    | A. flavus                    | Ascomycetes    | Phylloplane and rhizosphere               |
| 3.    | A. fumigatus                 | Ascomycetes    | Phylloplane and rhizosphere               |
| 4.    | Trichoderma harzianum        | Ascomycetes    | Phylloplane and rhizosphere               |
| 5.    | T. viride                    | Ascomycetes    | Phylloplane and rhizosphere               |
| 6.    | T. asperellum                | Ascomycetes    | Phylloplane and rhizosphere               |
| 7.    | Fusarium solani              | Ascomycetes    | Phylloplane and rhizosphere               |
| 8.    | Rhizopus stolonifer          | Zygomycetes    | Only Rhizosphere                          |
| 9.    | Mucor mucedo                 | Zygomycetes    | Phylloplane and rhizosphere               |
| 10.   | Cladosporium sphaerospermum  | Ascomycetes    | Phylloplane and rhizosphere               |
| 11.   | Penicillium sp.              | Ascomycetes    | Phylloplane and rhizosphere               |
| 12.   | Penicillium chrysogenum      | Ascomycetes    | Only Rhizosphere                          |
| 13.   | Curvularia lunata            | Ascomycetes    | Only Phylloplane                          |
| 14.   | Alternaria sp.               | Ascomycetes    | Only Phylloplane                          |
| 15.   | Colletotrichum gloeosporioides | Sordariomycetes | Only Phylloplane                          |
Table 2. Effects of rhizosphere mycoflora on plant growth parameters (120 days after planting) under greenhouse condition

| S. No. | Mycoflora               | Plant height (in cm) | Fresh leaf weight (in g) | Fresh root weight (in g) | Dry leaf weight (in g) | Dry root weight (in g) | Number of suckers per plant | Remarks                           |
|--------|-------------------------|----------------------|--------------------------|--------------------------|------------------------|------------------------|-------------------------------|----------------------------------|
| 1.     | Aspergillus niger       | 21.58                | 121.15                   | 6.99                     | 4.2                    | 1.27                   | 3.67                          | Good health                     |
| 2.     | Aspergillus sp.         | 21.04                | 115.08                   | 6.39                     | 3.4                    | 1.24                   | 4.67                          | Good health                     |
| 3.     | Trichoderma viride      | 26.33                | 185.85                   | 9.16                     | 3.38                   | 2.02                   | 3.33                          | Healthy plants with vigorous growth |
| 4.     | Aspergillus flavus      | 21.17                | 109.15                   | 6.66                     | 3.04                   | 1.02                   | 5.00                          | Good health                     |
| 5.     | Fusarium solani         | 19.14                | 93.68                    | 4.52                     | 2.48                   | 0.54                   | 4.33                          | Very small size plant, poor health |
| 6.     | Aspergillus fumigates   | 21.18                | 108.83                   | 5.81                     | 3.35                   | 0.98                   | 5.33                          | Healthy plants                  |
| 7.     | Trichoderma harzianum   | 28.09                | 187.87                   | 10.33                    | 7.12                   | 3.18                   | 10.00                         | Very healthy plants and vigorous growth |
| 8.     | Rhizopus stolonifer     | 22.02                | 157.61                   | 6.86                     | 4.1                    | 1.26                   | 8.00                          | Healthy plants                  |
| 9.     | Mucor sp.               | 21.88                | 138.39                   | 5.72                     | 3.95                   | 0.88                   | 6.00                          | Healthy plants                  |
| 10.    | Cladosporium sphaerospermum | 19.72            | 132.56                   | 5.76                     | 3.05                   | 0.85                   | 4.33                          | Comparatively poor growth compared to control |
| 11.    | Penicillium sp.         | 23.32                | 158.37                   | 8.03                     | 4.16                   | 1.76                   | 8.33                          | Healthy plants                  |
| 12.    | Penicillium chrysogenum | 25.02                | 174.91                   | 9.08                     | 4.25                   | 1.98                   | 9.33                          | Healthy plants                  |
| 13.    | Trichoderma asperellium | 26.11                | 182.23                   | 9.27                     | 5.02                   | 2.02                   | 8.67                          | Healthy plants with vigorous growth |
| 14.    | Alternaria sp.          | 18.32                | 118.25                   | 4.09                     | 2.96                   | 0.79                   | 6.33                          | Comparatively poor growth compared to control |
| 15.    | Control                 | 20.65                | 135.57                   | 4.25                     | 2.05                   | 0.93                   | 7.33                          | Healthy plants with normal growth |
| C.D.   |                        | 1.05                 | 0.52                     | 0.27                     | 0.18                   | 0.07                   | 1.41                          |                                  |
| SE (m) |                        | 0.36                 | 0.18                     | 0.09                     | 0.06                   | 0.03                   | 0.49                          |                                  |
| C.V.   |                        | 2.81                 | 0.22                     | 2.37                     | 2.86                   | 2.94                   | 12.32                         |                                  |
Table 3. Effect of phylloplane mycoflora on plant health (120 days after planting) under greenhouse condition

| S. No. | Mycoflora                | Plant height (in cm) | Fresh leaf weight (in g) | Fresh root weight (in g) | Dry leaf weight (in g) | Dry root weight (in g) | Effects on plants                              |
|--------|--------------------------|----------------------|--------------------------|--------------------------|------------------------|------------------------|-----------------------------------------------|
| 1.     | Aspergillus niger        | 21.35                | 119.13                   | 5.66                     | 3.50                   | 1.21                   | Normal growth, no symptoms.                   |
| 2.     | Aspergillus flavus       | 20.25                | 108.25                   | 5.81                     | 3.01                   | 1.02                   | Normal growth, no symptoms.                   |
| 3.     | Aspergillus fumigatus    | 20.20                | 107.69                   | 4.78                     | 2.85                   | 0.74                   | Normal growth, no symptoms.                   |
| 4.     | Trichoderma harzianum    | 26.36                | 183.78                   | 9.85                     | 4.25                   | 3.02                   | Good health, vigorous growth.                 |
| 5.     | T. viride                | 25.80                | 181.65                   | 8.88                     | 3.65                   | 2.94                   | Good health, vigorous growth.                 |
| 6.     | T. asperellum            | 24.75                | 179.86                   | 8.72                     | 3.35                   | 2.84                   | Good health, vigorous growth.                 |
| 7.     | Mucor sp.                | 20.12                | 132.35                   | 5.23                     | 3.12                   | 0.79                   | Normal growth                                 |
| 8.     | Penicillium sp.          | 21.25                | 136.15                   | 5.65                     | 2.98                   | 0.72                   | Good health.                                  |
| 9.     | Cladosporium sphaerospermum | 19.51          | 130.25                   | 5.41                     | 2.75                   | 0.74                   | Comparatively poor growth compared to control.|
| 10.    | Curvularia lunata        | 18.15                | 107.23                   | 3.65                     | 2.73                   | 0.68                   | Comparatively poor growth compared to control.|
| 11.    | Alternaria sp.           | 18.32                | 116.31                   | 3.96                     | 2.86                   | 0.82                   | Comparatively poor growth compared to control.|
| 12.    | Fusarium solani          | 18.00                | 93.25                    | 3.87                     | 2.75                   | 0.78                   | Comparatively poor growth compared to control and yellowing and curling of leaves. Leaf spot symptoms. |
| 13.    | Colletotrichum gloeosporioides | 18.45           | 110.39                   | 3.85                     | 2.85                   | 0.87                   | Poor growth compared to control and induced symptoms of black rot |
| 14.    | Control                  | 20.85                | 135.57                   | 4.25                     | 2.05                   | 0.93                   | Normal growth                                 |
|        |                          |                      |                           |                          |                        |                        |                                               |
|        | C.D.                     | 0.57                 | 0.68                     | 0.52                     | 0.47                   | 0.23                   |                                               |
|        | SE(m)                    | 0.20                 | 0.23                     | 0.18                     | 0.16                   | 0.08                   |                                               |
|        | C.V.                     | 1.63                 | 0.31                     | 5.41                     | 9.17                   | 10.35                  |                                               |
On the basis of results obtained from the effect of phylloplane and rhizosphere mycoflora on plant health under greenhouse conditions all mycoflora were categorized into beneficial or non-pathogenic and pathogenic ones (Table 4).

### 3.5 Antagonistic Activity of Beneficial Mycoflora Isolated from Phylloplane and Rhizosphere of Aloe vera against the Pathogen-Colletotrichum gloeosporioides

Antagonistic effect of beneficial mycoflora from phylloplane and rhizosphere were evaluated by dual culture technique. Based on the measurements of radial growth, it was observed that all the beneficial isolates of mycoflora were capable of strongly inhibiting the growth of pathogen in PDA medium by >65% (Table 5). Among the eight antagonists, the strongest inhibitory effect on pathogen growth was shown by *Trichoderma harzianum* (76.32%) followed by *T. viride* (73.68%), *Aspergillus niger* (72.42%) and *T. asperellum* (72.38%).

### 4. DISCUSSION

Results of this study showed that *Aloe vera* plant harbors a diverse group of phylloplane and rhizosphere mycoflora that belong to different genera, mainly within the phylum Ascomycota.

The Phylloplane mycoflora communities mainly belonged to *Aspergillus, Mucor, Alternaria, Curvularia, Fusarium, Cladosporium, Trichoderma* and *Penicillium*.

Earlier also presence of different fungi in rhizosphere and phylloplane of various herbs including *Aloe vera* have been reported. Domsch and Gams [10] remarked that spores of these fungi might have come in contact with *Aloe vera* plant by air movement. Dongo and Ayodele [11] found *Cladosporium, Fusarium* and *Mucor* as common fungal airspore of Abraka.

Thakur [12] reported *Aspergillus niger, T. harzianum, Penicillium frequentans* as regular parasitic taxa of *Rauwolfia serpentina*. Reshaya and Niladi [13] conducted a survey on the occurrence of phylloplane fungi from *Azadirachta indica, Centella asiatica, Justicia adhatoda, Ocimum tenuiflorum* and *Plecteranthus samboinicus* and reported 18 fungal species of thirteen genera of which *Aspergillus* had the highest incidence followed by *Cercospora, Cladosporium, Curvularia* and *Diplococcium*.

#### Table 4. Categorization of Phylloplane and rhizosphere as beneficial and pathogenic ones

| S. No. | Beneficial or Non – pathogenic isolates | Pathogenic isolates |
|--------|----------------------------------------|---------------------|
| 1.     | Aspergillus spp.                        | Fusarium solani     |
| 2.     | *Trichoderma* spp.                     | Cladosporium sphaerospermum |
| 3.     | *Penicillium* spp.                     | Curvularia lunata   |
| 4.     | Mucor sp.                              | Alternaria sp.      |
| 5.     | Rhizopus sp.                           | Colletotrichum gloeosporioides |

#### Table 5. Antagonistic effect of beneficial mycoflora on radial growth of *C. gloeosporioides*

| S. No. | Mycoflora       | Mycelial growth (mm*) | Per cent inhibition of mycelia growth (%) |
|--------|-----------------|-----------------------|------------------------------------------|
| 1.     | *Aspergillus niger* | 21.33                 | 72.42 (58.38)                            |
| 2.     | *Aspergillus flavus* | 22.00                 | 71.58 (57.83)                            |
| 3.     | *A. fumigatus*    | 23.67                 | 69.36 (56.45)                            |
| 4.     | *Trichoderma viride* | 20.33                 | 73.68 (59.19)                            |
| 5.     | *T. harzianum*    | 18.33                 | 76.32 (60.93)                            |
| 6.     | *T. asperellum*   | 21.33                 | 72.38 (58.35)                            |
| 7.     | *Penicillium* sp. | 26.33                 | 65.99 (54.37)                            |
| 8.     | *P. chrysogenum*  | 23.00                 | 70.19 (56.98)                            |
| 9.     | Control          | 77.33                 | 00.00 (0.57)                             |
| C.D.   |                 | 3.38                  | 4.14                                     |
| SE(m)  |                 | 1.13                  | 1.38                                     |
| C.V.   |                 | 6.93                  | 3.77                                     |

*Mean of three replications; Figure in parentheses indicate angular transformed values
The findings of present investigation showed the presence of some fungal isolates, in the rhizosphere and phylloplane of Aloe vera, having plant growth promoting effect as well as antagonistic effect on the pathogen.

The involvement of mycoflora in plant health promotion has also been reported by earlier workers. The Aspergillus species have been previously described as the best phosphate-solubilizing fungi on a variety of different substrates [14] and they are capable of increasing soluble phosphate, which is normally not available in soil for plants, so resulted in increased plant height, fresh and dry weight of leaves and root of Aloe vera plant.

In the present study, the phylloplane and rhizosphere mycoflora of Aloe vera such as Trichoderma harzianum, T. viride, T. asperellum, A. niger, and Penicillium sp. suppressed the growth of C. gloeosporioides under in vitro condition. In earlier studies also, several Aspergillus species including Aspergillus flavus and A. niger proved to have antagonistic activities against C. gloeosporioides. Evueh et al., [15] used phylloplane mycoflora as biocontrol agent against Colletotrichum leaf disease of rubber. They found that Aspergillus sp lyzed the cytoplasm of the pathogen on PDA. Trichophyton sp and Gliocladium sp antagonized by overgrowing and exhibited highest antagonistic activity against C. gloeosporioides. Trichoderma species can act by colonizing the soil rhizosphere and or parts of the plants, occupying a physical space and thus preventing the multiplication of the pathogens, producing cell wall degrading enzymes against the pathogens, producing antibiotics that can kill the pathogens, promoting the plant development and inducing the defense mechanisms of the plant. Its application enhances plant biomass by promoting plant growth [16,17].

5. CONCLUSIONS

In the present study, a total of 15 mycoflora were obtained from the phylloplane and rhizosphere of Aloe vera. Among these, three isolates of mycoflora showed plant growth promotion activity, two isolates showed normal plant growth without showing pathogenic effect whereas five isolates showed abnormal disease like symptoms that resulted in poor plant growth. The isolates of Trichoderma, Aspergillus and Penicillium were found to have strong antagonistic effect on the pathogen. The presence of these fungal isolates in the rhizosphere and phylloplane of Aloe vera plants may be exploited for evolving healthy crop management strategy for cultivation of Aloe vera crop.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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