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# Screening of rhizosphere microorganisms for antagonism against *Alternaria alternata* (Fr.) Keissler causing Alternaria leaf blight of Isabgol *(Plantago ovate)*

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**ABSTRACT**

*Isabgol (Plantago ovata) is a crucial medicinal plant valued for its high soluble fiber content, benefiting digestive health and cholesterol management. However, Alternaria leaf blight, caused by Alternaria alternata, poses a significant threat to Isabgol cultivation, impacting both yield and quality and necessitating effective disease management strategies. The experiment was conducted at the Department of Plant Pathology, College of Agriculture, Bikaner, Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, in dual culture techinique under controlled conditions to screen rhizosphere microorganisms for their antagonism against Alternaria alternata in Isabgol. The result showed that the significant variation in the reduction mycelial growth of pathogen in the presence of various microorganisms. Among all the tested antagonists, Trichoderma viride was the most effective, with maximum 83.9% mycelial growth inhibition of pathogen followed by Colletotrichum lindemuthianum (79.73%), Trichoderma harzianum (76.01%), Aspergillus flavus (73.98%), Rhizoctonia solani (72.16%), Fusarium spp. (71.30%), Trichoderma spp. (67.55%), Aspergillus parasiticus (67.23%) and Colletotrichum gloeosporioides (66.95%). These findings indicated that certain rhizosphere microorganisms possess substantial potential as bio control agents against A. alternata, and an environmentally friendly alternative to chemical control.*

***Keywords*:** *Alternaria alternata, in vitro*, Isabgol, microorganism, mycelial growth, Screening

## INTRODUCTION

*Plantago ovata* commonly known as 'Psyllium' in English and 'Isabgol' in Hindi belongs to the family of *Plantaginaceae*. It has gained a reputation as a naturally occurring medicinal herb. It is believed to have originated in Persia and introduced in India. It is predominantly grown in India, Pakistan, Bangladesh, Persia, Mexico and Mediterranean regions due to its seed mucilage, pharmaceutical, cosmetics and food grade properties. The genus Plantago includes over 200 species cultivated globally, but only 10 are commonly found in India. Among these, blond psyllium (*Plantago ovata* Forsk.), known for its high-quality husk.

India is dominating the world market in production and export of psyllium husk powder. It provides approximately 80% of psyllium husk powder in the world market. About 90-95% of India's isabgol production is exported. The area under isabgol cultivation in India is 4.5 lakh hectares with a production of 4.32 lakh metric tonnes (Anonymous, 2024).

Isabgol is cultivated under Rabi season and easily grown under hot weather and saline soil consitions but require cool and dry weather during its crop season. It takes roughly 120 days to mature (November to Feb-March) (Jat *et al.*, 2015). Psyllium is a naturally occurring substance that is water soluble and it has been traditionally used in

Indian and Chinese herbal medicine to treat various conditions such as skin irritations, high blood pressure, constipation, diarrhoea, chronic dysenteries of amoebic and bacilliary origin and ulcerated surface of intestinal mucosa (Fernandez., 2006). The swelling and gelatinous mass properties of psyllium make it suitable for use in specific drug delivery systems as well as absorption. Psyllium has also been reported to possess cholesterol-lowering abilities and wound healing properties (Taneja *et al*., 1989 and Tomar *et al.*, 2010).

One of the primary factors diminishing Isabgol yield is the attack of diseases such as leaf blight (*Alternaria alternata*), wilt (*Fusarium* sp.), damping off (*Pythium ultimum*), and downy mildew (*Peronospora plantaginis*), as reported by Patel *et al*. (1984), Russel (1975), Kapoor and Choudhary (1976), and Richardson (1990), respectively. Among these, leaf blight caused by *Alternaria alternata* has emerged as a particularly serious issue in recent years. It has been found that downy mildew affected crops are more prone to be attacked by *Alternaria alternata*. This disease causes considerable damage every year and sometimes becomes very severe, resulting the total yield loss. Leaf blight in psyllium affects the crop at all stages of growth. Initially, infected plants develop small spots with a loss of chlorophyll. These spots gradually expand, covering larger areas, and the affected portions change color from light brown to dark brown and black. Necrosis of the affected areas also occurs (Patel *et al*., 1984; Meena and Maharshi, 2013b).

The necrotrophic nature of *Alternaria* species often results in significant damage to plants and their harvest, with seedlings rarely surviving an attack (Humpherson-Jones, 1985; Rimmer and Buchwaldt, 1995; Mamgain *et al*., 2013; Dhaka *et al*. 2022b). *Alternaria* is easily identifiable by its conidia, which are large, ovoid to obclavate, dark-colored (melanized), and multicellular with both longitudinal and transverse septations (Barnett, 1998). These conidia are produced in chains, broadest near the base, and tapering gradually to an elongated beak (Dube, 2013). It is known that members of the genus *Alternaria* produce a variety of phytotoxic metabolites that affect many of the plants on which the fungi grow on (Bruce *et al.* 1984). These phytotoxins have a wide range of impacts on metabolism and biological processes include tenuazonic acid (TA), alternariol, alternariol monomethyl ether (AME), alternaric acid, altenuene, altenuic acid, tentoxin, AK-toxin and AAL-toxin (Nishimura and Kohmoto, 1983). Therefore, this study aims to identify and evaluate the efficacy of various rhizosphere microorganisms in inhibiting the growth of *Alternaria alternata*, with the ultimate goal of developing effective biological control strategies for managing Alternaria leaf blight in Isabgol.

## MATERIALS AND METHODS

All microorganism isolated from the rhizosphere of Isabgol were evaluated for their ability to show antagonistic effect against *Alternaria alternata* by Dual Culture Technique. Fungal microorganisms were screened for their antagonistic activity in dual culture on PDA in Petri plates (Johnston and Curl, 1972). Both microorganisms and pathogen were inoculated at the same time and 5 mm bit of young vigorously growing cultures were placed at the opposite points in Petri plates 40 mm apart from each other. In three replicates and incubated at 25± 2° C. The interactions were observed up to seventh day of incubation between rhizosphere fungal and bacterial microflora and the pathogen. In case of control, the Petri dishes were inoculated with mycelial disc of the test pathogen only. The mycelial growth of test pathogen was measured after 7 days of inoculation. Per cent growth inhibition was calculated by using the formula given by Vincent (1947).

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

Where, I = Inhibition per cent

C = Colony diameter (mm) in control plate

T = Colony diameter (mm) in treated plate

## RESULTS AND DISCUSSION

### Rhizosphere/rhizoplane microorganisms of Isabgol

A total of 17 fungi and 3 bacteria were isolated from rhizosphere /rhizoplane of Isabgol plants (Table 1). Fungi isolated were *Rhizoctonia solani, Drechslera spp, Aspergillus glaucus, Claviceps purpurea, Alternaria solani, Aspergillus flavus, Fusarium spp., Sclerotium rolfsii , Aspergillus parasiticus, Sclerotia sclerotiorum, Colletotrichum lindemuthianum, Aspergillus nidulans, Colletotrichum gloeosporioides,*

**Table 1: Microorganism isolated from the rhizosphere and rhizoplane of Isabgol**

| S. No. | Microorganisms isolated | S. No. | Microorganisms isolated |
|---|---|---|---|
| 1. | *Rhizoctonia solani* | 12 | *Aspergillus nidulans* |
| 2 | *Drechslera spp* | 13 | *Xanthomonas spp.* |
| 3 | *Aspergillus glaucus* | 14 | *Colletotrichum gloeosporioides* |
| 4 | *Claviceps purpurea* | 15 | *Trichoderma viridae* |
| 5 | *Alternaria solani* | 16 | *Trichoderma harzianum* |
| 6 | *Aspergillus flavus* | 17 | *Pseudomonas fluorescens* |
| 7 | *Fusarium spp.* | 18 | *Bacillus subtilis* |
| 8 | *Sclerotium rolfsii* | 19 | *Aspergillus niger* |
| 9 | *Aspergillus parasiticus* | 20 | *Trichoderma spp.* |
| 10 | *Sclerotia sclerotiorum* | 21 | Control |
| 11 | *Colletotrichum lindemuthianum* | | |

**Table 2 : Effect of microorganisms on the growth of *Alternaria alternata* in culture**

| S. No. | Microorganisms | Mycelial growth (mm)* | Mycelial growth inhibition (%) | Effect on pathogen |
|---|---|---|---|---|
| 1 | *Rhizoctonia solani* | 25.05 (30.02) | 72.16 | + |
| 2 | *Drechslera spp* | 33.42 (35.30) | 62.86 | - |
| 3 | *Aspergillus glaucus* | 37.42 (37.69) | 58.42 | - |
| 4 | *Claviceps purpurea* | 42.28 (40.54) | 53.22 | + |
| 5 | *Alternaria solani* | 54.53 (47.58) | 39.41 | - |
| 6 | *Aspergillus flavus* | 23.41 (28.91) | 73.98 | - |
| 7 | *Fusarium spp.* | 25.83 (30.53) | 58.42 | - |
| 8 | *Sclerotium rolfsii* | 37.10 (37.51) | 58.77 | + |
| 9 | *Aspergillus parasiticus* | 29.49 (32.87) | 67.23 | - |
| 10 | *Sclerotia sclerotiorum* | 32.26 (34.59) | 64.15 | + |
| 11 | *Colletotrichum lindemuthianum* | 18.24 (25.26) | 79.73 | + |
| 12 | *Aspergillus nidulans* | 35.69 (36.66) | 60.34 | - |
| 13 | *Xanthomonas spp.* | 32.18 (34.54) | 64.24 | - |
| 14 | *Colletotrichum gloeosporioides* | 29.74 (33.03) | 66.95 | - |
| 15 | *Trichoderma viridae* | 14.49 (22.36) | 83.9 | + |
| 16 | *Trichoderma harzianum* | 21.59 (27.67) | 76.01 | + |
| 17 | *Pseudomonas fluorescens* | 44.19 (41.64) | 50.9 | - |
| 18 | *Bacillus subtilis* | 39.35 (38.83) | 56.27 | - |
| 19 | *Aspergillus niger* | 33.42 (35.30) | 62.86 | + |
| 20 | *Trichoderma viridae* | 29.20 (32.68) | 67.55 | + |
| 21 | Control | 90.00 (71.53) | 0.0 | |
| | **S.Em±** | **0.460** | | |
| | **CD (*p=0.05*)** | **1.318** | | |

*Mean of three replications
Figures in parentheses are angular transformed values
+ = Microorganism over grew on pathogen (*A. alternata*) slightly
- = no effect on pathogen (*A. alternata*)

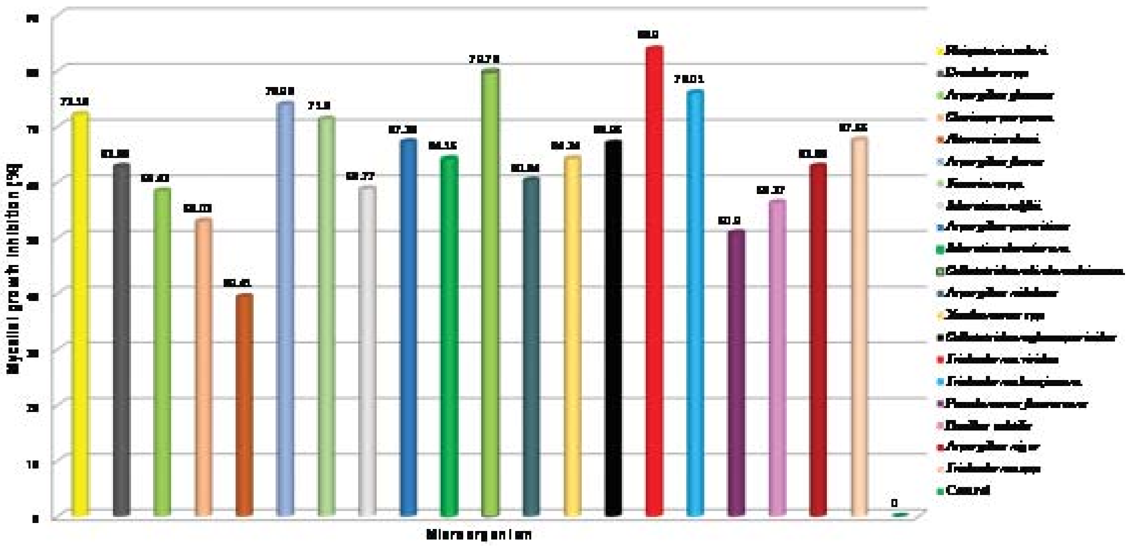

**Fig. 1 : Screening of rhizosphere/rhizoplane microorganism for antagonism against pathogen**

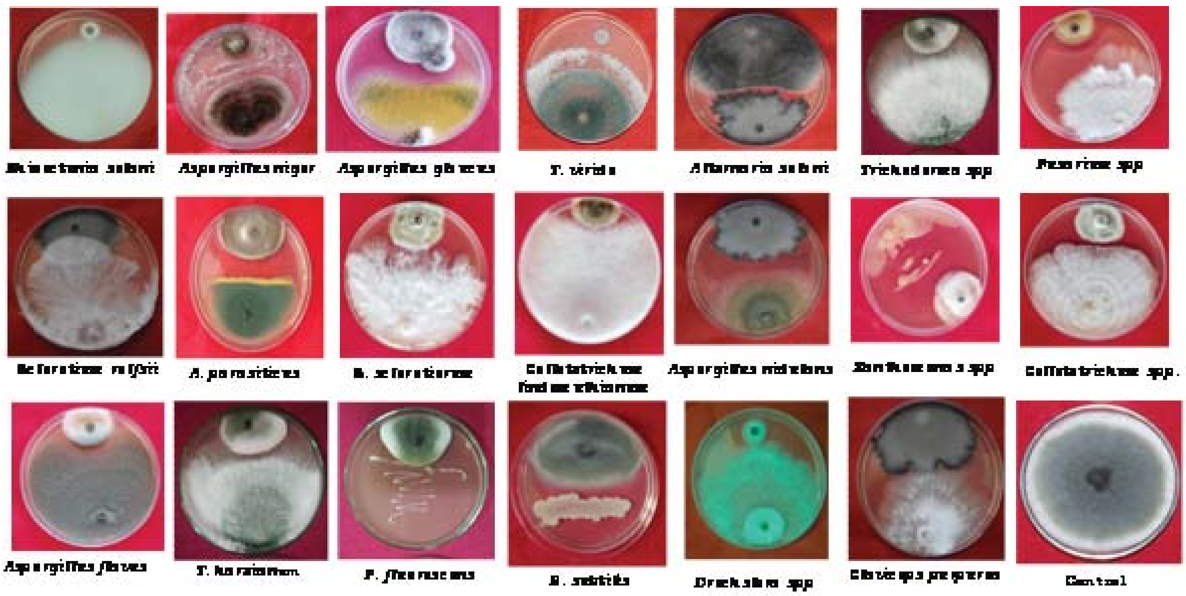

**Plate 1 : Screening of rhizosphere/rhizoplane microorganism for antagonism against pathogen**

*Trichoderma viridae, Trichoderma harzianum, Aspergillus niger, Trichoderma* spp. Bacteria were identified as *Bacillus subtilis, Xanthomonas* spp. and *Pseudomonas fluorescens*.

### Antagonistic effect of rhizosphere/rhizoplane microorganisms on pathogen *in vitro*

All the microorganisms isolated from the rhizosphere/rhizoplane of Isabgol were tested for their antagonistic effects against *A. alternata* by dual culture and streak plate method in case of fungi and bacteria respectively. Observations on types of interactions started from 2$^{nd}$ day of inoculation and continued up to 7$^{th}$ day. Results are summarized in (Table 2, Fig. 1 and Plate 1)

Results showed a significant variation in the reduction of the growth of the pathogen in the presence of microorganisms (Table 2). Among the antagonists, *Trichoderma viride* (83.9%) was found to be the most effective by recording the maximum inhibition of mycelial growth of the pathogen followed by *Colletotrichum lindemuthianum* (79.73%), *Trichoderma harzianum* (76.01%), *Aspergillus flavus* (73.98%), *Rhizoctonia solani* (72.16%), *Fusarium* spp. (71.30%), *Trichoderma* spp. (67.55%), *Aspergillus parasiticus* (67.23%) and *Colletotrichum gloeosporioides* (66.95%).

These fungi gave more than 65 per cent growth inhibition. Their growth rate was very fast and arrest and overgrew the pathogen within 7 days of incubation. Rest of the fungi was also antagonistic to pathogen but in their presence the growth inhibition of the *A. alternata* was less than 65 per cent and transparent inhibition zone was formed in two fungus between antagonistic fungi and the pathogenic fungus. The antagonists either overgrew the pathogen or just met at the margin. The three bacteria also inhibit the growth of the pathogen. Therefore, except the *Trichoderma harzianum, Fusarium oxysporium. Rhizoctonia solani, Trichoderma virens* and *A. niger* all other microorganism were discarded and not used in disease control studies.

Ngo *et al.* (2021) discovered that *A. candidus* and *A. montenegroi*, isolated from a marine environment, exhibit biocontrol potential for the first time. While various secondary metabolites and their biological activities have been documented from *A. candidus*, there is limited information on its efficacy in controlling plant diseases. Additionally, Choudhary *et al.* (2021) found that both *T. viride* and *T. harzianum* are highly effective in reducing the radial growth of *Alternaria solani*. All microorganisms isolated from rhizospheric soil show potential growth when treated with *Trichoderma* and other bioagents (Saran *et al*., 2021; Bairwa *et al*., 2022; Jangir *et al.*, 2022; Terki *et al*., 2023).

Although various workers have reported formation of clear cut inhibition zones by these soil microbes due to antibiosis or by the production of lytic enzymes (Wright, 1956; Smith, 1957; Papavizas, 1985; Upadhyay and Rai, 1987; Dhaka *et al*., 2022b). Philip *et al.* (2024) reported that *Trichoderma afroharzianum metabolites* inhibit *A. alternata* growth and induce tomato defense-related enzymes.

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## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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