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Phylloplane microflora diversity of cucumber and their antagonistic activity against powdery mildew

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Abstract

The diversity of phylloplane microflora of cucumber was studied during the 2021-2022 growing season. Eight microorganisms comprising *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp., *Trichoderma* sp., and three isolates of bacterial were recovered from the leaf surface of cucumber. Among these, *Fusarium*, *Aspergillus*, *Penicillium*, and the bacterial isolates exhibited the highest frequency of occurrence, whereas *Trichoderma* sp. appeared at a lower frequency. The antagonistic potential of the isolated microflora against the powdery mildew pathogen was evaluated under *in vitro* conditions using the slide spore germination technique. All culture filtrates significantly reduced conidial germination of the pathogen. The maximum inhibition of conidial germination (22.50%) was observed with *Trichoderma* sp., followed by *Alternaria* sp., *Fusarium* sp., and the bacterial isolates. The least inhibitory in recorded with *Aspergillus* sp. and *Penicillium* sp.

Keywords: Microflora, phylloplane, powdery mildew, cucumber

Introduction

Cucumber (*Cucumis sativus* L.), belonging to the family Cucurbitaceae, is an important vegetable crop cultivated extensively worldwide. The family comprises approximately 120 genera and 825 species exhibiting wide morphological diversity. Cucumber is believed to have originated in Southern Asia (Gangadhara *et al.*, 2019) [1], with India considered one of its primary centres of diversity. It is valued for its tender, soft fruits, which are commonly consumed fresh as salads, cooked as a vegetable, or pickled at the immature stage. The fruits possess cooling properties and are used as astringents and antipyretics, while both fruits and seeds are known to alleviate constipation, jaundice and indigestion. Cucumber fruits also contain nearly 95 per cent water, a range of antioxidants including carotenes, lutein and zeaxanthin, and provide 0.4 per cent protein and 3.63 g/100 g edible carbohydrates (Yadav *et al.*, 2020) [4]. Considerable variation exists in fruit shape, size and colour among cultivated varieties.

Globally, cucumber production reached approximately 94.7 million tonnes in 2022-23. China remains the largest producer, contributing nearly 83-84 per cent of global production with 72.77 million tonnes, whereas India ranks 27th worldwide (FAOSTAT, 2023) [2]. In India, cucumber is grown on 1.16 thousand hectares with an annual production of 2.05 million tonnes (Horticultural Statistics, 2021-2022). Haryana, Karnataka, Uttar Pradesh, Assam, Madhya Pradesh and Andhra Pradesh are among the leading cucumber-producing states (NHB, 2023).

Phylloplane microorganisms associated with cucumber foliage play a vital role in the ecological balance of the leaf surface and may directly influence foliar diseases, including powdery mildew. Natural antagonists present on the phylloplane are known to suppress powdery mildew fungi on several crops by exploiting mechanisms such as competition, antibiosis and mycoparasitism. Earlier studies have reported diverse antagonists including *Acremonium* spp., *Ampelomyces* spp., *Penicillium* spp., *Cladosporium* spp., *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp. and several yeasts effectively inhibiting powdery mildew pathogens on oak, dogwood and other hosts (Mmbaga *et al.*, 2008; Topalidou and Shaw, 2015) [6]. Significant *in vitro* inhibition of *Erysiphe polygoni* by *C. cladosporioides*, *A. alternata*, *Fusarium* spp. and *Aspergillus* spp. has also been reported (Suman, 2008) [7].

Given the increasing concerns over fungicide resistance, environmental hazards and the breakdown of host resistance, the study of phylloplane microflora has gained considerable importance for developing sustainable disease management strategies. Indigenous fungal and bacterial populations on leaf surfaces have been shown to effectively suppress pathogens by reducing inoculum potential and thereby minimizing disease severity (Maji, 2003) [8].

The present study was therefore undertaken to document the diversity and frequency of occurrence of phylloplane microflora associated with powdery mildew-infected cucumber leaves and to evaluate the antagonistic efficacy of culture filtrates of these isolates against the cucumber powdery mildew pathogen under *in vitro* conditions.

Materials and Methods

Isolation of Phylloplane Microflora from Powdery Mildew Infected Cucumber Leaves

Isolation of phylloplane microflora was carried out from powdery mildew infected cucumber leaves using the leaf wash method. The leaf washings were plated on potato dextrose agar (PDA), malt extract agar (MEA) and nutrient agar (NA) media in sterilized Petri plates. The plates were incubated at 25 ± 1°C for the development of microbial colonies. Fungal cultures were purified by the hyphal-tip method, while bacterial cultures were purified by repeated streaking. The purified fungal and bacterial cultures were maintained on PDA and nutrient agar slants, respectively and stored under refrigerated conditions for further studies.

Preparation of Culture Filtrates from Phylloplane Microflora: Culture filtrates were prepared from fungal isolates including *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp. and *Trichoderma* sp., as well as three bacterial isolates. Fungal isolates were cultured in potato dextrose broth (PDB) and incubated at 20-25°C for 15 days, while bacterial isolates were grown in nutrient broth (NB) for 3 days. Following incubation, the cultures were centrifuged at 10,000 rpm for 20 minutes to separate the biomass. The supernatant was collected, and the cell-free filtrate was sterilized by passing through a 0.2 µm membrane syringe filter. The resulting sterile culture filtrates were used for *in vitro* antagonistic assays.

In vitro Evaluation of Antagonistic Activity by Slide Spore Germination Test

A double-strength solution of each culture filtrate was prepared using sterilized distilled water. Simultaneously, spore suspension of the cucumber powdery mildew fungus (*Erysiphe cichoracearum*) was prepared by gently dislodging the conidia from freshly infected cucumber leaves, maintained at approximately 28-30 conidia per microscopic field. One drop of culture filtrate and one drop of spore suspension were mixed in the cavity of a cavity slide. The cavity slides were then placed on glass rods inside sterilized Petri plates containing sterilized distilled water at the bottom and sterilized moist cotton lining the inner surface of the lid to maintain humidity. The Petri plates were incubated in a growth chamber at 25 ± 1°C with 85 per cent relative humidity. The experiment was laid out in a Completely Randomized Design (CRD) with three replications for each treatment. Observations on conidial germination were recorded after 24, 36, 48, 60 and 72 hours using a light microscope. The percent inhibition of conidial germination was calculated by adopting the formula given by Vincent (1947) [9]:

$$I = \frac{C - T}{C} X\,100$$

Where, I = Per cent inhibition, C = Germination of conidia in control, T = Germination of conidia in treatment.

Results and Discussion

Frequency of Occurrence of Cucumber Phylloplane Microflora

Among the isolated phylloplane microorganisms, *Fusarium*, *Aspergillus* and *Penicillium* spp., together with the bacterial isolates as represented in Fig.1, recorded the highest frequency of occurrence and were consistently recovered from most of the cucumber leaf samples throughout the study period. Their repeated isolation indicates that these genera form a stable and dominant component of the cucumber phylloplane microbiome. In contrast, *Trichoderma* sp., although present, appeared much less frequently and was isolated only occasionally. This comparatively lower occurrence suggests that *Trichoderma* may constitute a minor yet functionally important part of the phylloplane community, particularly due to its known antagonistic and mycoparasitic abilities despite its lower population density.

These findings are consistent with previous studies reporting diverse phylloplane microorganisms, including Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium, and Trichoderma spp., isolated from various crops (Yeasmin & Shamsi, 2013) [11]. Tesfagiorgis and Laing (2010) [11] similarly documented extensive bacterial, fungal, and yeast diversity on the phylloplane of powdery mildew-infected zucchini plants. Additionally, several phylloplane fungi, including *Acremonium* sp., *Ampelomyces* sp. and *Trichoderma* sp., have been associated with powdery mildew suppression in oak (Topalidou & Shaw, 2015) [6].

In vitro Evaluation of Antagonistic Activity against *Erysiphe cichoracearum*

The antagonistic activity of isolated microorganisms was evaluated using a slide spore germination test, and the results are presented in Table 1. All culture filtrates exhibited varying levels of inhibition, with statistically significant differences among treatments. Trichoderma sp. demonstrated the highest conidial germination inhibition (22.50%), consistent with its well-documented role as a potent mycoparasite and antagonist through antibiotic production, lytic enzyme secretion, and competitive exclusion. Moderate inhibition was observed with Alternaria sp. (data not shown), followed by Fusarium sp. and Bacterial isolate 2. The lowest inhibition levels were recorded for Aspergillus sp. (2.50%), Bacterial isolate 3 (2.66%), Penicillium sp. (5.00%), Bacterial isolate 1 (6.83%), and Fusarium sp. (8.33%).

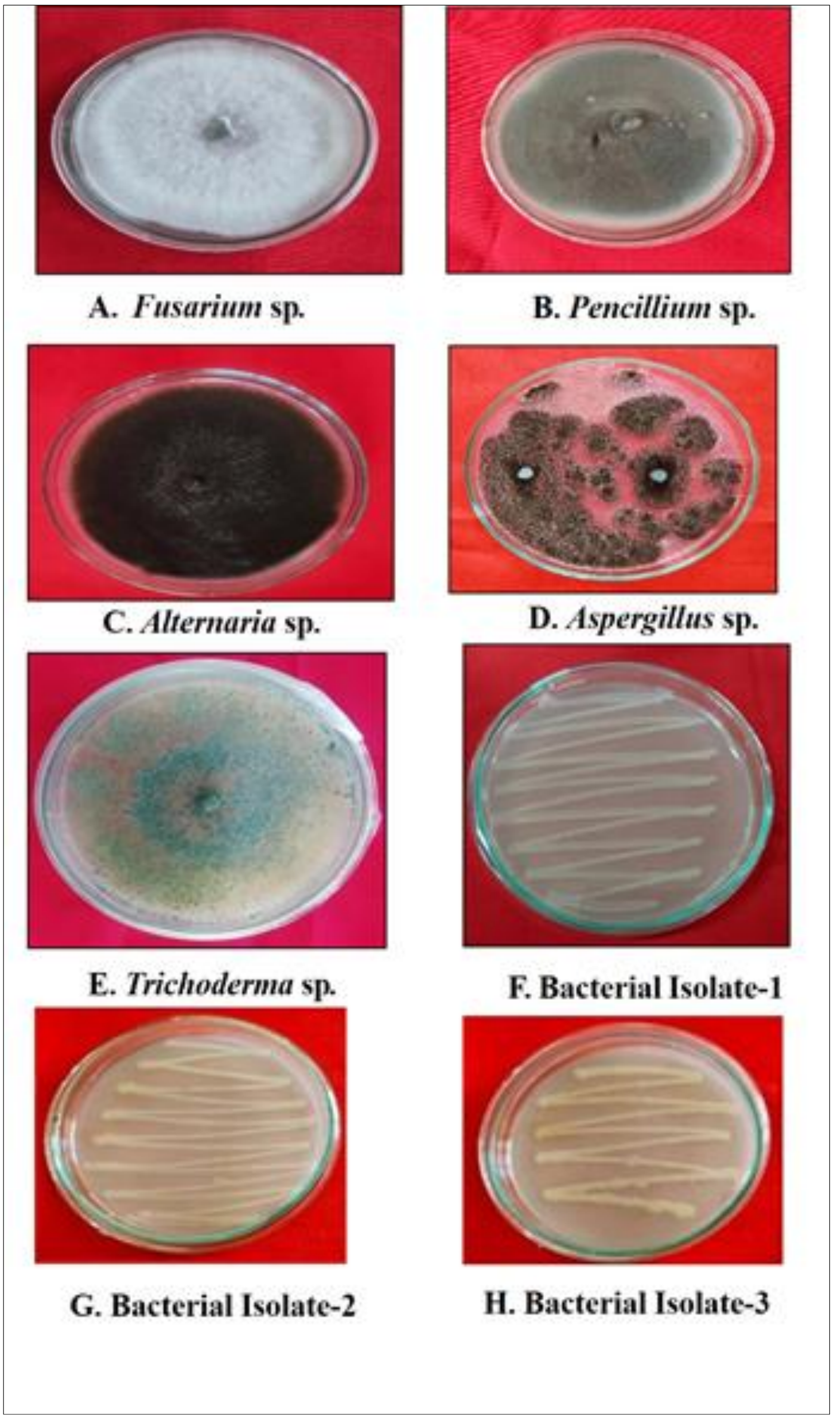

Fig 1: Microflora were isolated from the phylloplane of cucumber leaves

Table 1: *In vitro* evaluation of culture filtrate of isolated phylloplane microbiome on conidial germination inhibition (%)

Microflora	Conidia Germination inhibition (%)
Fusarium sp.	8.33 (3.04) *
Aspergillus sp.	2.50 (1.87)
Alternaria sp.	14.16 (3.89)
Trichoderma sp.	22.50 (4.84)
Penicillium sp.	5.00 (2.41)
Isolate 1 (Bacteria)	6.83 (2.79)
Isolate 2 (Bacteria)	11.45 (3.52)
Isolate 3 (Bacteria)	2.66 (1.91)
C.D.	0.39
SE(m)	0.13
SE(d)	0.18

*Figures in parentheses are square root transformed values

The findings of the present investigation are in close agreement with earlier reports on phylloplane microflora associated with powdery mildew. Tesfagiorgis and Laing (2010) [11] reported the isolation of nearly 2,000 fungal, yeast and bacterial isolates from the phylloplane of powdery mildew-infected zucchini plants. Similarly, Yeasmin and Shamsi (2013) [11] isolated a wide range of fungi, including *Trichoderma viride*, *Aspergillus flavus*, *A. terreus*, *Alternaria citrii*, *Colletotrichum* sp., *Fusarium nivale* and *Rhizopus stolonifer* from the phylloplane of Gerbera. Topalidou and Shaw (2015) [6] also isolated diverse microorganisms such as *Acremonium* sp., *Trichoderma* sp., *Ampelomyces* sp., *Leptosphaerulina* sp. and *Phoma* sp. from the phylloplane of oak plants infected with powdery mildew. Furthermore, Kumar and Chandel (2018) [13] reported the association of *Fusarium* sp., *Botrytis* sp., *Cladosporium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp., *Trichothecium* sp. and *Trichoderma* sp. with powdery mildew on rose and also studied their role in reducing disease severity. These earlier reports strongly support the diversity and functional importance of phylloplane microflora observed in the present study.

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