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Investigation of Phylloplane Microflora of Banana

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ABSTRACT: To fully understand the microbial potential of bioagents on the banana's phylloplane with the goal to use the right phylloplane microflora to manage banana diseases efficiently. The experiment featured a completely randomized design and had been carried out in a laboratory environment. To do this, the phylloplane microflora associated with healthy and diseased leaves were studied using a modified leaf washing strategy. The dual culture approach was used to evaluate the isolated phylloplane microflora's ability to combat banana pathogens in vitro. Two bacteria, Bacillus sp. and Pseudomonas sp., and four fungus, *Colletotrichum* sp., *Trichoderma* sp., *Aspergillus niger*, and *Penicillium* sp., were recovered from the phylloplane microflora of both healthy and diseased banana leaves. By using the dual culture method, five phylloplane microflora were tested for their antagonistic effect against *Colletotrichum* sp. The results indicated that the treatment of *Trichoderma* sp. (9.75 mm) resulted in the significantly least growth of the pathogen, as the *Trichoderma* sp. overgrew the *Colletotrichum* sp. colony, preventing further growth and sporulation. *Trichoderma* sp. treatment (88.95%) and *Aspergillus niger* treatment (81.30%) showed the greatest growth inhibition of *Colletotrichum* sp. Incredibly varied and diverse fungal species were found on leaf surfaces which can be used as effective as a bio control for effective management of plant diseases. So from this study it can be stated that *Trichoderma* sp. found from banana phylloplane were found effective bio agent against *Colletotrichum* sp.

Keywords: *Phylloplane Microflora, Banana, Trichoderma* sp., *Colletotrichum* sp.

INTRODUCTION

The impact of pathogen-induced disease on plants and plant parts is typically significant, resulting in a significant loss of both quantity and quality each year. Phylloplane denotes to the plant's leaf surface. Because of the physiochemical and structural characteristics of the leaf as well as the activity of other microorganisms, the environment on the leaf surface is extremely dynamic Esser *et al.* (2015). Leaf physiology and the microenvironment support the distribution and abundance of microorganisms on the leaf surface is described by the arrangement of leaf epidermal cells. The success or failure of plant diseases is mostly determined by the type and result of intra- and inter-specific competition among leaf surface microorganisms. Phylloplane microflora's ability to restrict pathogens has grown in importance and could aid in the management of plant disease. In order to effectively manage banana diseases by using the right phylloplane microflora, the current study is to explore the microbiological potential of bio agents on the banana's phylloplane.

MATERIAL AND METHODS

A. Collection of healthy and diseased leaves

Both healthy and diseased banana leaves from plants that were 6 to 8 months old banana field from Navsari Agricultural University, Navsari. Five plants were marked for both healthy and diseased leaves, and three leaves from each plant—the top, middle, and bottom—were carefully cut with sterile forceps before being placed in plastic bags and taken to the lab. The individual healthy leaves were incorporated to create a composite sample of healthy leaves, and the same procedure was used for the diseased leaves.

B. Isolation of phylloplane microflora from healthy and diseased leaves

Both healthy and diseased leaves have been analyzed for phylloplane microbes using a modified leaf washing method. Using a sterile cork borer, a disc with a diameter of one centimeter was randomly cut from both healthy and sick leaves. To create a uniform suspension, fifty discs from both healthy and sick leaves were put in a 250 ml conical flask with 100 ml of sterile distilled water. The flask was then shaken for 15 to 20 minutes. A sterile and solidified Petri plate containing Potato Dextrose Agar (PDA) for fungal isolation, nutritional agar for bacteria, and Actinomycetes agar for actinomycetes were filled with a 1 ml suspension that was previously pipetted out. Using a spreader, the suspension was evenly distributed throughout the Petri plates, which were then allowed to remain at room temperature for three to five days. Various bacterial and fungal colonies were seen during incubation, and pure cultures were prepared for further study.

C. Isolation of pathogen from infected leaves
The conventional tissue isolation technique was used to isolate the banana fungal pathogen. After being surface sterilized for one minute with 0.1% $HgCl_2$, small pieces of diseased leaves were aseptically added in sterile Petri plates with sterilized and solidified PDA after being rinsed three times with sterile distilled water. For six to seven days, the plates were incubated at room temperature. The fungal colonies were observed and kept in pure culture after incubation.

D. Identification of phylloplane microflora
Standard microbiological procedures were used to identify the microbial colonies that were isolated from phylloplane. The identification of microbes were based on morphological and cultural observations.

E. In vitro evaluation of isolated microflora from phylloplane against leaf pathogen of banana
By using the dual culture approach, isolated phylloplane microflora were evaluated in vitro against banana pathogens (Dennis and Webster 1971). The test organisms and pathogen were grown on their respective media separately. A 5 mm diameter disc of the test organism (phylloplane microflora) and pathogen were aseptically inoculated by positioning 5 mm diameter culture blocks 70 mm apart. For every treatment, three replications were kept, and the control group consisted of Petri plates containing only the pathogen. After 6-7 days of incubation at 28 + 2°C, the radial growth of the pathogen and phylloplane microflora were measured on each plate. The per cent growth inhibition (PGI) was calculated by using the formula given by Vincent (1927).

$$\text{PGI} = \frac{100(\text{DC} - \text{DT})}{\text{DT}}$$

Where,
PGI = Per cent growth inhibition,
DC = Average diameter of mycelial colony of control set
DT = Average diameter of mycelial colony of treated set

RESULTS AND DISCUSSION

A. Collection and isolation of phylloplane microflora from healthy as well as diseased leaves of banana and of pathogens
When compared to healthy banana leaves, the overall number of microbial communities on diseased banana leaves was greater (Fig. 1). From the phylloplane microflora of both healthy and diseased banana leaves, a total of four fungus and two bacteria were recovered. *Aspergillus niger*, *Colletotrichum* sp., *Penicillium* sp., and *Trichoderma* sp. were the four fungi that were isolated. Three of the four distinct fungi—*Aspergillus niger*, *Penicillium* sp., and *Trichoderma* sp.—were identified as phylloplane mycoflora. A single fungus, *Colletotrichum* sp., was identified as a banana pathogen. Two bacteria were identified as phylloplane bacteria: *Bacillus* sp. and *Pseudomonas* sp (Table 1 and 2).

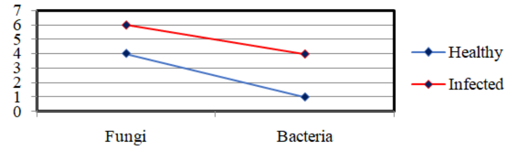

Fig. 1. Total number of colonie estimated as phylloplane microflora isolated from banana leaves.

Table 1: Total number of microbes/cm^2 of healthy and diseased leaves of banana.

	Fungi	Bacteria
Healthy	1.27	0.38
Diseased	2.29	0.76

Table 2: Microbial frequency (%) from each of the healthy and diseased sample of banana.

Microflora	Healthy	Diseased
Colletotrichum sp.	0	29.17
Trichoderma sp.	07.69	20.83
Aspergillus niger	30.77	12.50
Penicillium sp.	30.77	12.50
Bacillus sp.	07.69	08.33
Pseudomonas sp.	15.38	16.67

B. Identification and characterization of phylloplane microflora
The isolated fungi's microscopic and cultural traits were examined and contrasted with existing research (Table 3). Biolog was used for bacterial identification.

Table 3: Cultural and morphological characteristics of isolated phylloplane microflora from banana.

Sr. No.	Name of the phylloplane microflora	Cultural characteristics of microflora
1	*Aspergillus niger*	Colonies are white at first and soon becoming black upon conidial production. Back side of colony is pale yellow and generate radial pattern. Conidial heads up to 3 mm × 15 to 20 µm in diameter, conidia 3.5 to 5 µm
2	*Trichoderma* sp.	Colonies are initially white which turn into greenish in colour with concentric rings and granular. Conidia measured 4.0 × 3.5 µm with flask shaped phialides
3	*Penicillium* sp.	Colonies grow rapidly with dark green color and granular powdery. The back side of colony was yellow. Mycelium 2.5 µm to 4.5 µm, single celled conidia 3.5 µm to 5 µm in diameter
4	*Colletotrichum* sp.	Colonies are initially white which later turning black in colour. Conidia measured unto 51.35 to 95 × 4.5 to 7.1 µm
5	*Bacillus* sp.	Colonies are flat or slightly convex with irregular edges
6	*Pseudomonas* sp.	Colonies are small, rough, strongly cohesive

C. In vitro evaluation of isolated microflora against Colletotrichum sp.

Using the dual culture approach, the antagonistic effect of five phylloplane microflora against *Colletotrichum* sp. was evaluated *in vitro*.

According to the data shown in Table 4, *Trichoderma* sp. (9.75 mm) showed the pathogen's least amount of growth, and *Colletotrichum* sp. colony was overgrown by *Trichoderma* sp. *Aspergillus niger* (16.50 mm) and *Penicillium* sp. (40.50 mm) were next in merit. *Trichoderma* sp. (88.95%) showed the highest growth inhibition of *Colletotrichum* sp., followed by *Aspergillus niger* (81.30%) and *Penicillium* sp. (54.11%). According to the study, the best phylloplane mycoflora for preventing the mycelial growth of *Colletotrichum* sp. isolated from bananas is *Trichoderma* sp. In terms of comparison, the other phylloplane microorganisms were not as effective in preventing mycelial growth of *Colletotrichum* sp.

Balakrishnan Nair and Wilson (1976) observed that on the upper, middle, and lower leaves of infected plants, there were more fungus, bacteria, and actinomycetes overall than on the leaves of healthy plants during the monitoring period. El-Said, Ahmed (2001) found *Alternaria, Aspergillus, Chaetomium, Cladosporium, Cochliobolus, Curvularia, Giberella, Memnoniella, Mycosphaerella, Setosphaeria,* and *Stachybotrys* were the most prevalent fungus from banana phylloplane. Dewada *et al.* (2024) isolateda total of 28 morphologically distinct bacterial isolates in pure culture from collected samples of banana leaves.

Table 4: Evaluation of isolated phylloplane microflora against *Colletotrichum* sp. of banana in *In vitro.*

Sr. No.	Name of the Phylloplane microflora	*Colletotrichum* sp.	
		Average mycelium growth (mm)	Growth inhibition (%)
1.	*Aspergillus niger*	16.50	81.30
2.	*Penicillium* sp.	40.50	54.11
3.	*Trichoderma* sp.	9.75	88.95
4.	*Bacillus* sp.	70.00	20.68
5.	*Pseudomonas* sp.	60.00	32.01
6.	Control	88.25	
	S.Em.	0.84	
	CD	2.50	

CONCLUSIONS

Studies of the banana's phylloplane microflora revealed that there were more fungi isolated than bacteria. When comparing the phylloplane microflora of diseased and healthy banana leaves, the diseased ones had a significantly higher population than the other one. *Trichoderma* sp. demonstrated a high level of antagonistic activity in the phylloplane microflora's antagonistic activity against the banana pathogen, directly inhibiting the growth of the pathogen. Therefore, it is possible to use *Trichoderma* sp. as antagonists against banana foliar pathogens. Further these effective phylloplane microflora can be mass multiplied and can be assessed at field level.

FUTURE SCOPE

Phylloplane microbes can be used as bio agents because they have anti-bacterial and anti-fungal properties. In future, phylloplane microflora can be directly used to induce systemic resistance in plants.

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