



**ISOLATION OF SOIL MICROFLORA AND ANALYSIS OF THEIR
ANTIMICROBIAL ACTIVITY AGAINST PLANT PATHOGEN**

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ABSTRACT

The soil is the primary supply of food and energy for all living things. The soil is an inordinate source of microorganisms and they carry out one of the major duties of soil that is to start and enhance the growth of plants and crops. However, the harvests are often harmed and spoilt by plant infections in a large region that grows under ideal circumstances and during climate change. The most of the recent work has undertaken using soil bacteria to fight against plant infections. This might infer that, pathogenic fungi are the primary cause of many diseases that result in considerable output losses. The crop growers frequently employ synthetic fungicides to both avoid and treat plant diseases. The multiple available forms of fungicides have been overused and used carelessly, causing environmental damage. Therefore, there is a high growth in industries which provide agricultural goods that have been grown naturally, without the usage of chemical fertilizers

or pesticides. In the present research one of the biological approaches is explored to resist the growth of fungal plant pathogen. The various soil bacterial strains were isolated from the maize crop and tested to possess antimicrobial activity against *Aspergillus* genus strain, which is potent plant pathogen of tomato. *Aspergillus* causes Black rot diseases to tomato and so the fungal sample was isolated from the rotten tomato. To observe the antifungal properties against *Aspergillus* the *trichoderma*, *pseudomonas* etc... bacterial strains were used. The findings of study revealed that *Staphylococcus*, *Streptococcus* and *Pseudomonas* were capable to possess the high potential to resist and cidal activity against *Aspergillus*.

Keywords: Soil suppressiveness, Microbial analysis, *Aspergillus*

INTRODUCTION

Current research on soil-beneficial microbial species revealed that the addition of fungi, actinomycetes, and other bacteria boosted plant tolerance and growth as well as the soil's capacity to prevent soilborne plant illnesses [1, 2]. The most important functions in ecological systems are carried out by soil microorganisms, which are incredibly plentiful in soil. Many of these species offer soil with the vital quality of suppressively, or the capacity to prevent the establishment of harmful agents [3]. All across the world, there exist agricultural soils that are resistant to soil-borne plant diseases, and certain of these soils' biological foundations have been established. Regardless of a powerful pathogen and a weak host are present, suppressive soils only moderately promote disease growth [4]. Suppressiveness is influenced by both anatomical and inanimate components of the soil environment. Many soils share traits with

regard to the microorganisms that fight illness, while others are exclusive to certain pathogen suppressive soil systems [4, 5]. Organic soils contain fluorescent *Pseudomonas* species and *Fusarium* species that is not hazardous and prevent plant pathogen wilt. The soil-borne pathogens *Phytium*, *Aspergillus*, and *phytoinsects* are a few examples that cause plant diseases and contribute significantly to the pollution of the environment in agriculture [5]. Plant pests are among the most important biotic agents harming agricultural cultivation, including pathogenic organisms, parasitic weeds, and diseases. *Aspergillus* species are responsible for a multitude of issues with plants and plant-based products [6]. They have the ability to contaminate agricultural products at a number of stages, including the period before harvesting, gleaning, manufacturing, and handling. Pigmentation, staining, rotting, the emergence of off-odors,

and off-flavor are several of the sensory, nutritive, and qualitative impacts of *Aspergillus* species deteriorating. They are opportunistic pathogens since they are typically found on plant-based products as storage mold [6, 7].

Fungus that grows on the stored items and the mycotoxins are a major source of worry for the agricultural and herbal medicine sectors. Despite the fact that many herbs and plants have been employed as medicines and food preservation for centuries, some of them exhibit antibacterial characteristics when combined and are used as alternatives to conventional antimicrobial agents. Because these substances are so hard to break down, animals are becoming more deadly as a result of their concentration or buildup in food chains [8]. To avoid fungus-related plant diseases, synthetic pesticides have been employed extensively. However, this use has resulted in the breeding of fungicide-resistant genotypes in addition undesirable effects on ecosystems and non-target organisms [9]. Biocontrol agents could reduce the demand for chemical products because they do not produce hazardous byproducts. The release of substances by soil microbes that interrupt the life cycle of the target based phytopathogens can be referred to as antagonism. Circumstances in a greenhouse and a field,

numerous *Trichoderma* isolates significantly reduce plant diseases brought on by pathogens such *Aspergillus*, *Gaeumannomyces graminivore*, *Sclerotium rolfsii*, *Pythium aphanidermatium*, *Fusarium oxysporum*, and *Rhizoctoniasolani Tritici* [9, 10, 11]. In this regard, both science and agriculture are very interested in the process of alternate strategies for dealing with plant infections [12, 13]. The aim of this study was to extract soil microorganisms and screen them for the capacity to block or limit the progression of fungal soilborne plant diseases using the genera *Aspergillus*, isolated from spoiled tomato was carried out [14].

MATERIALS AND METHODS:

Soil Sample Collection for non-pathogenic microbes

The loamy soil sample was collected from the maize crop farm situated in Balapur village, Rajkot, Gujarat, India (26.8403° N, 83.3542° E) during the rainy season to isolate the microorganisms which can resist the phytopathogens.

Isolation of non-pathogenic microbes

The non-pathogenic microbes from the loamy soil were isolated over the nutrient agar medium using serial dilution followed by consecutive spreading and streaking. The environmental factors were also considered and maintained. The temperature of the

incubation was 37°C and pH of media preparation was 7.5. The medium was incubated for 24 hours on the rotating shaker.

Isolation of Phytopathogenic Fungi

The pure culture of *Aspergillus niger* species was isolated from rotten tomato which was incubated under the moist atmosphere for 10 days for the development of black mold on it. The fungal sample was picked up from the tomato and grown on SDA medium for 7-10 days at room temperature for the isolation of pure culture.

Antifungal activity of bacterial isolates

The antifungal activity of isolates was carried out on Potato Dextrose Agar. The bacterial cultures were first spread on the media which was followed by inoculation of pathogenic fungi on the same plate using a sterile needle. Later, the plates were incubated at room temperature 4-7 days. The observation for change was done in every 24 hours.

Fungal identification

The primary identification of phytopathogenic fungi was carried out by Lactophenol cotton blue method in which Lactophenol cotton blue stain was utilized to stain the fungal isolate which was further studied under the microscope. Additionally, the Cellotape method was also used in which the cellotape inserted inside the pure colony of fungus with one finger and stucked this cellotape to the

slide properly and observe under the microscope [15].

RESULTS AND DISCUSSION

Isolation of non-pathogenic microbes

The non-pathogenic microorganisms which may resist the growth of fungi were isolated on nutrient agar plate. The bacteria isolated from the collected soil sample were recognized to belong to the species of *Pseudomonas*, *Escherichia*, *Bacillus*, *Staphylococcus*, and *Streptococcus* as per their Gram's staining characteristics (Table 1) and biochemical properties (Table 2).

Isolation and Identification of Phytopathogenic Fungi

The phytopathogenic fungal identification was carried out using the techniques Lactophenol cotton blue and Cellotape methods. When the fungi were grown over the PDA medium the fungal colonies were observed to be as brown rusty to black in color. The margins were irregular and sharp (Figure 1) [18]. The microscopic observation of fungal structure was done using Lactophenol cotton blue and Cellotape methods. The similar type of fungal structure was spotted under the microscope in both the experiments. The clear observation of messy bunch of hyphae were observed along with microsporangium and spores (Figure 2).

These physical and microscopic observations of fungal structures revealed that the fungi may belong to the species of *Aspergillus*. The *Aspergillus* is well known plant pathogen to cause the black mold diseases to legumes, fruits and vegetables in plants. Later, the activity of soil microorganisms was analyzed against the *Aspergillus* infection to the plants.

Antimicrobial activity of soil microbes on *Aspergillus*

The soil microbes possess antimicrobial activity against many phytopathogens. The

various microbes isolated from soil sample were able to resist the growth of potent phytopathogen belong to *Aspergillus* species. As shown in **Figure 3**, it was found that species of *Streptococcus*, *Staphylococcus*, *Pseudomonas* were potent strains to inhibit the growth of *Aspergillus*. However, the strains of *E. coli*, *Bacillus sp.* and *Salmonella sp* were unable to resist the growth of pathogenic fungi.

Table 1: Gram’s Staining of Microbial Strains isolated from Soil Sample

Sr. No.	Name of Isolate	Gram’s staining Result
1.	<i>Pseudomonas aeruginosa</i>	Gram-negative
2.	<i>Escherichia coli</i>	Gram-negative
3.	<i>Bacillus subtilis</i>	Gram-positive
4.	<i>Staphylococcus aureus</i>	Gram-positive
5.	<i>Streptococcus aureus</i>	Gram-positive
6.	<i>Salmonella spp.</i>	Gram-negative

Table 2: Biochemical Characterization of Microbial Strains isolated from Soil Samples.

Sr. No.	Biochemical Test	<i>Streptococcus spp.</i>	<i>Staphylococcus spp.</i>	<i>Bacillus</i>	<i>Escherichia</i>	<i>Salmonella spp.</i>	<i>Pseudomonas spp.</i>
1.	Voges Proskauer	Positive	Positive	Positive	Negative	Negative	Negative
2.	Methyl Red	Positive	Positive	Negative	Positive	Positive	Negative
3.	Catalase Test	Negative	Positive	Positive	Positive	Positive	Positive
4.	Simmons Citrate	Positive	Positive	Positive	Negative	Positive	Positive
5.	Oxidase Test	Negative	Negative	Positive	Negative	Negative	Positive
6.	Indole Test	Negative	Positive	Negative	-	Negative	Negative
7.	Sucrose Fermentation	-	-		Variable	Negative	Negative
8.	Lactose Fermentation	Positive	Positive	Negative	Positive	Negative	Negative
9.	Dextrose Fermentation	-	-	Positive	Positive	Positive	Negative



Figure 1: Aspergillus species of Fungi on Potato Dextrose Agar medium

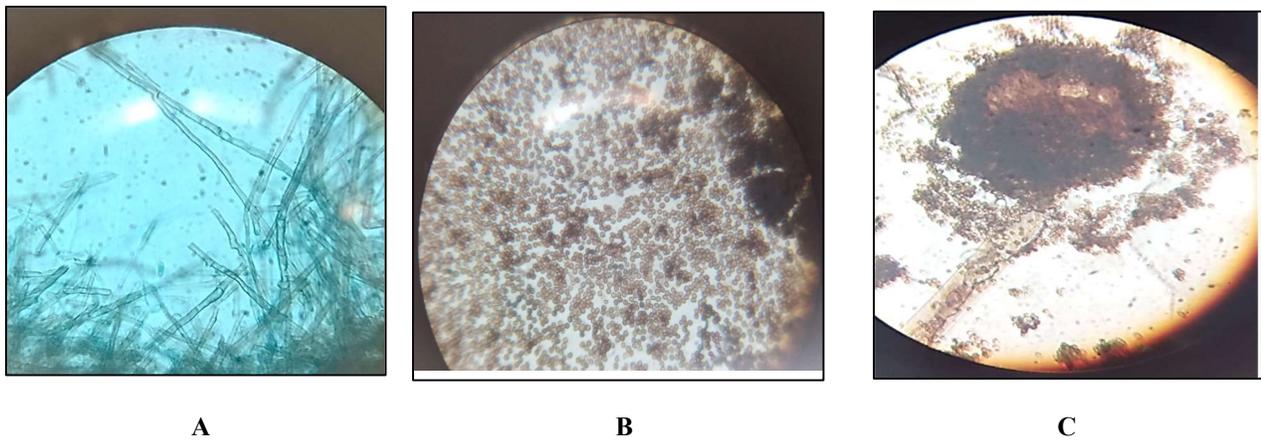


Figure 2: Microscopic observation of Aspergillus Fungi A: Fungal hyphae by Lactophenol staining B. Fungal Spores C. Fungal Hyphae with microsporangium and spores

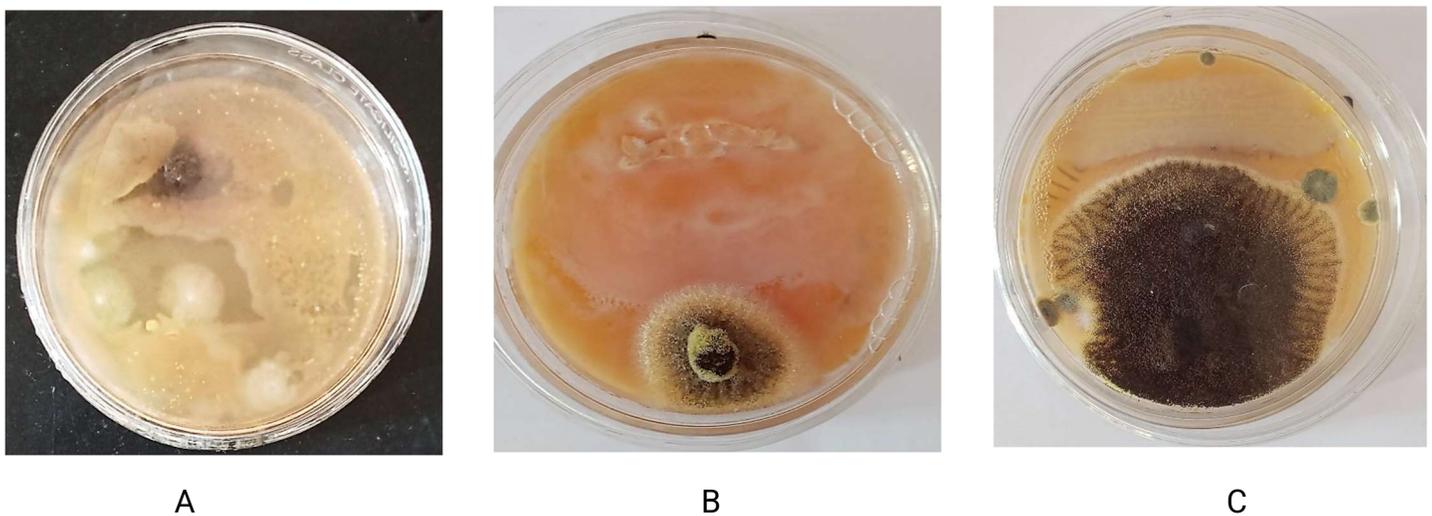


Figure 3: Antifungal activity of soil microorganism: A. *Streptococcus* B. *Staphylococcus* C. *Pseudomonas*

The mechanism of inhibiting the growth of phytopathogenic fungi by the soil bacteria may be the production of toxic substances which interferes in the metabolism of fungi and leads to the death of fungi. Additionally, the bacterial species *Streptococcus*, *Staphylococcus* and *Pseudomonas* are involved in the hinderance of evolution of *Aspergillus* strains due to the consistent inhibition.

CONCLUSION

The phytopathogens play a vital role in decreasing the plant population and hampering the crops which thereby affects the economy. This study presented that some soil microorganisms are potent to inhibit the growth of plant pathogens in a natural and cost-effective method. In the present study the wild type soil microorganisms were isolated from the soil sample collected from Balapur village, Rajkot. The antimicrobial activity of these isolates was analyzed against the most potent phytopathogenic fungi *Aspergillus*. Finally it was observed that some microorganisms of sample were potentially able to resist the growth of phytopathogens. Therefore, by this study it can be concluded that the phytopathogens can be resisted or killed in ecofriendly manner which does not show any hazardous effects on plant and soil.

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