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Isolation And Characterization Of Microorganisms From Pesticide Treated Soils Of Different Regions Of Telangana

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Abstract

This investigation is carried out to identify the microorganisms for the pesticide treated soil samples collected from different places of Warangal District, Telangana. The identification was based on morphological, physiological and biochemical. Randomly five samples from one village haven selected. The type and amount of pesticide used by the farmers have been noted. With reference to the results of morphological, physiological and biochemical, we identified 10 bacterial genus namely, Pseudomonas, Bacillus, Streptomyces, Rhizobium, Klebsiella, Staphylococcus, Streptococcus, Azatobacter Azospirillum, Actinomycetes and 06 fungal genus namely, Aspergillus, Penicillium, Trichoderma, Fusarium, Rhizopus and Vesicular Arbuscular Mycorrhiza have been predominantly identified from all the samples collected.

Keywords: morphological, physiological and biochemical, Warangal, pesticide

1.0 INTRODUCTION

Pesticides are chemical compounds extensively used in agriculture to control pests and increase crop yields. Pests can include insects, weeds, fungi, bacteria, rodents, and other organisms that can cause harm or damage to crops, livestock, structures, or human health. While these chemicals have proven effective in enhancing agricultural productivity, they can also have adverse effects on the environment, including soil microbial communities (Cederberg *et al*., 2019; Koli *et al*., 2019; Mandal *et al*., 2020). Soil microorganisms play vital roles in nutrient cycling, organic matter decomposition, and maintaining soil health. Therefore, understanding the impact of pesticides on soil microbial diversity and function is crucial for sustainable agriculture and environmental conservation.

Telangana, a region in India known for its diverse agricultural practices, employs various pesticides to protect crops from pests and diseases. As the use of pesticides continues to rise, it becomes imperative to investigate how these chemicals affect the composition and activities of microorganisms in the soil. Isolating and characterizing microorganisms from pesticide-treated soils in different regions of Telangana can shed light on their response to pesticide exposure and their potential to degrade or interact with these agrochemicals

Isolation and screening of microorganisms from diverse habitats have historically been an important strategy in the discovery of new antibiotics. Many antibiotics, such as penicillin and streptomycin, were originally

isolated from microorganisms. By exploring the microbial diversity and their natural products, scientists can identify novel compounds that have the potential to combat drug-resistant pathogens and provide new avenues for drug development.

Overall, the isolation of microorganisms is fundamental to advancing our understanding of microbial life, exploring their potential applications, and addressing various scientific, medical, environmental, and biotechnological challenges. Isolation of microorganisms from pesticide-treated soil involves the process of extracting and cultivating microorganisms that exist in the soil environment after it has been exposed to pesticides. This process allows for the identification and study of these microorganisms and their potential roles in pesticide degradation, as well as their overall impact on soil health and ecosystem functioning.

Microorganisms are a significant source of biodiversity in soils and an essential component of terrestrial ecosystems. They help with major biological functions such nutrition and gas cycling, biogeochemical processes, and organic matter degradation and transformation. Fungi are very abundant in soil, accounting for up to 80% of soil microbial biomass. According to the investigations made by Major of agriculture pesticide application besides serving its purpose instead, gets dispersed through the air, soil, and water. Seven main classes of microorganisms in soil are, Bacteria, Fungi, Actinomycetes, Protozoa, Blue-green algae (cyanobacteria), Viruses and Nematodes. Bacteria far outnumber and out type all other soil microorganisms. There are more than a few million, and potentially even a few billion, of them in only a single gram of soil.

2.0 MATERIALS AND METHODS

2.1 SOIL SAMPLING

Soil sampling equipment (shovels, spades, or soil corers) were sterilized to avoid contamination. This is done by heating the equipment or using disinfectants like ethanol. Care was taken to ensure that the equipment is dry before use. For sample collection surface debris was removed to expose the sampling area of the soil. The sample is typically taken from the topsoil (0-15 cm).

Multiple subsamples were collected from different locations within the sampling area to capture spatial variation. Care was taken to avoid contamination from nearby sources. A clean container (sterile plastic bag) was used to store the collected soil. Each container was labeled with relevant information like sample location, date, and depth. The collected samples were handled appropriately to preserve the microbial community structure and activity. For this, the samples were preserved in cool temperatures and exposure to high temperatures or direct sunlight. Sample disturbance was minimized to maintain the microbial community composition. Excessive handling was also avoided.

Same procedure was followed for collection of control samples. These control samples were collected from agricultural farms where organic farming is being practiced for the last 14 years.

2.2 ISOLATION OF BACTERIA FROM SOIL

Isolation of bacteria from soil involves a series of steps to separate and culture individual bacterial species. The serial dilution technique is commonly used for isolating microorganisms from soil samples (50.No) treated with synthetic compounds and control samples (06.No) where organic farming was practiced.

2.3 PLATING

0.1 ml of soil suspension was taken from each dilution and plated onto Tryptic soy agar (Tryptone - 15.0 g/l, Soybean Meal - 5.0 g/l, Sodium Chloride - 5.0 g/l, Agar - 16.0 g/l), suitable for isolation of bacteria and Rose bengal agar (Dextrose - 10.0 g/l, Peptone – 10.0 g/l, Rose Bengal - 0.05 g/l, Agar – 16.0 g/l), suitable for isolation of fungi.

2.4 INCUBATION

The agar plates were incubated at the 37^0C temperature and 25^0C for bacteria and fungi respectively. Incubation time maintained for bacteria was 24 to 48 hours and 7 days for fungi.

2.5 COLONY COUNTING

After incubation, the agar plates were observed, and visible colonies were counted. Plates with a countable number of colonies (generally 30 to 300 colonies) were selected for further analysis and isolation.

2.6 IDENTIFICATION BY MORPHOLOGICAL CHARACTERISTICS

2.6.1 COLONY MORPHOLOGY

The appearance of bacterial and fungal colonies grown on agar plates was observed. Characteristics such as colony shape, color, size, elevation, and texture were noted to provide initial clues for identification.
For fungi, the appearance of colonies grown on agar plates was examined. Characteristics such as colony shape, color, texture, and growth pattern like, radial or filamentous were noted.

2.6.2 CELL MORPHOLOGY

Bacterial cells were examined under a microscope to determine their shape and arrangement. Common cell shapes, including cocci (spherical), bacilli (rod-shaped), and spirilla (spiral-shaped), which were very rare, were identified.

2.6.3 HYPHAL MORPHOLOGY

The structure and arrangement of fungal hyphae under a microscope was observed. The characteristics such as hyphal diameter, septation, branching patterns, and presence of specialized structures like conidiophores or sporangia were noted.

2.6.4 GRAMS NATURE

Bacteria were commonly classified based on their Gram nature, which refers to their response to the Gram staining technique developed by Hans Christian Gram. They are differentiated as Gram positive or Gram negative based on the type of stain they retained during Gram staining

2.6.5 ENDOSPORE

The staining technique commonly used for visualizing spore-forming bacteria is Schaeffer-Fulton method. The bacteria were differentiated as Spore forming bacteria and Non – spore forming bacteria based on the staining solution they retained during Schaeffer-Fulton staining.
For fungi, the shape, size, color, and arrangement of spores produced was examined. Spores are observed using microscopy and are differentiated as unicellular (yeast-like) or multicellular (filamentous).

2.6.6 CAPSULE STAINING

Bacteria were identified based on the presence of capsules using the capsule staining technique. Capsule staining allowed for the visualization and identification of bacterial capsules, which are slimy and gelatinous structures surround some bacterial cells. Capsules can be an important virulence factor in certain pathogenic bacteria.

2.6.7 FLAGELLA STAINING

Bacteria were identified based on the presence and arrangement of flagella using the flagella staining technique. Flagella staining allowed for the visualization and identification of bacterial flagella, which are responsible for bacterial motility. The staining technique provided information about the presence, number, and arrangement of flagella on bacterial cells.

2.7 IDENTIFICATION BY PHYSIOLOGICAL CHARACTERISTICS

2.7.1 GROWTH CONDITIONS

Bacteria were incubated at different temperatures to assess their optimal growth range. Incubation temperatures varied, including common ranges such as 25°C, 37°C, depending on the bacteria being studied.
For fungi, the optimal temperature range was determined by incubating the fungi at different temperatures (e.g., 20°C, 25°C, and 30°C) and assessing their growth or lack thereof. The temperature preferences or limitations are noted.

2.7.2 OXYGEN REQUIREMENT

Bacteria's oxygen requirements were determined by culturing them in various conditions, such as aerobic (with oxygen), anaerobic (without oxygen), or facultative anaerobic (capable of growing with or without oxygen).

2.7.3 IDENTIFICATION OF FUNGI BASED ON CARBON UTILIZATION

The reference method for testing fungi for carbohydrate fermentation is the Filamentous Fungi Carbohydrate Utilization Test. This test assesses the ability of fungi to utilize various carbohydrates as carbon sources for

growth and metabolism. The test was performed by preparation of media enriched with specific carbohydrate, Inoculation of the isolates and incubation of plates at 25-30°C, Observation and interpretation of the plates for fungal growth and colony morphology, Fungal growth on the specific carbohydrate. Positive results indicate the ability of the fungus to utilize the tested carbohydrate as a carbon source, resulting in visible growth. Negative results suggest that the fungus did not utilize the tested carbohydrate, and growth may be limited or absent on the corresponding plates.

2.7.4 IDENTIFICATION OF FUNGI BY PIGMENTATION

The coloration of fungal colonies or structures when grown on different types of media was observed as some fungi produce pigments that can be used for identification

2.8 IDENTIFICATION BY BIOCHEMICAL CHARACTERISTICS

Bacteria were commonly identified based on their biochemical characteristics using a series of tests. A panel of biochemical tests (Catalase, Oxidase, Indole production test, Citrate utilization test, carbohydrate fermentation test, Urease test) was chosen based on the target bacteria and the available test options. Common biochemical tests included carbohydrate fermentation, enzyme activity, gas production, pH change, and utilization of specific substrates. Bacterial isolates were inoculated into appropriate media or test tubes containing specific substrates or indicator systems. The cultures were incubated under appropriate conditions, such as temperature and oxygen requirements, for a suitable duration. After incubation, the cultures were observed for changes in color, pH, gas production, or any visible growth. Specific reactions, such as acid or gas production, color change, or precipitation, were noted. Results from the biochemical tests were compared with known patterns or reference databases to determine the identity of the bacteria. Patterns of positive or negative reactions were analyzed to identify characteristic biochemical profiles. Common biochemical tests used for bacterial identification included carbohydrate utilization tests (e.g., glucose fermentation), catalase test, oxidase test, indole production test, urease test, citrate utilization test, and many others. These tests provided valuable information about the metabolic capabilities and pathways of the bacteria, helping in their identification.

For Fungi, A panel of biochemical tests (Carbohydrate assimilation, Nitrate assimilation, Urease activity, Catalase activity, Phenoloxidase activity and Starch hydrolysis) was chosen based on the target bacteria and the available test options. Common biochemical tests included was amylase, cellulase enzyme production tests. These tests were performed to gather further information about the fungi.

2.9 CHARACTERIZATION OF PREDOMINANT BACTERIA AND FUNGI

By following the methods involved in identification of bacteria based on morphological, physiological and biochemical characters those bacterial genus which were found in majority of soil samples were selected. For Fungi the methods of selection of most predominant genus in majority of soil samples were also the same which were employed for bacteria. The predominant bacteria and fungi in the soil were successfully characterized and identified, providing valuable insights into the microbial composition of the soil ecosystem

3.0 RESULTS

3.1 ENUMERATION OF BACTERIAL AND FUNGAL COLONIES

The number of bacterial and fungal colonies obtained on agar plates were manually counted using a colony counter or by visual estimation, taking into account the Shape, Margin, Elevation, Size, Color, Texture, and Opacity for bacteria and Shape, Margin, Texture, Color, Growth Rate, Surface and Spore Formation for Fungi and the result was expressed in Colony forming units (CFU) applying the following formula,

$$\text{Colony count} = (\text{Number of colonies counted}) / (\text{Dilution factor}) \times (\text{Reciprocal of the plated volume})$$

Where,

"Number of colonies counted" refers to the actual count of visible colonies on the agar plate. "Dilution factor" represents the cumulative dilution achieved through the series of dilutions.

It is calculated by multiplying the dilution factors of each dilution step. If the original sample was diluted 1:10 in each step and a total of three dilution steps were performed, the dilution factor would be 1:10 × 1:10 × 1:10 = 1:1000. "Reciprocal of the plated volume" refers to the inverse of the volume (in milliliters) of the diluted sample that was spread onto the agar plate. For example, if 0.1 ml of the diluted sample was plated, the reciprocal of the plated volume would be 1/0.1 = 10. The number of bacterial and fungal colony forming units is represented in table 1.1 and 1.2

Table 1.1Enumeration of Bacterial and Fungal colonies

S.No	Soil Sample Code	No of Bacterial colonies in CFU	No of Fungal colonies in CFU
01	WAR-01	3.3×10^5	1.6×10^4
02	WAR-02	3.6×10^5	1.8×10^4
03	WAR-03	3.5×10^5	1.1×10^4
04	WAR-04	3.8×10^5	1.4×10^4
05	WAR-05	3.1×10^5	1.5×10^4
06	NAR-01	3.2×10^5	1.2×10^4
07	NAR-02	4.1×10^5	1.5×10^4
08	NAR-03	3.8×10^5	1.1×10^4
09	NAR-04	3.6×10^5	1.4×10^4
10	NAR-05	3.9×10^5	1.6×10^4
11	PAR -01	4.2×10^5	1.8×10^4
12	PAR-02	3.9×10^5	1.2×10^4
13	PAR-03	4.3×10^5	1.4×10^4
14	PAR-04	4.0×10^5	1.9×10^4
15	PAR-05	4.3×10^5	1.3×10^4

Table 1.2 Enumeration of Bacterial and Fungal colonies contd...

S.No	Soil Sample Code	No of Bacterial colonies in CFU	No of Fungal colonies in CFU
16	GHA-01	3.2×10^5	1.6×10^4
17	GHA-02	4.1×10^5	1.2×10^4
18	GHA-03	3.6×10^5	1.1×10^4
19	GHA-04	3.9×10^5	1.4×10^4
20	GHA-05	3.1×10^5	1.5×10^4
21	SOM-01	4.0×10^5	1.8×10^4
22	SOM-02	3.2×10^5	1.6×10^4
23	SOM-03	3.6×10^5	1.1×10^4
24	SOM-04	4.1×10^5	1.8×10^4
25	SOM-05	3.9×10^5	1.5×10^4
26	HAS-01	4.3×10^5	1.2×10^4
27	HAS-02	3.6×10^5	1.1×10^4
28	HAS-03	3.1×10^5	1.6×10^4
29	HAS-04	4.0×10^5	1.5×10^4
30	HAS-05	4.3×10^5	1.8×10^4
31	MAM -01	3.6×10^5	1.6×10^4
32	MAM-02	3.9×10^5	1.1×10^4
33	MAM-03	4.1×10^5	1.2×10^4
34	MAM-04	3.6×10^5	1.6×10^4
35	MAM-05	3.1×10^5	1.1×10^4
36	RAY-01	4.1×10^5	1.8×10^4
37	RAY-02	3.9×10^5	1.6×10^4
38	RAY-03	4.3×10^5	1.1×10^4
39	RAY-04	3.2×10^5	1.5×10^4
40	RAY-05	3.1×10^5	1.2×10^4
41	GUD-01	4.1×10^5	1.1×10^4

42	GUD-02	4.0 x 10^5	1.8 x10^4
43	GUD-03	3.1 x 10^5	1.6x10^4
44	GUD-04	3.6 x 10^5	1.1 x10^4
45	GUD-05	4.0 x 10^5	1.5 x10^4
46	WRT-01	3.1 x 10^5	1.1 x10^4
47	WRT-02	4.3 x 10^5	1.2 x10^4
48	WRT-03	3.3 x 10^5	1.8 x10^4
49	WRT-04	4.0 x 10^5	1.1 x10^4
50	WRT-05	3.6 x 10^5	1.5 x10^4

3.2 CHARACTERIZATION OF PREDOMINANT BACTERIA AND FUNGI

Microbial communities can vary significantly based on factors such as soil type, climate, land use, and management practices. The composition of bacterial and fungal populations in soil can also change over time due to environmental factors and human activities. Various bacterial taxa have been identified as predominant in soil ecosystems. These include members of genera such as Pseudomonas, Bacillus, Streptomyces, Rhizobium, Klebsiella, Staphylococcus, Streptococcus, Azatobacter Azospirillum and Actinomycetes have been frequently observed in soil samples. Fungal communities in soil are diverse and play crucial roles in nutrient cycling and organic matter decomposition. The dominant fungal genera encountered in soil samples were Aspergillus, Penicillium, Trichoderma, Fusarium, Rhizopus and Vesicular Arbuscular Mycorrhiza.

3.3 IDENTIFICATION OF BACTERIA BASED ON MORPHOLOGICAL CHARACTERISTICS

The predominant isolates were grown on Trypticase soy agar exhibited different colony morphology (Smooth, Powdery, Round and fuzzy as listed in table 1.3

The isolates when stained and observed under microscope for cell morphology, Grams nature, Endospore formation, Capsule possession and for the presence of Flagella exhibited variations among each genus. The result is shown in table 1.3

Table 1.3 Identification by Physiological characteristics

Bacterial Genus	**Colony Morphology**	**Cell Morphology**	**Grams Nature**	**Endospore**	**Capsule**	**Flagella**
Pseudomonas	Smooth	Monobacilli	-ve	-ve	+ve	Lopho trichous
Bacillus	Powdery	Streptobacilli	+ve	+ve	+ve	Varying
Streptomyces	Powdery	Filamentous	+ve	-ve	-ve	-ve
Rhizobium	Round	Monobacilli	-ve	-ve	+ve	Lopho trichous
Klebsiella	Smooth	Short bacilli	-ve	-ve	+ve	-ve
Staphylococcus	Small	Staphylo coccus	+ve	-ve	-ve	-ve
Streptococcus	Round	Strepto coccus	+ve	-ve	+ve	-ve
Azatobacter	Round	Diplobacillus	-ve	-ve	+ve	Lopho trichous
Azospirillum	Smooth	Spiral	-ve	-ve	-ve	Lopho trichous
Actinomycetes	Fuzzy	Filamentous	+ve	-ve	Variable	-ve

3.4 IDENTIFICATION BY PHYSIOLOGICAL CHARACTERISTICS

Predominant bacterial inoculated into their growth specific media and were incubated at different temperatures to assess their optimal growth range Bacteria's oxygen requirements were determined by culturing them in various conditions, such as aerobic (with oxygen), anaerobic (without oxygen) (Table 1.4).

Table 1.4 Identification by Physiological Characteristics

Bacterial Genus	Temperature in ^{0}C	O_2 Requirement
Pseudomonas	25-37	Aerobic
Bacillus	25-40	Facultatively anaerobic
Streptomyces	25-30	Aerobic
Rhizobium	20-30	Microaerophilic
Klebsiella	25-37	Facultatively anaerobic
Staphylococcus	30-37	Facultatively anaerobic
Streptococcus	35-37	Facultatively anaerobic
Azatobacter	25-37	Aerobic
Azospirillum	20-30	Aerobic/Facultative anaerobes
Actinomycetes	20-30	Aerobic

3.5 IDENTIFICATION BY BIOCHEMICAL CHARACTERISTICS

Common biochemical tests were for bacterial identification they include Carbohydrate utilization tests (e.g., glucose fermentation), catalase test, oxidase test, indole production test, urease test, citrate utilization test and citrate utilization tests. Bacterial isolates were inoculated into appropriate media or test tubes containing specific substrates or indicator systems. The cultures were incubated under appropriate conditions, such as temperature and oxygen requirements, for a suitable duration. After incubation, the cultures were observed for changes in color, pH, gas production, or any visible growth. Specific reactions, such as acid or gas production, color change, or precipitation, were noted. These tests provided valuable information about the metabolic capabilities and pathways of the bacteria, helping in their identification. The results are as shown in table 1.5

Table 1.5 Identification by Biochemical Characteristics

Bacterial Genus	Glucose Fermentation	Catalase test	Oxidase test	Urease test	Indole test	Citrate Test
Pseudomonas	-ve	+ve	+ve	+ve	-ve	+ve
Bacillus	+ve	+ve	+ve	+ve	-ve	-ve
Streptomyces	-ve	+ve	-ve	-ve	+ve	-ve
Rhizobium	+ve	+ve	+ve	+ve	+ve	-ve
Klebsiella	+ve	+ve	-ve	+ve	-ve	+ve
Staphylococcus	+ve	+ve	-ve	+ve	-ve	-ve
Streptococcus	+ve	-ve	-ve	-ve	-ve	+ve
Azatobacter	+ve	-ve	-ve	-ve	-ve	+ve
Azospirillum	+ve	+ve	+ve	+ve	-ve	-ve
Actinomycetes	+ve	+ve	-ve	+ve	-ve	+ve

3.6 IDENTIFICATION OF FUNGI BASED ON MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS

The appearance of fungal colonies grown on agar plates was examined. Characteristics such as colony shape, color, texture, and growth pattern were examined. The structure and arrangement of fungal hyphae under a microscope was observed. The characteristics such as hyphal diameter, septation were examined.

The type of spores produced by the fungi was examined. Spores are observed using microscopy and are differentiated.

The optimal temperature range for fungal growth was determined by incubating the fungi at different temperatures (e.g., 20°C, 25°C, and 30°C) and assessing their growth. The reference method for testing fungi for carbohydrate fermentation is the Filamentous Fungi Carbohydrate Utilization Test. This test assesses the ability of fungi to utilize Glucose as carbon sources for growth and metabolism was examined (Table 1.6).

Table 1.6 Identification of fungi based on Morphological and Physiological characters

Fungal Genus	Colony Morphology	Hyphal Morphology	Spore Morphology	Glucose Utilzation	Pigmentation
Aspergillus	Thick & Green	Septate	Conidiospores	+ve	Green
Trichoderma	White	Septate	Conidiospores	+ve	Light Yellow
Fusarium	Cottony	Septate	Conidiospores	+ve	Cream
Rhizopus	Fluggy	Septate	Sporangiospores	+ve	Black
VAM	-	Septate	Globose	Mutualist	-

3.7 IDENTIFICATION OF FUNGI BASED ON BIOCHEMICAL CHARACTERS

Fungal isolates were inoculated into appropriate media or test tubes containing specific substrates or indicator systems. The cultures were incubated under appropriate conditions, such as temperature and oxygen requirements, for a suitable duration. After incubation, the cultures were observed (Table 1.7).

Table 1.7 Identification of bacteria based on Biochemical characters

Fungal Genus	Carbohydrate Assimilation	Nitrate Assimilation	Urease	Catalase	Phenol oxidase	Starch hydrolysis
Aspergillus	+ve	+ve	+ve	+ve	+ve	+ve
Trichoderma	+ve	+ve	+ve	+ve	+ve	+ve
Fusarium	+ve	+ve	+ve	+ve	+ve	+ve
Rhizopus	+ve	+ve	+ve	+ve	+ve	+ve
VAM	Indirect	Enhances	+ve	+ve	+ve	+ve

4.0 DISCUSSION

Isolation, identification, and characterization of microorganisms are essential steps in microbiological research and various applications, including biotechnology, environmental studies, and medical microbiology. These processes involve obtaining pure cultures, determining their identity, and studying their characteristics. Microorganisms can be isolated from various sources such as soil, water, plants, animals, or clinical specimens. Proper sampling techniques and aseptic conditions are crucial to ensure representative and uncontaminated samples. The isolation of microorganisms involves obtaining pure cultures from different sources through sample collection, dilution, and plating on appropriate culture media. This step provides the basis for further study and analysis.

Identification of microorganisms involves macroscopic and microscopic examination, biochemical and physiological tests, and molecular techniques. By examining their morphological characteristics, metabolic capabilities, and genetic makeup, microorganisms can be identified at different taxonomic levels. Colonies with distinct morphological characteristics are observed macroscopically, while microscopic examination involves observing stained or unstained cells for their shape, size, arrangement, and staining properties. Various biochemical and physiological tests are conducted to determine the metabolic capabilities of the microorganism, such as sugar fermentation, enzyme production, and utilization of specific substrates. Growth rate, temperature range, pH tolerance, and oxygen requirements are determined by growing microorganisms under different conditions. Microorganisms are evaluated for their ability to utilize different carbon and energy sources, produce specific metabolites or enzymes, and their response to specific environmental factors.

5.0 CONCLUSION

In accordance to our results here we conclude that, 10 bacterial genus namely, Pseudomonas, Bacillus, Streptomyces, Rhizobium, Klebsiella, Staphylococcus, Streptococcus, Azatobacter Azospirillum, Actinomycetes and 06 fungal genus namely, Aspergillus, Penicillium, Trichoderma, Fusarium, Rhizopus and Vesicular Arbuscular Mycorrhiza have been predominantly identified from all the samples collected.

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CONFLICT OF INTEREST

The authors disclose no conflict of financial or nonfinancial interest.

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