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**SEASONAL VARIATION OF PHYLLOSHERIC MICROFLORA OF  
SUGARCANE CROP IN AMBEDKAR NAGAR DISTRICT, U.P.**

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**Abstract-**The phyllosphere, representing the aerial surfaces of plants, harbors diverse microbial communities including bacteria, fungi, and yeasts. These microorganisms play a crucial role in plant growth, health, and productivity. The present study investigates the seasonal variation of phyllospheric microflora associated with sugarcane (*Saccharum officinarum* L.) in Ambedkar Nagar district, Uttar Pradesh. Leaf samples were collected during three major seasons (summer, rainy, and winter) and analyzed using standard microbiological techniques. The results revealed significant variation in microbial population density and diversity across seasons, with maximum microbial load observed during the rainy season and minimum during winter. The study highlights the influence of climatic factors such as temperature, humidity, and rainfall on phyllospheric microbial dynamics.

**Introduction-** The aerial surfaces of plants, collectively referred to as the phyllosphere, represent one of the largest microbial habitats on Earth, covering an estimated surface area of approximately  $6.4 \times 10^8$  km<sup>2</sup> (Phyllosphere). This unique ecological niche is colonized by a diverse assemblage of microorganisms, including bacteria, fungi, yeasts, and actinomycetes, which interact dynamically with their host plants and surrounding environment (Vorholt, 2012; Lindow & Brandl, 2003). Among these, bacteria are the most dominant inhabitants, often reaching densities of  $10^6$ – $10^7$  cells per cm<sup>2</sup> of leaf surface, followed by fungi and yeasts in comparatively lower numbers (Whipps et al., 2008).

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Phyllospheric microorganisms play a crucial role in plant physiology and ecosystem functioning. They contribute to nutrient cycling, nitrogen fixation, and the synthesis of phytohormones such as indole-3-acetic acid (IAA), thereby promoting plant growth and development (Vacher et al., 2016). In addition, several phyllosphere-associated microbes exhibit antagonistic activity against plant pathogens, thus serving as natural biocontrol agents and enhancing plant resistance to diseases (Lindow & Leveau, 2002). The interactions between plants and their phyllospheric microbiota are often mutualistic, although some microorganisms may act as opportunistic pathogens under favorable conditions.

The composition and structure of phyllospheric microbial communities are influenced by a wide range of abiotic and biotic factors. Environmental parameters such as temperature, relative humidity, solar radiation, and rainfall significantly affect microbial colonization, survival, and proliferation on leaf surfaces (Remus-Emsermann & Schlechter, 2018). Seasonal variation, in particular, has been identified as a key determinant of microbial diversity and abundance in the phyllosphere. Changes in climatic conditions across seasons alter the availability of nutrients, moisture, and microhabitats, thereby influencing microbial dynamics (Copeland et al., 2015).

Several studies have demonstrated that microbial populations tend to increase during humid and warm conditions, especially in the rainy season, due to enhanced moisture availability and nutrient leaching from leaf tissues (Peñuelas & Terradas, 2014). Conversely, extreme temperatures and low humidity during winter can limit microbial activity and reduce population density. Seasonal shifts not only affect microbial abundance but also lead to changes in community composition, with certain microbial taxa becoming dominant under specific environmental conditions (Redford et al., 2010).

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Sugarcane (*Saccharum officinarum* L.) is one of the most important commercial crops in India, particularly in the state of Uttar Pradesh, which is a major contributor to national sugar production. The crop provides a suitable habitat for diverse microbial communities due to its broad leaves, high sugar content, and prolonged growth period. The phyllosphere of sugarcane supports a wide range of microorganisms, including beneficial bacteria such as *Pseudomonas* and *Bacillus*, as well as fungi like *Aspergillus* and *Fusarium*, which may influence crop productivity and health.

Despite the agricultural significance of sugarcane, limited information is available regarding the seasonal dynamics of its phyllospheric microflora, especially in eastern Uttar Pradesh regions such as Ambedkar Nagar. This district is characterized by a subtropical climate with distinct seasonal variations, including hot summers, humid monsoons, and cool winters, which may significantly influence microbial diversity and distribution on leaf surfaces.

Understanding the seasonal variation of phyllospheric microflora is essential for developing sustainable agricultural strategies. Knowledge of microbial diversity and their functional roles can be utilized in enhancing crop productivity, improving disease management, and promoting eco-friendly farming practices. Therefore, the present study aims to investigate the seasonal variation of phyllospheric microflora associated with sugarcane crops in Ambedkar Nagar district, Uttar Pradesh, with a focus on microbial diversity, abundance, and environmental influences.

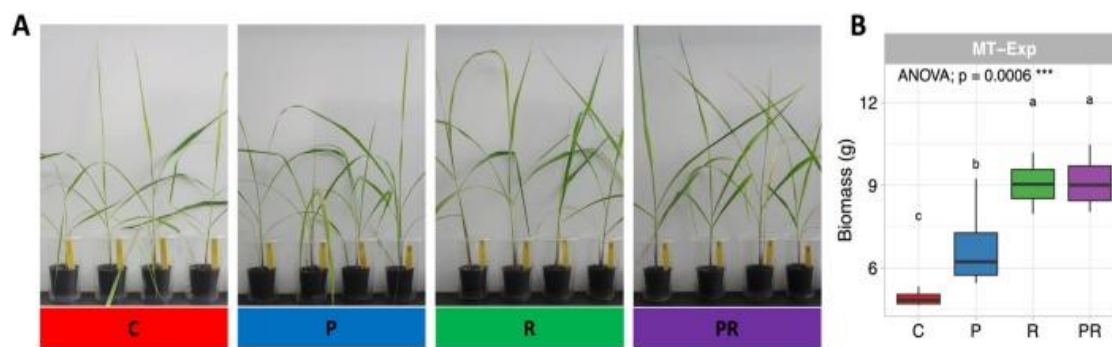
## **Material and Method-**

### **Study area-**

The present investigation was carried out in sugarcane-growing fields of Ambedkar Nagar. The district is located in eastern Uttar Pradesh and lies between 26.30°N latitude and 82.50°E longitude. The region experiences a subtropical climate with

three distinct seasons: summer (March–June), rainy/monsoon (July–September), and winter (November–February).

The average annual rainfall ranges between 900–1100 mm, with maximum precipitation occurring during the monsoon season. The temperature varies from approximately 8–12°C in winter to 40–45°C in summer. The soils are predominantly alluvial, fertile, and suitable for the cultivation of *Saccharum officinarum*.



**Fig-1** Map of Ambedkar Nagar and Phyllosphere image of Sugarcane

### Experimental design and sampling strategy-

A field-based observational study was conducted during one complete cropping cycle of sugarcane. Sampling was performed in three different seasons:

- 1-Summer: April–June
- 2-Rainy (Monsoon): July–September
- 3-Winter: December–February

Three different agricultural fields were selected randomly within the district to ensure variability. From each field, replicate leaf samples (n = 5) were collected at each sampling time.

**Collection of leaf samples-** Healthy, disease-free leaves of sugarcane were selected for sampling. Leaves were collected from the middle portion of the plant to maintain uniformity. Sampling was carried out in the morning hours (8:00–10:00 AM) to avoid fluctuations due to temperature and sunlight.

Leaves were cut using sterile scissors and transferred into pre-sterilized polyethylene bags. Each sample was labelled properly and transported immediately to the laboratory under aseptic conditions for further analysis.

#### **Isolation of Phyllospheric microorganism-**

##### **Leaf Washing Technique**

The phyllospheric microflora was isolated using the standard leaf washing method (Lindow & Brandl, 2003):

- Approximately 1 g of leaf material was taken and cut into small pieces
- The sample was transferred into 100 mL sterile distilled water
- The suspension was shaken vigorously for 20–30 minutes using a rotary shaker to dislodge surface microorganisms.

**Serial dilution-** The leaf wash suspension was subjected to serial dilution up to  $10^{-6}$  dilution level using sterile distilled water.

##### **Plating Technique-**

From appropriate dilutions, 0.1 mL aliquots were spread on culture media:

- Nutrient Agar (NA): for isolation of bacteria
- Potato Dextrose Agar (PDA): for isolation of fungi (supplemented with streptomycin to inhibit bacterial growth)

Plates were incubated at:

- $28 \pm 2^\circ\text{C}$  for 24–48 hours (bacteria)
- $25 \pm 2^\circ\text{C}$  for 3–5 days (fungi)

### **Bacterial Identification-**

Bacterial isolates were identified based on:

- Colony characteristics (shape, size, color, margin)
- Gram staining
- Biochemical tests:
  1. Catalase test
  2. Oxidase test
  3. Indole production
  4. Methyl Red (MR) test
  5. Voges-Proskauer (VP) test
  6. Citrate utilization test

Identification was carried out using standard Bergey's Manual of Systematic Bacteriology.

### **Fungal Identification-**

Fungal isolates were identified based on:

- Colony morphology (texture, color, growth pattern)
- Microscopic examination using Lactophenol Cotton Blue staining
- Observation of spore structure and hyphal arrangement

Identification keys by standard mycological manuals (Barnett & Hunter, 1998) were used.

### **Statistical Analysis-**

All experiments were conducted in triplicates, and results were expressed as mean  $\pm$  standard deviation (SD).

- One-way Analysis of Variance (ANOVA) was used to determine significant differences among seasons
- Significance level was considered at  $p < 0.05$

Statistical analysis was performed using standard software such as SPSS or MS Excel.

### **Result and discussion-**

#### **Seasonal Variation in Microbial Population-**

The population density of phyllospheric microorganisms associated with *Saccharum officinarum* varied significantly across seasons. Both bacterial and fungal populations showed clear seasonal trends, with maximum abundance during the rainy season and minimum during winter.

**Table-1** Seasonal Variation in Microbial Population (CFU/cm<sup>2</sup> of Leaf Surface)

Season	Bacterial Count (CFU/cm <sup>2</sup> )	Fungal Count (CFU/cm <sup>2</sup> )
Summer	$4.2 \times 10^5$	$2.8 \times 10^4$
Rainy	$7.8 \times 10^5$	$5.6 \times 10^4$
Winter	$2.1 \times 10^5$	$1.5 \times 10^4$

The highest microbial population was observed during the rainy season, while the lowest was recorded in winter.

#### **Diversity of Phyllospheric Microorganisms-**

A variety of bacterial and fungal species were isolated from the leaf surface.

**Table 2:** Diversity of Phyllospheric Microflora in Different Seasons

Microorganism	Summer	Rainy	Winter
<i>Bacillus spp.</i>	+	+++	+
<i>Pseudomonas spp.</i>	++	+++	+
<i>Micrococcus spp.</i>	+	++	+
<i>Staphylococcus spp.</i>	+	++	-
<i>Aspergillus niger</i>	++	+++	+
<i>Penicillium spp.</i>	+	++	+
<i>Fusarium spp.</i>	+	+++	-
<i>Alternaria spp.</i>	+	++	+

### Diversity Index-

**Table 3:** Shannon Diversity Index (H') Across Seasons

Season	Shannon Index (H')
Summer	1.85
Rainy	2.63
Winter	1.21

The highest diversity index was recorded in the rainy season, indicating greater species richness and evenness.

### Influence of Environmental Factors-

**Table 4:** Seasonal Environmental Parameters

Season	Temperature (°C)	Humidity (%)	Rainfall (mm)
Summer	38–42	40–50	Low
Rainy	25–30	75–90	High
Winter	10–20	50–60	Very low

Increased humidity and rainfall during the rainy season correlated positively with microbial growth.

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### **Discussion-**

The present investigation revealed significant seasonal variation in phyllospheric microflora associated with *Saccharum officinarum* cultivated in Ambedkar Nagar. The observed variations in microbial population and diversity are strongly influenced by environmental factors such as temperature, humidity, and rainfall.

The results indicate that the rainy season supports the highest microbial load ( $7.8 \times 10^5$  CFU/cm<sup>2</sup> for bacteria and  $5.6 \times 10^4$  CFU/cm<sup>2</sup> for fungi). This can be attributed to increased moisture availability, which facilitates microbial colonization and survival on leaf surfaces. High humidity enhances nutrient diffusion and reduces desiccation stress, thereby promoting microbial proliferation. Similar findings have been reported by Vorholt (2012), who emphasized that moisture availability is a key factor regulating phyllosphere microbial communities.

In contrast, the winter season recorded the lowest microbial population. Lower temperatures and reduced humidity during winter create unfavorable conditions for microbial growth, leading to decreased microbial activity. This observation is consistent with the findings of Redford et al. (2010), who reported reduced microbial abundance in colder seasons.

The diversity analysis revealed that bacterial genera such as *Bacillus* and *Pseudomonas* were dominant across all seasons, indicating their adaptability to varying environmental conditions. These genera are known for their plant growth-promoting properties, including nitrogen fixation, phosphate solubilization, and production of growth hormones. Their consistent presence suggests a stable association with the sugarcane phyllosphere.

Fungal species such as *Aspergillus niger* and *Fusarium spp.* were more abundant during the rainy season. The high moisture conditions during this period favor spore germination and fungal growth. Fungi play important roles in decomposition and

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nutrient cycling but may also include potential plant pathogens, indicating a dual role in plant health.

The Shannon diversity index further confirmed that microbial diversity was highest during the rainy season ( $H' = 2.63$ ), reflecting greater species richness and evenness. Seasonal fluctuations in diversity are likely due to changes in environmental conditions and resource availability on the leaf surface. Copeland et al. (2015) also reported similar seasonal shifts in phyllospheric microbial communities.

The correlation between environmental parameters and microbial population clearly indicates that humidity and rainfall are the most critical factors influencing microbial dynamics. Temperature also plays a significant role, but extreme heat or cold can limit microbial survival.

Overall, the study demonstrates that seasonal variation significantly affects the structure and function of phyllospheric microflora in sugarcane. Understanding these dynamics is essential for exploiting beneficial microorganisms in sustainable agriculture and improving crop productivity.

### **Conclusion-**

The study concludes that the phyllospheric microflora of sugarcane in Ambedkar Nagar district shows significant seasonal variation. The rainy season supports maximum microbial diversity and population, followed by summer, while winter exhibits the lowest microbial activity. Environmental factors such as temperature, humidity, and rainfall play a crucial role in shaping microbial communities.

Understanding these seasonal dynamics is essential for developing sustainable agricultural practices, including the use of beneficial phyllospheric microbes for crop improvement and disease management.

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