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Research Article

# Isolation and Enumeration of Bacteria from Fresh Flowers of The Religious City of Ayodhya in the State of Uttar Pradesh in India

**Ranjan Singh[1]*, Prashant Singh[1]*, Ajad Patel[1], Vinay Singh Baghel[2], Vijay Kumar Shukla[3], Soni Tiwari[1], Prabhash Kumar Pandey[4], Laxmi Kant Pandey[5], Enosh Philip[5], Nitin Swamy[5], Femina Sobin[6], Neeraj Khare[7], Abhishek Vashishtha[8], Ashutosh Kumar[1] and Ashutosh Tripathi[1]**

[1]*Department of Microbiology, Dr. Rammanohar Lohia Avadh University, Ayodhya, Uttar Pradesh, India*

[2]*Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India*

[3]*Department of Biochemistry, Dr. Rammanohar Lohia Avadh University, Ayodhya, Uttar Pradesh, India*

[4]*Centre for Advance Research, Faculty of Medicine, King George's Medical University, Lucknow, Uttar Pradesh, India*

[5]*Department of Biotechnology, St. Aloysius College (Autonomous), Jabalpur, Madhya Pradesh, India*

[6]*Department of Microbiology, St. Aloysius College (Autonomous), Jabalpur, Madhya Pradesh, India*

[7]*Nims Institute of Allied Medical Science and Technology, Nims University, Jaipur, Rajasthan, India*

[8]*Department of Microbiology, Maharaja Ganga Singh University, Jaisalmer Road, Bikaner, Rajasthan, India*

***Corresponding Author:** Ranjan Singh and Prashant Singh, Department of Microbiology, Dr. Rammanohar Lohia Avadh University, Ayodhya, Uttar Pradesh, India.

**E-mail:** ranjan.singh13@gmail.com

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## Abstract

Flowers are the good mood refreshment and main parts of the decoration, used for various religious and cultural activities in all over the world. In the Indian state, Uttar Pradesh, the city Ayodhya is globally famous for the temple of Hindu god Lord Rama and that's why flowers play various roles in the events of religious and cultural activities. Due to its regular use, flowers should be away from microbial contamination. The flowers were collected from the main garden of Avadh University (Latitude 26°45'11.8"N, Longitude 82°08'32.7"E), Ayodhya from January to March 2024. Objective of this research was to isolation and enumeration of the pathogenic bacteria from the flowers that may harm humans, animals and environment. The results revealed that flowers contain harmful bacteria, which is a threat to the human and animal health prospective.

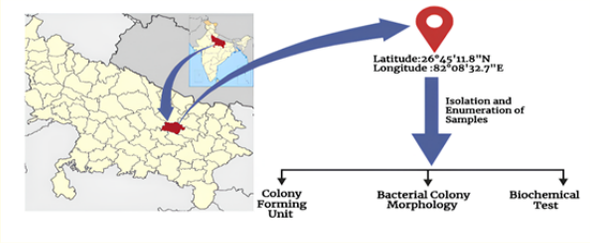


**Figure a**

**Keywords:** Decoration; Religious; Flower; Harm; Pathogenic Bacteria; Bacterial Contamination

## Abbreviation

UV: Ultra Voilet; EMB: Eosin Methylene Blue Agar; NA: Nutrient Agar; BOD: Biological Oxygen Demand; CFU: Colony Forming Unit; N: Negative; P: Positive

## Introduction

With their colour and scent, flowers are a delightful creation of nature. There are over 2.4 lakh flowering plant species known to exist worldwide. It is among the diverse groupings of microorganisms' ecological niches. The research of flower microbial diversity is still in its early stages and continues to pique the interest of microbiologists. Microorganisms are employed extensively nowadays to produce various colours and fragrances in response to the growing demand from humanism [1].

The term "phyllosphere" refers to the aerial sections of plants, such as buds, fruits, and other above-ground components like leaves and stems, that serve as a home for microorganisms [2]. It is a unique and dynamic habitat that is thought to contribute to erratic and occasionally significant variations in temperature, UV radiation, and leaf humidity [3]. Microbes are present on various plant segments as endophytes within the plant tissues and as epiphytes on the surface. Although bacteria are the most major microbial occupants in the phyllosphere, other groups of microorganisms, including filamentous fungus, yeasts, and archaea, are also thought to be important [2,4]. These bacteria, which affect the health of the host plant as well as people and animals, include both pathogenic and non-pathogenic populations [4,5].

Flowers are a complex mechanism for conspecific people to share pollen. The plants use several vectors, including insects and wind, to disseminate and receive pollen in order to achieve sexual reproduction rather than moving around in search of a possible mate. Consequently, under natural circumstances, flowers are similarly susceptible to microbial infestation [6,7]. Microbes can live in a variety of environments on flowers, which draw pollinators with their nectar, which is high in sugars and frequently contains additional nutrients like lipids and amino acids [8]. The development chamber for pollen tubes is attached to the stigma, which serves as a germination bed for pollen grains. Stigma's role is to maintain the nutrients and humidity required for pollen tube formation [7], which would also be beneficial for microbes.

Numerous species' flowers in a variety of settings have incredibly diverse microbial communities, according to recent research employing high-throughput sequencing [9-11]. Many viruses enter through flowers, particularly through the stigma and nectary. Fruit abortion and systemic infection are frequent outcomes of their blossom infection [7,12]. Four different kinds of flower samples were selected for the current investigation in order to isolate and count the bacteria present on the surface of the flowers. Samples of flowers included jasmine, marigold, sunflower, and hibiscus.

The family *Malvaceae* includes the well-known genus *Hibiscus* (*Hibiscus* spp.), which is made up of over 400 species of flowering plants that are indigenous to warm temperate, sub-tropical, and tropical climates worldwide. The *hibiscus* flower is considered the national flower of both Malaysia and Hawaii. In the Philippines, hibiscus has become more and more popular as an ornamental plant due to its beautiful blossoms. *Hibiscus* has long been produced commercially in a number of nations, including China, Thailand, Sudan, Egypt, Nigeria, Mauritius, Madagascar, Sri Lanka, Fiji, the Hawaiian Islands, and the Pacific Islands. India is one of the world's leading *Hibiscus* growers. Hibiscus is used for feed, food, industrial, and medical applications in addition to its religious and decorative significance. Its main bioactive components include flavonoids, terpenoids, glycosides, and saponins [13].

The plant known as marigold (*Tagetes erecta L.*) belongs to the *Asteraceae* family. In many parts of the world, it is frequently planted as a decorative plant and is well-known for its antibacterial and therapeutic qualities [14]. In many nations throughout the world, including Europe and India, marigold is grown not only for the pharmaceutical business but also for the food and cosmetics industries [15].

A significant oilseed crop in contemporary agriculture, sunflowers (*Helianthus annuus*) are utilized for a variety of industrial and culinary applications [16]. The sunflower's short growth cycle allows it to withstand drought, salt, and barrenness [17]. The majority of nations, including Turkey, India, Russia, Argentina, Ukraine, and South Africa, cultivate it [18]. Nevertheless, a lot of sunflower straw is produced when a lot of seeds are given. Statistics show that more than 26.5 million hectares of sunflowers were planted worldwide in 2017, producing between 80 and 186 million tons of leftover straw [19].

The jasmine flower is a highly valuable commodity that may be used not only as a decorative plant pot and garden but also as a tea deodorizer, a raw material for the perfume industry, cosmetics, traditional medicine, and a supplement in traditional ceremonies. Species *Sambac jasmine* the popular its species is used extensively for tea scents and floral arrangements, and it has been designated as Indonesia's national flower. Essential oils are typically made from jasmine blossoms and are highly costly. Chemicals in jasmine have the ability to reduce stress and despair and can boost self-esteem [20].

According to [21] that throughout production, harvesting, and distribution, fruit, vegetables, and flowers are subject to microbial contamination from soil, water, and air, among other sources. Additionally, humans, animals, and insects can all infect them. Flowers are a great substrate for many types of microbes because of their high water and nutritional content (Figure 1).

The current study was carried out in the microbiology department of Dr. Rammanohar Lohia Avadh University in Ayodhya, Uttar

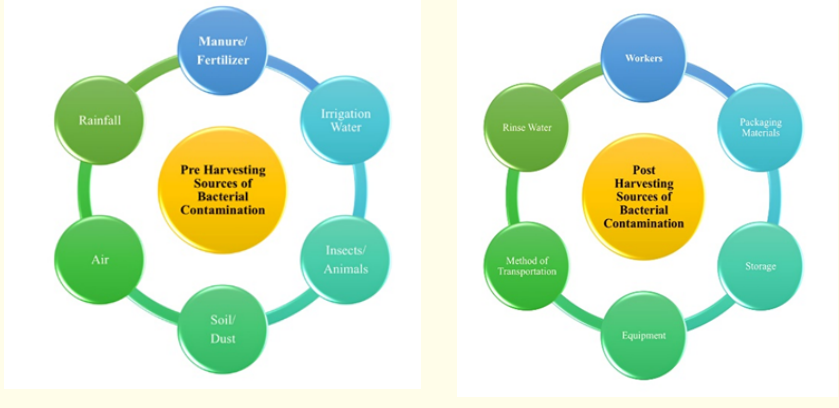


**Figure 1:** Pre and Post Harvesting Modes of Microbial Contamination of the Aerial Parts of the Plants [21,22].

Pradesh, India. Isolating and identifying the bacteria from fresh flowers (Jasmine, Marigold, Sunflower, and Hibiscus) at the RMLA University Campus of India City in Ayodhya is the primary goal of the narrative.

## Materials and Methods

All of the samples were taken from four different sites at the University Garden of Dr. Rammanohar Lodia Avadh University (Latitude 26°45'11.8"N, Longitude 82°08'32.7"E) in Ayodhya, India, in a single day (8 AM to 10 AM). Each sample was 20 meters away from the others. The flower samples included the following varieties: jasmine (J), marigold (M), sunflowers (S), and hibiscus (H). Each sample was labelled H, M, S, and J, in that order. The samples were collected in sterile plastic bags, brought to Dr. Rammanohar Lodia's microbiology department lab at Avadh University, and examined within a day.

Every piece of media utilized in the assessment, including NA, EMB, and MacConkey. The five bacterial stain chemicals (Crystal Violet, Iodine, Decolorization, Ethanol or Acetone, and Safranin) that come with the staining kit are used to make microscope slides that are used to identify bacteria. Serial dilution is a series of periodic dilutions used to reduce the dense culture of cells to a more manageable concentration. The bacterial concentration will decrease by a specific amount with each dilution ($10^5$ and $10^6$ dilution factor). A 0. 1µ sample was applied using the spread plate method to NA, MacConkey agar, and EMB agar, in that sequence, following serial dilution of each sample. This process involves creating the medium, chilling it, and then adding it to the petri dishes that have been disinfected.

After the media has solidified, the plate is inverted and BOD is incubated for a full day at 37°C. To isolate the bacteria, 100 millilitres of NA, 100 millilitres of MacConkey Agar, and 100 millilitres of EMB Agar were prepared and autoclaved at 15 pounds for 15 minutes at 121°C. The media are left to cool after autoclaving. They

are then put onto sterilized petri plates. Divide the medium between two plates. The plates are inverted and incubated for a full day at 37°C after being allowed to harden. This technique involves adding a 0. 1µ sample to the hardened plate and spreading it out with a spreader. After spreading the plates, they were wrapped in parafilm and kept in a BOD incubator set at 37°C for a full day. The colonies observed after 24 hours were re-streaked from mixed colonies to get pure culture colonies. CFU can be computed using the formula below:

$$\text{CFU/ml} = \frac{\text{Number of colonies} \times \text{Dilution Factor}}{\text{Volume of culture plate}}$$

The colonies from every test tube were re-inoculated onto nutrient agar in order to attain a pure growth. Bacteria were detected using the pure culture on the nutrient agar plate. Bacterial species are classified using this method into two main groups: Gram positive and Gram negative. Identifying peptidoglycan, which is present in the cell wall of Gram-positive bacteria, is necessary to differentiate between gram-positive and gram-negative bacteria as well as to ascertain the size, shape, organization, and other features of each bacterial cell. Gram staining separates bacteria according to the chemical and physical properties of their cell walls [5,23-25].

## Result and Discussion

Following the evaluation of every flower sample, the findings indicated in Table 1 and 2, are presented. Different levels of bacterial contamination are indicated by the pathogenic bacteria data from flower samples collected from Avadh University's main garden. The S sample exhibited a higher CFU, whereas the same sample also showed a lower CFU at serial dilution factors of 10-5 and 10-6, respectively. Following the staining procedure, the results showed that every sample had rod-shaped, pink stain, which is an indication of harmful bacteria. Table 3 displays the results of several biochemical tests.

Certain bacterial taxa are prevented from establishing themselves by the particular microhabitats that flower organs offer [9]. Flowers' distinct morphological features and metabolic profiles may make it impossible for certain bacteria to establish themselves [26]. Certain bacterial taxa are prevented from establishing themselves by the particular microhabitats that flower organs offer [27,28]. With differing relative abundances on each plant species, *Pseudomonas, Enterobacteriaceae,* and *Sphingomonas* dominated the flowers. With varying frequencies per plant species, *Sphingomonas*, *Methylobacterium*, and *Hymenobacter* were the most prevalent bacterial taxa on leaves. By creating a biological barrier on the surface of flowers and leaves, members of these taxa may be crucial in passively protecting host plants from diseases [29]. Floral structures such as nectaries may have sugar-rich secretions that limit bacterial microhabitats to those that can withstand osmotic stress [28]. Hydrophobic surfaces of petals likely limit bacterial growth, and flowers emit specific volatile compounds that could inhibit the growth of bacteria [27]. The only substantial difference in alpha diversity between host species was in phylogenetic diversity [9]. Early on in our work, we looked into the bacterial microbiomes linked to flowers. We halted this investigation further due to a lack of funding.

**Table 1:** Colony Forming Unit/100ml of Different Samples.

| S. No. | Sample | Dilution Factor | CFU/c□² |
|---|---|---|---|
| 1. | H | $10^{-5}$ | $6.45X10^{8}$ |
| | | $10^{-6}$ | $2.94X10^{9}$ |
| 2. | M | $10^{-5}$ | $5.60X10^{8}$ |
| | | $10^{-6}$ | $2.72X10^{9}$ |
| 3. | S | $10^{-5}$ | $7.64X10^{8}$ |
| | | $10^{-6}$ | $1.83x10^{9}$ |
| 4. | J | $10^{-5}$ | $2.88x10^{8}$ |
| | | $10^{-6}$ | $4.04X10^{9}$ |

**Table 2:** Bacterial Colony Morphology of Different flower Samples.

| S. No. | Sample | Gram staining | |
|---|---|---|---|
| 1. | H | Rod | Pink |
| 2. | M | Rod | Pink |
| 3. | S | Rod | Pink |
| 4. | J | Rod | Pink |

H= Hibiscus, M= Marigold, S= Sunflower, J= Jasmine.

**Table 3:** Biochemical Test of the Samples.

| S. No. | Test | Hibiscus | Marigold | Sunflower | Jasmine |
|---|---|---|---|---|---|
| 1. | Catalase | + | + | + | + |
| 2. | Methyl Red | - | + | - | - |
| 3. | Indole | - | - | - | - |
| 4. | Citrate | + | - | + | - |
| 5. | Urease | - | - | + | - |
| 6. | Sucrose | - | + | + | + |
| 7. | Lactose | - | - | + | + |

Positive (+); Negative (-).

## Conclusion

According to the findings from the current study, it is clear that flowers have up to $7.64\times10^8$ $cm^2$ of bacteria on their surfaces. When flowers are not properly cleaned, harmful bacteria are added and can cause contamination. Airborne dust and swarming house and flower flies can potentially be sources of infection, as can the use of unsanitary water preservation methods without refrigeration. Therefore, learning about flower safety and hygienic procedures is crucial. There were notable differences in abundance between the several flower species, according to the counting of bacterial CFUs. This variation implies that environmental variables, nectar content, and floral architecture all have a significant impact on how bacteria colonize and multiply on the surface of flowers.

## Acknowledgement

Not Applicable.

## Conflict of Interest

The authors collectively affirmed the absence of any conflicts of interest.

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