

SEASONAL AND GEOGRAPHICAL VARIATION IN THE ANTIBACTERIAL EFFICACY OF BEE BREAD COLLECTED FROM CHANDIGARH, INDIA

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(Received 24 March, 2025; Accepted 27 May, 2025)

Keywords: Bee bread, Antibacterial activity, Seasonal variation, Chandigarh flora

Abstract–Bee bread is a fermented product derived from bee pollen, enriched with enzymes and microorganisms that enhance its nutritional and medicinal properties. This study investigates the antibacterial efficacy of 20% (MIC) bee bread aqueous extract (BBAE) collected from various sub-divisions of Chandigarh across different seasons. The extract was tested against four pathogenic bacteria: *Pseudomonas aeruginosa* (MTCC 2453), *Bacillus subtilis* (MTCC 121), *Streptococcus pneumoniae* (MTCC 655), and *Salmonella typhimurium* (MTCC 3231) using the agar disc diffusion method. Results showed that the antibacterial effectiveness varied depending on the sub-division and season of collection. The most significant inhibition zones were observed in Sub-division 5 during autumn against *P. aeruginosa*, Sub-divisions 3 and 4 during winter against *B. subtilis*, Sub-division 3 during autumn against *S. typhimurium*, and Sub-division 2 during winter against *S. pneumoniae*. These findings suggest that the antibacterial properties of bee bread are influenced by its botanical origin and the time of collection. Study highlighting its potential as a natural antimicrobial agent and its relevance in alternative medicine against antibacterial resistance and sustainable apiculture.

INTRODUCTION

Honey bees (*Apis mellifera*), belonging to the family Apidae, are essential pollinators that support agricultural ecosystems by pollinating over 80% of flowering crops worldwide (Hung *et al.*, 2018). Among the many valuable products of the beehive, bee bread stands out for its superior nutritional and therapeutic properties. Bee bread is produced when bees mix pollen with nectar and their enzymatic secretions, followed by microbial fermentation inside the hive (Iorizzo *et al.*, 2020).

This fermentation process, which involves bacteria and yeasts, enhances the bioavailability of nutrients and breaks down complex compounds in pollen, resulting in a more digestible and stable food source (Herbert and Shimanuki, 1978; Vasquez and Olofsson, 2009). The resulting product is rich in carbohydrates, enzymes, vitamins B and K, and organic acids such as lactic acid, which lowers its pH and contributes to its antimicrobial effects (Kieliszek *et al.*, 2018).

Bee bread also contains phenolic compounds with antioxidant and anti-inflammatory properties (Pulido *et al.*, 2000), and lactic acid bacteria (LAB), such as *Lactobacillus kunkeei* and *L. plantarum*, which produce bacteriocins that contribute to its antimicrobial activity (Pelka *et al.*, 2021). These properties make bee bread a promising candidate for therapeutic applications, particularly in combating antimicrobial resistance.

This study aims to evaluate the seasonal and regional differences in the antibacterial activity of bee bread collected from five horticulturally defined sub-divisions of Chandigarh. The goal is to identify the most potent samples and explore bee bread's potential as an alternative antimicrobial agent.

MATERIALS AND METHODS

Study Location and Sampling Sites

The research was conducted across five sub-divisions of Chandigarh, categorized by the

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Horticulture Department based on vegetation type and planting patterns (Table 1).

Seasonal Classification

Bee bread samples were collected in four distinct seasons:

- **Spring:** Mid-February to early April
- **Summer:** Mid-April to late June
- **Autumn:** Late September to mid-November
- **Winter:** Mid-November to mid-February

Collection and Storage of Bee Bread

Bee bread was extracted directly from comb cells using sterilized spatulas and stored in airtight glass containers at "20 °C for further analysis.

Physical Evaluation

Each sample was assessed for color, texture, and consistency. Observed differences reflected the influence of floral sources and climatic conditions on bee bread composition (Table 3).

Preparation of Bee Bread Aqueous Extract

Following the modified protocol by Nagai *et al.* (2004), 3 g of bee bread was mixed with 30 ml of distilled water, shaken at 20°C for 24 hours, and centrifuged at 5000 rpm for 1 hour. The supernatant was collected and stored at "20°C.

Antibacterial Activity Testing

The antibacterial activity of the 20% BBAE (MIC)

was tested using the disc diffusion method against the following pathogens:

- *Pseudomonas aeruginosa* (MTCC 2453)
- *Bacillus subtilis* (MTCC 121)
- *Streptococcus pneumoniae* (MTCC 655)
- *Salmonella typhimurium* (MTCC 3231)

Test conditions, including temperature and incubation periods, were based on MTCC guidelines (Table 4). Standard antibiotic discs (Tetracycline and Streptomycin) served as positive controls.

Data Analysis

All tests were conducted in triplicate. Results were expressed as the mean diameter of the inhibition zone (in cm) ± standard deviation (SD).

RESULTS AND DISCUSSION

Physical Characteristics of Bee Bread

Seasonal changes influenced the physical properties of bee bread. Winter samples were typically brittle and dark in color, while summer samples were more varied, with some showing a lime-like aroma and a softer texture.

Antibacterial Activity Overview

The antibacterial potential varied with both the collection site and season, likely due to differences in flora and associated microbial communities. The summary below highlights key findings:

Table 1. 5 sub- divisions of Chandigarh

S.No.	Subdivisions	Area Details
1	Sub-division 1	SECTOR- 25, 36, 37, 38, 38, 39, 40, 41, 42
2	Sub-division 2	SECTOR- 27, 28, 30, 31, 32, Sec 29 Industrial area phase 1, Sanjay Colony (SLUM), Industrial area phase 1 and Industrial area (phase 1 Colony no. 4), Industrial area and phase II
3	Sub-division 3	SECTOR- 1 to 11, 12, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24
4	Sub-division 4	SECTOR- 26, Bapudham tragt Camp II Phase II Sec- 26E, Transport area Sec- 26-E, EWS Housing Board
5	Sub-division 5	SECTOR- 20, 33, 34, 35, 43, 44, 45, 49, 50, 51, 52 (Nizam Pur Burail), 51, and Sec- 55, 56, 60, 61, 63

In each sub-division (sub- div.), *Apis mellifera* colonies were maintained in Langstroth hives (Table 2).

Table 2. Details of honey bee colony installation

Sr.No.	Sub- division	Honey bee colony installation area	Date of installation
1	Sub- division 1	Post Graduate Govt. College for Girls, Sector-42, Chandigarh	02.02.2022
2	Sub- division 2	Sector- 29 (Nursery)	02.02.2022
3	Sub- division 3	Sector- 23 (Nursery)	05.02.2022
4	Sub- division 4	Central Reserve Police Force Camp	07.02.2022
5	Sub- division 5	Sector- 33 (Nursery)	05.02.2022

Pseudomonas aeruginosa

- **Highest inhibition:** Autumn, Sub-division 5 (0.96 ± 0.05 cm)
- **Lowest inhibition:** Spring, Sub-division 1 (0.56 ± 0.11 cm)

Bacillus subtilis

- **Highest inhibition:** Autumn, Sub-division 3 (1.00 ± 0.00 cm)
- **Lowest inhibition:** Winter, Sub-division 5 (0.53 ± 0.47 cm)

Salmonella typhimurium

- **Highest inhibition:** Summer, Sub-division 4 (1.03 ± 0.15 cm)
- **Lowest inhibition:** Spring, Sub-division 5 (0.40 ± 0.34 cm)

Streptococcus pneumoniae

- **Highest inhibition:** Autumn, Sub-division 1 (1.00 ± 0.05 cm)
- **Lowest inhibition:** Winter, Sub-division 1 (0.46 ± 0.41 cm)

These results indicate that the antibacterial efficacy of bee bread is significantly influenced by the floral origin and environmental conditions at the time of collection.

Comparison with Previous Studies

Previous research has shown that higher concentrations of pollen extracts yield greater antibacterial activity, largely due to increased phenolic content (Eswaran and Bhargava, 2014). Similarly, in this study, even the 20% BBAE demonstrated substantial antibacterial potential,

Table 3. Physical evaluation of bee bread collected from 5 sub- divisions of Chandigarh during different seasons

S. No.	Seasons	Colour	Nature and consistency
1	Winter	Light yellow, Dark yellow, Orange, Creamish white, Greyish white, Greenish yellow, Dark brown, Purple, Grey	Brittle nature Soft and brittle with greasy nature White bee bread has lime smell with soft nature but other have soft and dusty nature Dusty and brittle
2	Spring	White, Yellow, Orange, Light brown, Purple	
3	Summer	Orange, Yellow, White, Light brown, Black, Grey, Greyishgreen, Dark green	
4	Autumn	Yellow, Orange, White, Creamish white	

Table 4. Anti-bacterial efficacy of 20% bee bread aqueous extract against *Pseudomonas aeruginosa*

Seasons	Sub- div. 1	Sub- div. 2	Sub- div. 3	Sub- div. 4	Sub- div. 5
Winter	0.63± 0.5	0.66± 0.15	0.73± 0.15	0.73± 0.20	0.86± 0.05
Summer	0.53± 0.11	0.83± 0.05	0.86± 0.15	0.66± 0.15	0.73± 0.15
Autumn	0.83± 0.05	0.63± 0.15	0.73± 0.05	0.9± 0	0.96± 0.05
Spring	0.56± 0.11	0.7± 0	0.9± 0.1	0.86± 0.5	0.66± 0.15

Table 5. Anti-bacterial efficacy of 20% bee bread aqueous extract against *Bacillus subtilis*

Seasons	Sub- div. 1	Sub- div. 2	Sub- div. 3	Sub- div. 4	Sub- div. 5
Winter	0.73± 0.11	0.8± 0.2	0.96± 0.05	0.96± 0.05	0.53± 0.47
Summer	0.73± 0.11	0.93± 0.05	0.76± 0.05	0.9± 0	0.6± 0.1
Autumn	0.9± 0.17	0.66± 0.15	1± 0	0.8± 0.1	0.83± 0.20
Spring	0.83± 0.11	0.8± 0.2	0.93 ± 0.05	0.83± 0.05	0.66± 0.11

Table 6. Anti-bacterial efficacy of 20% bee bread aqueous extract against *Salmonella typhimurium*

Seasons	Sub- div. 1	Sub- div.2	Sub- div. 3	Sub- div. 4	Sub- div. 5
Winter	0.83± 0.11	0.8± 0.2	0.96± 0.05	0.66± 0.11	0.66± 0.05
Summer	0.86± 0.11	0.8± 0.52	0.6± 0	1.03± 0.15	0.9± 0.37
Autumn	0.66± 0.05	0.7± 0.05	0.96± 0.15	0.6± 0.52	0.7± 0
Spring	0.63± 0.05	0.8± 0.2	0.76± 0.20	0.73± 0.15	0.4± 0.34

Table 7. Anti-bacterial efficacy of 20% bee bread aqueous extract against *Streptococcus pneumoniae*

Seasons	Sub- div.1	Sub- div. 2	Sub- div. 3	Sub- div. 4	Sub- div. 5
Winter	0.46± 0.41	0.93± 0.05	0.86± 0.15	0.83± 0.20	0.86± 0.15
Summer	0.9±0.1	0.8± 0.2	0.9± 0.1	0.63±0.05	0.83± 0.20
Autumn	1±0.05	0.76± 0.2	0.7± 0.20	0.73± 0.15	0.83± 0.11
Spring	0.6±0	1±0.1	1± 0.05	0.9± 0	0.8± 0.2

particularly against *P. aeruginosa* and *S. typhimurium*. This underscores bee bread's natural efficacy and suggests its effectiveness can be further enhanced with higher concentrations.

CONCLUSION

This study confirms that bee bread possesses considerable antibacterial activity, influenced by seasonal variation and local flora. Autumn and winter samples, particularly from Sub-divisions 3 and 5, demonstrated the highest efficacy. The presence of beneficial lactic acid bacteria and bioactive compounds in bee bread supports its use as a natural antimicrobial agent.

The findings emphasize the importance of regional plant diversity and its impact on bee products. Bee bread shows strong potential as a complementary treatment against bacterial infections and can play a significant role in addressing antimicrobial resistance. Furthermore, promoting beekeeping and the cultivation of bee-friendly flora can enhance local economies and public health.

ACKNOWLEDGEMENT

The authors would like to express sincere thanks to Director of Origin life Healthcare solutions & Research Centre LLP, Sector- 38, Chandigarh, 160036 for providing necessary facility and the financial support.

Conflict of Interest Disclosure Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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