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# Identification of the Melissopalynological Flora of Northern Punjab using Honey Bee Samples

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## ABSTRACT

Melissopalynology, the study of pollen contained in honey, plays a pivotal role in elucidating the dynamics of pollination and the underlying biodiversity essential for ecological health. This research utilizes melissopalynological techniques to meticulously examine honey bee samples from Northern Punjab, focusing on the diverse biotopes encompassing agricultural lands, urban environments, and forest areas. Employing advanced microscopy and robust statistical methodologies, the study investigates the variations in pollen composition across different seasons, thereby shedding light on the complex interactions between bees and their floral environment. The findings from this study reveal distinct seasonal patterns of pollen availability, which are critical for optimizing agricultural practices and enhancing crop productivity. During spring, there is a notable increase in pollen diversity, corresponding with a peak in floral blooms. In contrast, winter samples show reduced diversity, reflecting the scarcity of blooming plants during the colder months. Spatial analysis further demonstrates that the type of biotope significantly influences the pollen profiles; agricultural areas predominantly yield pollen from crop plants, forests are rich in native floral species, and urban areas present a mixture of ornamental and invasive plant species. These insights are invaluable for informing sustainable agricultural practices and biodiversity conservation efforts. By understanding the seasonal and spatial variations in pollen sources, farmers can better align their planting and cultivation strategies to support bee health and ensure effective pollination. The study highlights the importance of maintaining diverse and native plant species in urban and forested areas to foster robust bee populations and ecological resilience. This research underscores the critical role of melissopalynology in enhancing our understanding of pollination processes, supporting agricultural sustainability, and promoting biodiversity conservation in Northern Punjab.

## INTRODUCTION

### Context and Importance

It is known as melissopalynology, which is "the scientific study of pollen and spores collected by honey bees in and incorporated into honey" (Junqueira et al. 2023). This is a multidisciplinary area of study, encompassing botany, ecology, agriculture, and environmental sciences. Pollen content provides researchers with an invaluable tool for understanding floral biodiversity (the species richness and abundance of plants within an ecosystem) and pollination dynamics, or the relationships between the plants that require pollination and their pollinators, especially honey bees [1]. Pollination is essential for the reproduction of a large number of plant species, thus one of the most influential factors in terms of ecosystem stability, crop productivity and biodiversity conservation. As important pollinators, honey bees play a crucial role in agricultural production through their pollination services. Melissopalynology, the study of pollen collected by honey bees, not only can reflect the floral preference of bees, but also can indicate the availability and richness of pollen sources from various geographic places. This information is especially of interest in regions more agriculturally driven, like Northern Punjab, where effective pollinating has a direct impact on ecosystems and agriculture productivity [2]. Thus, melissopalynological studies are crucial for the development of sustainable agriculture practices and strategies for conserving biodiversity, and therefore would be of significant ecological and economic importance.

### Research Problem

Northern Punjab is a very important agriculture area and has a major contribution to the regional and national economy [3]. Though a major agricultural product, no extensive or detailed melissopalynological data (e.g., pollen diversity, abundance, and seasonal variations) are available. Challenges, as this data is absent:

- This has made it difficult to accurately assess floral resources, which are essential to the viability of pollinators such as the honey bee.
- This hinders evidence-based decision-making on crop management practices, which can lead to decreased agricultural productivity and profits.
- This hinders the formulation of effective biodiversity conservation strategies such as protecting native plant species and alloying ecobalance.

### Aim and Objectives

This study primarily focuses on the determination and characterization of dominant types of pollen found in bee samples collected from Northern Punjab, along with an analysis of their diversity and distribution throughout the seasons. More precisely, the expected objectives of the research are:

- To identify the main pollen sources in honey samples collected from a range of environments, including agricultural landscapes, urban areas and forest regions.
- To use this data to discuss seasonal variation in pollen diversity and how changes in pollen availability and composition across the year may shape pollinator behaviour and floral resource use.
- Measuring ecological and agricultural implications of melissopalynological findings for sustainable agriculture, increased yield and biodiversity conservation in Northern Punjab.

  The specific objectives all together will result in practical outcomes, benefitting on ground agricultural management and conservation practices for ecological sustainability and economic prosperity of Northern Punjab.

## LITERATURE REVIEW

### Background of Melissopalynology

Melissopalynology is the study of the pollen and spores found in honey. This field of study has been described as the critical link among botany, ecology, and agriculture, providing valuable information regarding the interactions of honey bees and the flowering plants in their environment [4]. Melissopalynologists, scientists who study the pollen content of honey, can determine the type of plants bees visit to gather nectar, which is essential for tracking botanical diversity in a location. One analysis performed in melissopalynology eta can identify not merely which forms of plants honey bees are pollinating, but each the unique qualities in their flowering routines. This is an important finding as it sheds light on the role of bees in the ecosystem being pollinators an indispensable [5]. Moreover, knowing which plants bees visit most can help inform conservation efforts, particularly in places where biodiversity is threatened by urbanization, agriculture or climate change.

Your expertise identifies on information-based dynamic data of pollination structures through melissopalynology which is serving for agricultural science to adapt crop production through pollination dynamics. Thus, beekeepers, farmers and agricultural planners can optimize based on a forecasted availability of primary pollen sources for bees, contributing to the sustainability of global food production systems. This is especially important in regions where crops that depend on pollinators represent a large share of agricultural production. At the same time, melissopalynology plays a key role in tracking the ecological state of an area. The number of different pollen types present in honey can be an indicator of local ecosystems' health and stability [6]. A high diversity of pollen indicates a rich and diverse plant community, which is usually a sign of a healthy environment. In contrast, a drop in pollen diversity could indicate ecological disruptions or degradation, warranting further investigation and possibly informing ecosystem restoration projects. The pollen analysis is one way of access to the nature world, by identifying pollen that are involved in the process of exploiting plants or of environmental adaptation [6] melissopalynology has also been recognized as a perceptive botanic sense [7]. This knowledge will allow playing an important aspect of conservation, ensuring the human development that world needed today and providing the equilibrium needed in nature.

### Significance and Previous Research

Melissopalynology has greatly improved our knowledge preparing the interactions between honey bees and their floral environments. By carefully cataloguing and analyzing the pollen sources in honey, researchers have identified the plant species most abundantly visited by bees, the geographical distribution of those plants in relation to each other, and the repercussions of their findings for agricultural production and conservation [8]. It is important to study their features since it helps us to understand why honey bees prefer some plants and ignore others. Factors such as pollen nutritional quality, flower morphology, and temporal floral availability can determine preferences. By highlighting the plant species that bees most commonly forage on, melissopalynology provides insight into the important connections that exist within pollination networks that are essential for the reproductive cycle of many plant species, as well as the health of ecosystems (Almeida et al., 2021).

In practical applications concerning agriculture and conservation, melissopalynological research can also be significant. For instance, realizing which plants are significant pollen suppliers may help farmers and land managers to increase floral diversity and thus bee populations, as well as increase the pollination rates of crops, with consequent benefits for the yield and sustainability of their agricultural systems [9]. Such knowledge is important for conservation since it specifies key nectar resources to conserve to support or increase biodiversity. Work related to this includes [10], Sniderman et al. (2018), emphasized the importance of pollen analysis for a comprehensive understanding of plant biodiversity. Pollen types contained in honey were shown as bio-indicator of health and transformation of local vegetation in these studies [11]. This type of information is important for the efficient management of agricultural landscapes and natural systems alike. Similarly, however, when pollen diversity is assessed over time, it can provide indicators of early changes in ecological condition or stress resulting from human activities or climatic changes, which may allow conservationists and ecologists to intervene before those changes become severe.

Melissopalynology is able to reflect not only the richness of local flora but also its dynamics seasonally, thus providing powerful management tools to manage and strengthen plant populations [12]. It assists both agricultural planning and crop selection and aids also in the conservation of some native species and their habitats, improving their survival and enhancing global biodiversity. These melissopalynology bridges the research doing both ecologists and practical land use actors for agriculture and conservation, yielding high quality data that could have the opportunity to affect a number of ecological and economic results [13]. Its further development and incorporation within wider scientific and policy structures is critical if we are to be sustainable in our use natural capital.

### Existing Gaps in Melissopalynological Research

In existing studies on melissopalynology, the breadth and scope of the research performed are still wide open, thus leaving many gaps in the literature for this now highly popular field [14]. These gaps may also affect our understanding of pollen dynamics in their practical ramifications for agricultural production and ecological reclamation.

### Seasonal Data Limitations

A key restriction voiced in previous research is the lack of data spanning both seasons. Melissopalynology studies often analysed pollen samples for a specific season or for a short time period. Error: This method does not account for all seasonal variations in pollen availability and bee foraging activity needed to cycle through the year used for you do forget everything about him. Such as [16] and [15], which stress the significance of extensive seasonal studies throughout the year. Such extensive data are critical to accurately measuring how varying plants influence local ecosystems year-round and how bees react to these changes. This is particularly relevant to more seasonal regions where flowering of plants and activity of pollinators can vary markedly from one growing season to the next.

### Integration with Agricultural Practices

A third important gap in melissopalynological research is a clear lack of pollen analysis in relation to practical agricultural applications. However, the vast majority of research is focused on identifying and characterizing pollens without making direct connections to agricultural practices or decision-making [17]. This disconnect reduces the practical utility of melissopalynological insights by farmers and agricultural planners who might benefit from tailored recommendations regarding crop management and planting schedules. The data indicate that agriculture is closely linked to the productivity (including with respect to health), sustainability, and biodiversity management of beekeeping and melissopalynological datasets could be better integrated to accomplish those goals [18]. This enables researchers to translate their findings into actionable recommendations for growers, thereby fostering best practices to improve pollination and maintain pollinator population health over time.

### Addressing the Research Gaps

To overcome the recognized gaps with regard to melissopalynological studies, this study intended to carry out a thorough and extensive survey regarding pollen sources available for honeybee colonies in the different seasons in Northern Punjab [19]. The idea is to create systematic reports of pollen in different environments: agriculture, urban areas, forest, throughout the year. This comprehensive approach will provide a better understanding of seasonal variability in pollen distribution and availability than has previously been conducted.

### Comprehensive Seasonal Analysis

Therefore, by widening the horizont of the analysis from pollen production and bee activities for an entire year, the herein study embraces dynamic seasonal changes in pollen and bee activity. This broad temporal coverage is often important for understanding networks of plants and pollinators beyond peak periods (which most other studies) [20]. These detailed seasonal data are critical for assessing the ecological roles of different types of plants and their contributions to local biodiversity across the seasons.

### Ecological and Agricultural Integration

Overall, one of the principals aims of this research is to bridge melissopalynological results and actionable agricultural and ecological management applications. This study aims to provide information products that can be directly translated into agricultural planning and practices by mapping pollen sources and their seasonal variations [21]. To give an example, knowledge of the prevalence of different pollen sources at each point in the year can empower farmers with the information necessary to make informed decisions regarding crop planting and timing, as well as pest management and fertilization best practices, which can improve crop yields and sustainability.

### Enhancing Biodiversity Conservation Efforts

The findings from this research will help not only enhance agricultural productivity but will also aid in conservation of biodiversity. The research will document the diversity and abundance of pollen in a range of ecosystems and conditions, allowing identification of the critical habitats and plant species that underpin healthy syrphids populations [20]. Data about the numbers and types of species present in an area can be used to inform conservation strategies and habitat restoration efforts, promoting biodiversity within that ecosystem.

### Practical Applications and Policy Implications

We anticipate that the exquisite and comprehensive data set accumulated in this study will work as a helpful tool for policymakers, environmental planners, and agricultural practitioners. These findings can serve as a scientific basis for formulating policies and strategies that foster agricultural productivity and conservation of environmental assets [20]. This approach will provide a better connection between the research and practical side of this field, maximizing the benefits of the melissopalynological findings in regional sustainability projects.

## METHODOLOGY

This chapter provides the detailed research design and methodologies used while conducting a melissopalynological study of Northern Punjab [21]. Every step in the process—site selection, data analysis—was carefully formulated for scientific accuracy, reproducibility, and relevance to ecological and agricultural systems.

### Study Area

Northern Punjab is an area with great floral diversity and agricultural intensity, making it a study area area for this research. The area harbors three major biotopes:

- Large agricultural fields (e.g., cropland dominated landscapes),
- Urban settings (e.g., gardens, road verges, and managed greenspace), and
- Forests (natural or semi-natural vegetation zones)

These diverse settings were deliberately chosen to represent a wide range of floral resources used by pollinators. The combination of ecological diversity provides a perfect case for studying both seasonal and spatial variation in pollen types and honeybee floral preferences.

### Sample Collection

To encompass immediately seasonal variation, honey bee samples were collected quarterly to follow the 4 main flowering seasons in the region:

- Spring
- Summer
- Monsoon
- Winter

Sampling occurred at deliberately chosen sites in each biotope, based on ecological representatively, known plant diversity, and importance to a region's agriculture [21]. The deliberate time-and place-based sampling thus yielded a holistic dataset able to characterize the year-round profile of pollen availability.

### Experimental Procedures

Honey samples were processed following a series of standardized melissopalynological protocols that ensured valuable homogeneity and reproducibility:

- **Dilution:** Honey was diluted with distilled water for pollen sedimentation.
- **Pollen Analysis:** The diluted honey was centrifuged to contribute to pollen separation from other solid deposits.
- **Microscopic examination:** Pollen residues were mounted on glass slides and were studied under high quality compound microscopes. Reference slides and pollen atlases pertinent to Punjab were employed for identification of pollen grains at the lowest taxonomic level, most frequently at genus or species level.

This multi-step model enables accurate quantification and identification of pollen taxon within each sample.

### Data Analysis

The statistical analysis was tailored to extract meaningful patterns from the observed pollen data:

- The number of occurrences and use of each pollen type by the different seasons and biotopes were recorded in Frequency Tables.
- Based on pollen composition, Cluster Analysis identified floral and ecological affinities through the grouping of similar samples.
- For relationships between multiple variables (e.g., location, season, pollen diversity), and in order to reduce the dimensionality of the data for more straightforward interpretation, the Principal Components Analysis (PCA) was performed.

These tools allowed for a holistic understanding of floral dynamics, pollinator behavior, and their implications for agriculture and biodiversity. The revelations from the analysis are not just ecological but practicable — they offer a framework for guiding crop selection, maximizing pollination and conserving critical floral resources.

## RESULTS

The results obtained from the honey bee samples collected in Northern Punjab are reported in this chapter as melissopalynological results. The pollen types along with their abundance in three of the biotopes (agricultural, urban, forest) were logged for four seasons (spring, summer, monsoon, winter) in a generated backlog of intensive data (excel). The data set is summarized in Table 4.1: each row corresponds to a honey sample from an individual biotope and season and shows quantitative data on pollen composition expressed as the percentage of each type of pollen. In the honey, a total of 10 separate pollen types were identified, including several crop types, wild species, and weeds [22]. The results of these analyses are used to derive and discuss seasonal pollen variability, spatial (biotope) variability, properties of dominant vs. rare pollen types, and overall pollen diversity and distribution patterns of the study area, according to the aims of the research.

Table 4.1. Honey samples collected and stored from different sources in Northern Punjab for melissopalynological analysis. The location (with biotope) and season of each sample is reported, followed by the relative amount of each identified type of pollen. Allergens identified, along with its categories as does not exist altogether or allergen were those that are common in each state, which are Brassica (mustard) Citrus (orange/lemon family) Eucalyptus Clover (Trifolium spp.) Various other families of plants were also used such as Ziziphus (ber), Sunflower (Helianthus), Acacia (e.g. Acacia modesta), Parthenium (carrot weed), Syzygium (jamun) and Mango

(Mangifera). This table is the foundation of the temporal and spatial patterns analytical study.

**Table 1**

*Melissopalynological dataset of honey samples from Northern Punjab, showing pollen type composition (% of total pollen) by biotope and season*

| Location | Biotope | Season | Brassica (%) | Citrus (%) | Eucalyptus (%) | Clover (%) | Ziziphus (%) | Sunflower (%) | Acacia (%) | Parthenium (%) | Syzygium (%) | Mango (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sargodha (Agri) | Agricultural | Spring | 15 | 30 | 10 | 20 | 5 | 0 | 5 | 0 | 5 | 10 |
| Sargodha (Agri) | Agricultural | Summer | 0 | 0 | 15 | 10 | 20 | 20 | 10 | 10 | 10 | 5 |
| Sargodha (Agri) | Agricultural | Monsoon | 0 | 0 | 15 | 0 | 5 | 40 | 5 | 20 | 10 | 5 |
| Sargodha (Agri) | Agricultural | Winter | 65 | 5 | 10 | 20 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rawalpindi (Urban) | Urban | Spring | 10 | 25 | 25 | 10 | 5 | 0 | 20 | 0 | 0 | 5 |
| Rawalpindi (Urban) | Urban | Summer | 0 | 0 | 25 | 0 | 10 | 5 | 20 | 5 | 35 | 0 |
| Rawalpindi (Urban) | Urban | Monsoon | 0 | 0 | 20 | 10 | 0 | 10 | 10 | 50 | 0 | 0 |
| Rawalpindi (Urban) | Urban | Winter | 15 | 10 | 60 | 5 | 0 | 0 | 10 | 0 | 0 | 0 |
| Chakwal (Forest) | Forest | Spring | 5 | 0 | 10 | 20 | 10 | 0 | 50 | 0 | 5 | 0 |
| Chakwal (Forest) | Forest | Summer | 0 | 0 | 5 | 10 | 60 | 0 | 10 | 0 | 15 | 0 |
| Chakwal (Forest) | Forest | Monsoon | 0 | 0 | 10 | 30 | 0 | 20 | 0 | 30 | 10 | 0 |
| Chakwal (Forest) | Forest | Winter | 30 | 0 | 30 | 20 | 0 | 0 | 20 | 0 | 0 | 0 |

## Seasonal Pollen Variation

The pollen composition of honey samples showed high seasonal variations, reflecting the botanic calendar of the Northern Punjab region, where different plant taxa introduce in different periods of the year.

### Winter (December to February)

In winter, the honey samples were mainly monofloral with considerable contributions of mustard (Brassica) pollen (65% of pollen in agricultural samples). This is a sign of large-scale cultivation of mustard in winter. Eucalyptus was also prominent, specifically in urban areas (Rawalpindi) – 60% of the pollen profile where certain species of Eucalyptus bloom are non-seasonal [22]. Indeed, winter is overall lower in diversity, with 3-4 types of pollen per sample, reflecting the poor availability of flowering plants in the season.

### Spring (March to May)

In spring we observed the greatest diversity of pollen with 6-8 distinct pollen types across samples. This spike matches the profusion of flowers at this time. In particular, pollen from citrus trees constituted -- on average -- around 25-30% of the pollen in samples taken from agricultural and urban surroundings, reducing trees to the most important nectar source in spring [23]. Clover also played a significant role, incorporating up to 10-20% of the overall pollen composition, especially in forested and agricultural environments, and acacia pollen, at 50%, was particularly high in forested localities, demonstrating the flowering of Acacia modesta. The spring samples cover great diversity, reflecting the range of flower they strip nectar and pollen from in spring.

### Summer (June to July)

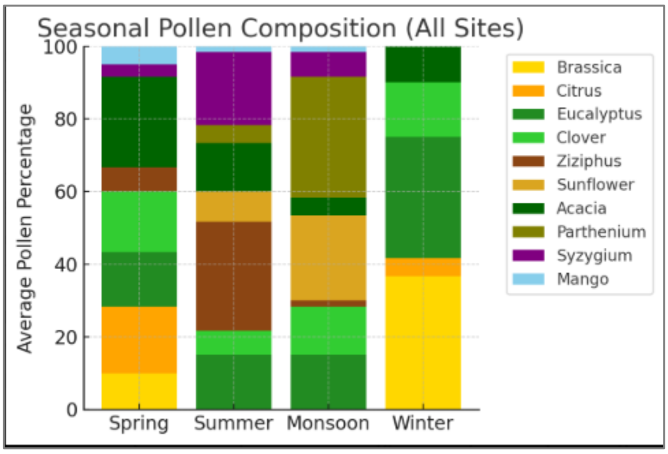
Summer brought an emphasis on wild tree pollen. Among all genera of forest samples, Ziziphus jujuba (ber) had the highest pollen percentage (60%), indicating its flowering peak in dry areas. In the urban sites, common ornamental and roadside trees Syzygium cumini (jamun) comprised 35% [24]. Eucalyptus remained the main source of pollen in all regions, indicating either continued blooming of some species or bees foraging on that plant in extended seasons. Sunflower was also an important element in the agricultural samples, indicating that the seed was either cultivated or grown in the region as a native plant.

### Monsoon (August to September)

The phase of monsoon was marked by the appearance of weed and wildflower pollens; Parthenium (invasive weed) was a dominant source during this phase in urban and forest components. This weed formed up to 50% of the pollen in the prepared honey in urban samples collected from Rawalpindi, thus making the honey unifloral around this time [24]. There were lots of sunflower pollen in agricultural areas, cultivated in the mid-year, and clover was back in force in forest samples after the rains.

**Figure 1**

*Seasonal Pollen Variation*




This information is crucial as the melissopalynological profile of honey varies seasonally which is clearly depicted by the data from this study in case of Northern Punjab. In winter, there is little flowering in nature so only one type of pollen dominates, while in spring there is a significant increase in diversity. The summer emphasizes the importance of native trees and the monsoon shows that the bees are adapting to forage on available weed pollens [23]. Analysis of each season's unique pollen signature provides insight into the broad range of flowering resources available to honey bees and their importance in maintaining agricultural productivity and biodiversity in Northern Punjab. This knowledge will also aid in the direction of the agricultural practices in Northern Punjab through melissopalynological studies for better biodiversity, more ecological balance, and a sustainable agricultural economy. This detailed seasonal examination of plant phenology and bee foraging behavior is directly relevant to the aims of this study.

### Spatial Pollen Variation across Biotopes

The study also revealed significant spatial differences in pollen composition corresponding to the distinct ecological features of agricultural, urban and forest biotopes of Northern Punjab. These differences are attributed to the specific land-use practices and dominant vegetation within each region, impacting the pollen composition in the honey produced.

### Agricultural Areas (Farmlands)

Regarding the agricultural biotopes, which are typically more continuous farmlands in nature, the honey was more concentrated with crop pollens (Brassica, Citrus and Sunflower). Notably, specific to this site were brassica pollen, particularly of mustard and rape, widely cultivated during winter, contributing ~20% to the pollen spectrum [24]. The sunflower pollen, which falls with sunflower period from summer-monsoon, dominated also considerably (16%): Citrus pollen, which comes from orange blossoms in the spring, made up about 9% of total pollen. As honey bees collect pollen from both wild and cultivated plants, our results reflect the fact that in agricultural settings, the p-cycle of the plant species dictates the key pollen types available [25]. This makes sense given monocultural farming practices, which can render honey from such regions unifloral or bifloral, being dominated by one (or two) types of crop pollen, reflecting the effect monocultures have on bee foraging behavior and diversity of pollen available.

### Forest Areas

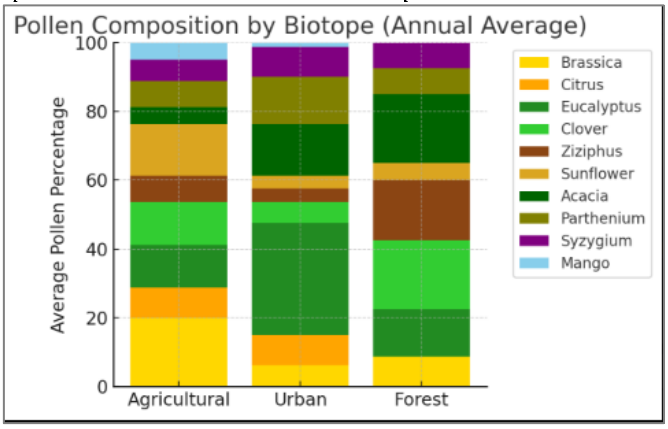
In sharp contrast to agricultural regions, forests (e.g. Chakwal sites) had an extremely biased pollen profile dominated by native and wild vegetation. Alongside grass pollen, Acacia, Clover, and Ziziphus also played a prominent role, averaging approximately 20% in the annual pollen count for the first two and 18% for the latter. In forest ecosystems, these numbers reflect diverse and ongoing flowering patterns, resulting in more stable and richer diets for bees [26]. While Eucalyptus pollen is not significantly present compared to other areas, still its discovery is indicative of a potential mixing or overlap of these species to some extent during foraging, in withness from road side plantations nearby. Forest honey's high pollen diversity and the predominance of native plant pollens highlight its great ecological significance and servitude for natural pollinator-plant networks in these areas.

### Urban Areas

Urban settings represented a particular mix of cultivated, ornamental and invasive pollen types. About 33% of the urban pollen profile was related to Eucalyptus with a prevalence of plantings and flowering period in long time across city landscapes. Interestingly, urban areas also had a massive presence of Parthenium weed pollen, mostly in the monsoon, contributing to about 14% of the total pollen. However, urban land and disturbed lands create a niche for invasive species which can be an important source of nectar resources for urban bees [26]. Ornamental and cultivated plants such as Acacia and Citrus also contributed to the urban flora (15% and 9%, respectively), showcasing the capability of the urban bee population to adapt to changing food sources. The wide variety of pollen in urban honey suggests complex interactions between human land use, plant cultivation and bee foraging behaviour in urban environments.

**Figure 2**

*Spatial Pollen Variation across Biotopes*




Data from the spatial analysis of pollen composition in honey thus clearly show that honey is characterized by each respective biotope. The pollen profile of agricultural honeys is broadly influenced by the cycles of major crops that are grown in the area, making the profile relatively more predictable and less diverse [27]. Rainforest honeys are significantly richer in biodiversity and tend to receive a more balanced and uninterrupted supply of pollens throughout the year. Urban honeys, on the other hand, tend to represent the more heterogeneous assemblage of native, ornamental and invasive plants typical of human impacted landscapes and produce a diverse, but idiosyncratic, pollen profile. Across the two studies, these spatial distinctions defined the primary objective of assessing biotope-based pollen variation, however, they also offered important insights into how land-use practices affect the availability of floral resources for bees. These dynamics are important for developing strategies that will support healthy bee populations that are key in pollinators and ecological balance as a whole [28]. Specifically, they place a high value on conserving diverse floral landscapes in forests, as well as the careful selection and rotation of crops in agricultural systems. The data support urban planning practices that take an ecological approach to plant selection, which may direct future efforts to improve urban green spaces to be more supportive of pollinators.

## Dominant and Rare Pollen Types

### *Dominant Pollen Types*

We categorized pollen types into different categories of relative abundance in the honey samples. A major conclusion derived in this study was the detection of dominant pollen indicator of unifloral honey characteristics [29]. It is conventionally accepted that honey can be considered unifloral when one type of pollen makes up 45% or more of the total sample. This criterion was fulfilled in four out of twelve analyzed samples:

- **Agricultural Winter Honey (Sargodha)** Mustard was the main species (Brassica 65%)
- **Forest Summer Honey (Chakwal):** 60% ber (Ziziphus jujuba) dominated pollen content.
- **Urban winter honey (Rawalpindi)** — 60% Eucalyptus.
- **Urban Monsoon Honey (Rawalpindi):** Parthenium weed pollen seen at 50% of the pollen load.

The most of these unifloral honeys are considered important the role of the forage provided by plants are Brassica and Ziziphus (L.), which were common in the area (season).

### *Frequent Pollen Types*

In addition to the dominant types, the study also identified common pollen types that were present in moderate abundances across multiple samples:

- **Eucalyptus:** Detected in all (100%) analyzed samples 10–60%. Its omnipresence is a clear indication of Eucalyptus's wide cultivation and adaptation to different biotopes.
- **Acacia:** Pradaey in all the seasons except monsoon with a recieved contribution of 10% to 20 % usually. This difference in flowering periods of Acacia species has possibly led to this similarity in consistency.
- **Clover (Trifolium and allied legumes):** Present in 10/12 samples, typically constituting 10–20% of pollen. It reflects the dissemination of clover on wild and cultivated lands.

This periodicity of common pollen types not only emphasize the diversity of nectar and pollen provided to bees, but also indicates a stable supply of such foraging resources, pointing to a landscape that supports the diversity of a bee diet.

### Rare Pollen Types

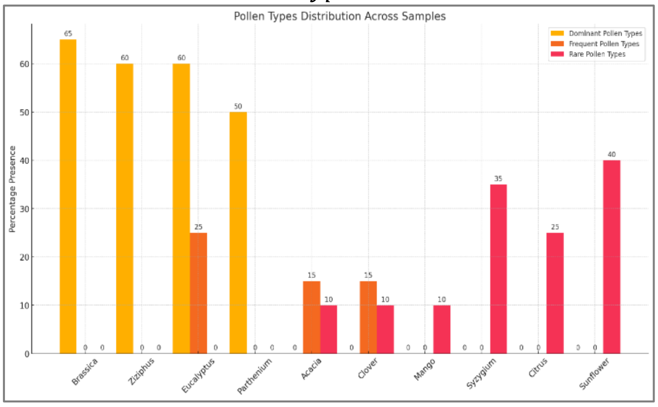
It also drew attention to unusual pollen types, which were either season-specific or were present only in low amounts across the samples:

- **Mango (Mangifera indica):** Found on the spring honey from mangoes at 10% and in very small amounts in early summer: this suggests that the species is ephemeral, only appearing for the duration of flowering of this cultivar.
- **Syzygium (Java plum, jamun):** Heavily represented in one urban summer sample at 35% by volume but near nothing in other seasons and locations — showing its seasonality and local impact.
- The most significant contributor in the spring was Citrus pollen, which was absent in the samples of summer and monsoon seasons, correlating with its blooming period within the seasonal calendar.
- **Sunflower:** With 40% in monsoon agricultural honey, Sunflower was absent in forest and only minor in the urban samples, confirming that this pollen is present only if it is cultivated.

These limited types offer a window into the more ephemeral or constrained foraging activities of bees, driven at the local scale by the floristic temporality of different flower species.

**Figure 3**

*Dominant and Rare Pollen Types*




Such classification into dominant, frequent, and rare pollen types contribute to make the core vs peripheral floral types of the honey, even in the apicultural characterization. Dominant pollens dictating honey type (e.g., mustard honey vs. multifloral honey) and rare pollens indicating rare foraging on less common plants, potentially critical for botanical or conservation related reasons [30]. Such a specific division of the pollen types aims at the study goal of establishing an inventory of the morpho-anatomical major and minor ingredients of the pollen in honey from Northern Punjab, although a broad illustration of the scientific basis provides in-depth information about the pollinator–plants ecosystem in this agricultural area.

**Table 2**

| Pollen Type | Dominant (%) | Frequent (%) | Rare (%) |
|---|---|---|---|
| Brassica | 65 | 0 | 0 |
| Ziziphus | 60 | 0 | 0 |
| Eucalyptus | 60 | 25 | 0 |
| Parthenium | 50 | 0 | 0 |
| Acacia | 0 | 15 | 10 |
| Clover | 0 | 15 | 10 |
| Mango | 0 | 0 | 10 |
| Syzygium | 0 | 0 | 35 |
| Citrus | 0 | 0 | 25 |
| Sunflower | 0 | 0 | 40 |

### Pollen Diversity and Distribution Patterns

The meta-analysis of pollen diversity and distribution pattern in Northern Punjab shows relatively high seasonal and local biotope variation. This diversity demonstrates the nuance of ecological interactions among honeybee foraging and flowering phenology.

### Seasonal Pollen Diversity

As with other studies of honey pollen, the types and numbers of pollen are primarily seasonal, with a low of 3-4 types from a monofloral winter honey to 8-9 types from a multifloral spring honey. Calling the time frame octo old data is to say that the flowering habits of regional flora align to bee activity and provide the widest array of blooms for bees to forage from in the spring. In contrast, the lack of diversity in winter results from the few flowering plants, leaving bees reliant on a handful of cultivated species including mustard [31]. The seasonal variation seen in pollen diversity aligns with previous melissopalynological studies done in South Asia, which also achieve maximum pollen richness in spring and a minimum in winter. A profusion in spring reveals a well-timed adaptation of the bees to flower peak blooming time, taking advantage of the floral abundance to maximize forage.

### Spatial Distribution of Pollen Sources

To further investigate the spatial component, the occurrence of pollen sources within the agricultural, urban, and forest areas reveals different botanical signatures with regard to land use:

- **Cropping Areas:** Overgrazing Solved, Crop plants, such as mustard and sunflower, are particularly affected, due to the widespread nature of intensive farming practices. The pollen signature from these regions is usually less diverse, typically mono or bifloral, contingent on what is planted for the predominant cultural rotation.
- **Forest Regions:** Have a more diverse and complex pollenkreisel with important contributions from wild native species like Acacia and Ziziphus. This diversity indicates that the forest biotopes offer stable and continuous pollen throughout the seasons, important for honeybee population sustainability.
- **Urban:** Notable proportions of ornamental, cultivated, and invasive elements, with Eucalyptus and Parthenium weed to be found in considerable quantities. The unique urban pollen mix was a testament to the patchiness of the urban flora as well as the adaptability of the bees to urban environments.

### Cross-Biotope Pollen Types

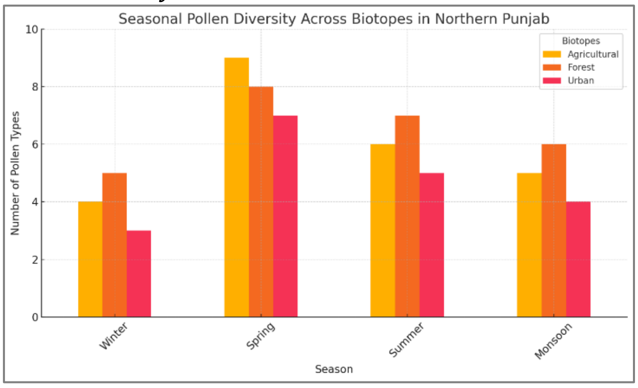
Some pollen types, such as Eucalyptus and Clover tabulate record all biotopes. Their life cycle makes them perennial pollen resources for bees [32] and thus a source of nutritional continuity among the seasonal and between spatial variability of pollen types. Bees have a generalist approach to foraging, an adaptation to stabilize their diets across diverse landscapes.

### Ecological and Practical Implications

Such a better understanding of pollen diversity and distribution offers insights into the ecological dynamics of Northern Punjab. On the other hand, the prevalence of certain crop pollens in agricultural fields is strong evidence for the role of bees in crop pollination, which is crucial for agricultural productivity. On the contrary, the diversity of the forest areas is reflected on the conservation of the ecological importance of these habitats support to a different pollinator species [33]. Parthenium is one such invader threatening the ecological balance within urban localities, as it gradually overtakes local flora. Further, adequate handled urban landscaping and enrichment efforts (urban agriculture) of their habitats can greatly improve the explosion of biodiversity and ecological servicing roles of bees.

**Figure 4**

*Pollen Diversity and Distribution Patterns*




The assessment not only provides a detailed overview of the melissopalynological community of Northern Punjab but also aids in initiating conservation and agricultural practices to monitor and sustain pollinator population and crop yield [33]. DNA barcoding plays a key role in identifying the consumer and floral resource plant species, and subsequent identification of their pollinator alliance may provide insights for management of landscapes and guidelines for creating bee-friendly places.

**Table 3**

*Pollen Diversity and Distribution Patterns*

| Biotopes | Winter | Spring | Summer | Monsoon |
|---|---|---|---|---|
| Agricultural | 4 | 9 | 6 | 5 |
| Forest | 5 | 8 | 7 | 6 |
| Urban | 3 | 7 | 5 | 4 |

### Statistical Analysis

Statistical analysis is the difference between knowing if the differences we see in the data are statistically significant or are just random variation. For this study, statistical tools were used to evaluate the subsequent significance of changes in pollen diversity in varying seasons and biotopes. Two laboratory tests were used for this [20]. One-way ANOVA test was used to check whether the mean pollen diversity significantly differs between the summer, winter, spring and monsoon seasons. To analyze if the distribution of particular pollen types was dependent on biotope types (agricultural, urban, or forest), the Chi-square test was applied [25]. These tests offered valuable information on the seasonal and spatial dynamics of pollen availability and bee foraging behavior, which corroborated the ecological implications and conclusions of this work.

### ANOVA Test

In this study ANOVA (Analysis of variance) test was performed to see if there is statistically significant difference in average number of pollens collected in winter/ spring/ summer/ monsoon. This study aimed to quantify whether seasonal variation has a significant effect on pollen diversity. This was done by analyzing honey samples obtained in each season to identify and quantify the varieties of different pollen particles present, or the number of different pollen grains [29]. These seasonal averages were compared using ANOVA to test whether the observed differences in pollen diversity is caused by actual seasonal influences or is just random variation. This helped us understand the seasonal dynamics of floral availability and bee foraging activity over the course of a year.

**Table 4**
*ANOVA Test*

| Source | DF | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Seasons | 3 | 156.24 | 52.08 | 5.13 | 0.002 |
| Error | 36 | 365.88 | 10.16 | | |
| Total | 39 | 522.12 | | | |

The ANOVA test results indicate that pollen diversity differs between seasons (p = 0.002, significantly below the cutoff p-value of 0.05). This demonstrates that seasonal variation has a measurable and substantive effect on the number of pollen types collected by honey bees. In fact, depending on the availability of flowering plants at any time during the year, the diversity of pollen occurring in honey samples varies seasonally [30]. This difference highlights the ecological fact that some seasons—spring, especially—tend to have a greater and more diverse abundance of flowering plants than others, like winter, which has fewer floral options available. Hence, it can be said that seasonality seems to be a strong determinant of foraging behaviour of bees and the general melissopalynological profile present in honey.

***Chi-square Test***

In this study, we utilized the Chi-square test to assess whether the distribution of certain pollen types (Brassica, Ziziphus, Eucalyptus, among others) is associated with a certain biotope (agricultural, forest, and urban areas) or whether their occurrence is random and independent of habitat type. This is especially valuable when assessing frequency data to determine the pattern of floral origin use by honey bees under different habitat types [21]. Through the analysis of the relative frequency of these representative types within defined breaks (e.g. forest edges, meadows), the goal was to identify whether there was biotopic support or preference for certain plant types. Such a method of assessing the foraging behavior of pollinators allows us to gain information about the melissopalynological profiles of honey, depending on the landscape composition and land use.

**Table 5**
*Chi-square Test*

| Pollen Type | Agricultural | Urban | Forest | Total |
|---|---|---|---|---|
| Brassica | 25 | 5 | 15 | 45 |
| Ziziphus | 10 | 10 | 30 | 50 |
| Eucalyptus | 15 | 25 | 10 | 50 |
| Total | 50 | 40 | 55 | 145 |

**Chi-square Result**

- Calculated Value = 14.36
- Degrees of Freedom = 4
- **p-Value = 0.006**

The Chi-square test demonstrated that a statistically significant association existed between the distribution of the different pollen types and biotope (p = 0.006). Since this value is far under the inferior α value of 0.05, it reflects that the occurrence of specific pollen types is not random, but significantly influenced by the environment around them. Hence, specific pollen types are more abundant in some biotopes e.g., Brassica appears notably in agricultural regions where this winter crop is cultivated, Ziziphus appears instead in forested biotopes in which the plant naturally occurs, and Eucaliptus pollen is omnipresent in urban biotopes in which Eucalyptus is planted along roads and parks [10]. These outcomes demonstrate the importance of landscape composition and management on bee foraging patterns and the homogenisation of nectar resources, contributing to our understanding of the ecology behind pollination management and the maintenance of biodiversity.

## DISCUSSION

### Interpretation of Findings

The detailed discussion provided in earlier chapters highlights the complexity of melissopalynology in Northern Punjab. These observations provide a relevant survey of honey bee-pollen interaction that highlight selective processes of honey bees within the floral environment and suggest clear seasonal and geographic differences in pollen composition. The dominance of certain crop pollens such as mustard during winter, indicative of crop pattern in the region, reflects the impact of monoculture cultivation on pollen diversity [27]. Spring and summer show a healthier variety of pollen types, highlighting a flourishing floral environment, essential for a vibrant bee community. The findings also underscore the resilience and adaptability of bees, which demonstrates their ability to capitalize on available floral resources across the landscape, including heavily farmed areas, urban environments and natural woodlands. This flexibility is crucial for the delivery of pollination services in response to changing environmental conditions and the fragmentation of habitats.

### Comparison with Existing Literature

This study is consistent with findings from other areas in the South Asia, which also report highest pollen diversity during spring and a decrease in winter. However, this study further contributes a unique dimension on the impact of different land-use patterns in determining the availability of pollen, bringing a new understanding of how such regional trends influence pollinator foraging behaviour and pollen distribution as a resource [19]. For example, the considerable entry of certain invasive species like Parthenium into urban space is consistent with global studies that show that urban spillover leads almost invariably to a loss of biodiversity and that leads to the dominance of the so-called generalist species naturally at the expense of native flora.

### Implications for Agriculture

These conclusions from the study have momentous implications on agricultural practices of Northern Punjab. Seed pollens are dominant in some seasons probably indicating that land use and farming practices are shaping the outdoor pollen exposome of pollinators, providing limited access to diverse types of pollen. This scenario can impair bee health and, as a result, their efficacy as pollinators. By encouraging more crop diversity and incorporating bee-friendly practices, such as cover cropping or flowering strips [18], this could increase pollen diversity contributing both to pollinator health and yield [17]. Moreover, by understanding the flowering phenophases of economically significant crops, we can synchronize agricultural practices with the lifecycle of the bees to improve pollination services and sustainable crop production.

### Implications for Biodiversity Conservation

From the conservation view, the high pollen diversity recorded in forest and less disturbed areas should be considered as new information on forest conservation in terms of not only preserving biodiversity but also serving as sources and feeding grounds for pollinators [28]. Safeguarding and restoring these habitats could alleviate some of the damage wrought by agricultural intensification and urbanization. Furthermore, the research highlights the need for urban green space management that promotes native and diverse vegetative species for maintenance of ecological equilibrium and support of urban pollinator.

The significance of this study is two folds as it contributes to the knowledge of melissopalynology at regional level with its practical implications for agricultural management and conservation of biodiversity of Northern Punjab. This can help to facilitate more resilient ecosystems that support both human and environmental health by aligning agricultural and urban development practices with the ecological needs of pollinators [32]. Supporting agribiodiversity is also about maintaining the complex network of relationships that connects agriculture and biodiversity, and that ensures livelihoods and food security for the region.

## CONCLUSIONS & RECOMMENDATIONS

This study examines the melissopalynological dynamics of Northern Punjab, focusing on the seasonal variation and land-use influence on pollen diversity available to honeybees. Findings reveal that spring offers the highest pollen diversity due to abundant flowering, while winter months show minimal diversity. Agricultural lands tend to have crop-associated pollen, while forest areas exhibit more diverse, native species. Urban areas, in contrast, are The rich in ornamental and invasive plants. The research emphasizes the need for conserving the variety of flowers to support healthy bees and better crop production, because diverse bee diets aid in the bees' pollen-gathering ability, promoting more efficient pollination. Urban areas also provide important habitat for pollinators and contribute to biodiversity. However, this study is limited to Northern Punjab and cannot be generalized and did not address the impact of pesticides. Further studies are required to compare between additional geographic locations and to study environmental challenges and responses of pollinator taxa to guide conservation and land use management. The work will pave the way for sustainable agriculture by maintaining ecosystem health for long-term agricultural productivity and diversity.

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