

EXPLORING POLLEN RESOURCES OF HIMALAYAN BUMBLEBEES: A SCANNING ELECTRON MICROSCOPY ANALYSIS OF ANGIOSPERM INTERACTIONS

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Abstract

This study investigates the role of the bumblebees in sustaining plant-pollinator networks, genetic diversity and ecosystem resilience in the Indian Himalayan Region (IHR). Sixteen species were documented across eight angiosperm families with Asteraceae as the dominant floral resource. Foraging patterns revealed both specialized and generalist strategies while pollen morphology and species specific distributions highlighted the adaptability of some taxa and the vulnerability of high altitude specialists. Using Scanning Electron Microscopy (SEM), pollen grains attached to different body parts especially the hind legs were identified and linked to plant families revealing intricate pollination networks. These findings demonstrate bumblebees' capacity to pollinate multiple plant species, facilitating cross-pollination and enhancing ecosystem stability. Conservation efforts should focus on protecting diverse floral resources particularly Asteraceae and Balsaminaceae, while long-term monitoring and advanced analytical approaches are essential to assess climate change impacts and support effective pollinator conservation in the mountain ecosystems.

Introduction

The conservation of plant genetic diversity is crucial for ecological stability, agricultural resilience and adaptation to environmental challenges (Smith *et al.* 2012, Ollerton 2021, Rather *et al.* 2023). In mountain ecosystems, pollinators such as bumblebees (*Bombus* spp.), leafcutter bees (*Megachile* spp.), and mining bees (*Andrena* spp.) drive genetic exchange through cross-pollination (Thorpe and Kevan 2000). Among them, bumblebees excel due to their ability to forage in low temperatures and visit a diverse floral spectrum, positioning them as key pollinators in pollination networks of the Indian Himalayan Region (IHR) (Smith *et al.* 2015, Rather *et al.* 2023). This study employed Scanning Electron Microscopy (SEM) to decode plant-pollinator interactions by analysing pollen grains retrieved from different body parts, primarily focusing on the hind legs of bumblebees. Fine forceps and acetolysis techniques facilitated the precise collection, while high-resolution imaging revealed intricate pollen textures, apertures and exine structures. This approach identified key plant families linked to bumblebee-mediated pollination, reinforcing their ecological significance. Pollinator fluctuations can directly influence plant reproduction and genetic variation, underscoring the urgency of conservation strategies. The findings provide crucial baseline data, advocating for habitat protection and sustainable pollination management in the IHR. Future research integrating molecular techniques with SEM will refine pollen identification, offering deeper insights into pollination dynamics in fragile mountain ecosystems (Smith *et al.* 2015, Burkle *et al.* 2021, Dalsgaard *et al.* 2021, Rather *et al.* 2023).

Materials and Methods

The study was carried out in the Indian Himalayan Region (IHR) across alpine meadows and low - density forests in Union Territory of Jammu and Kashmir (Gulmarg, Razdan Pass, Gurez

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Valley), Ladakh (Zaskar, Drass), Himachal Pradesh (Sangla, Pin Valley), Uttarakhand (Joshimath, Ghanghariya) and Arunachal Pradesh (Zemithang and Sela Pass). Bumblebee samples were collected using hand nets and malaise traps from 2020 to 2022 during blooming period (May-August). Only 16 out of 44 recorded species were selected for the SEM analysis based on their dominance, abundance and availability of worker individuals. Garmin 64SCGPS was used to record the Geo-coordinates of the study sites. Bees were euthanized with ethyl acetate, preserved, and transported for identification. Pollen samples were collected from the hind legs and stored for analysis. Specimens were pinned, dried, and examined for morphological traits such as thoracic colour patterns, wing venation and genitalia structure using taxonomic keys (Williams 2022). SEM was done at IIT Jodhpur involving pollen mounting, gold/platinum coating, and high-resolution imaging to examine pollen morphology in detail. Pollen identification was carried by the Botanical Survey of India (BSI) and were classified based on surface textures and apertures, enabling precise identification of plant families as per available literature (Weber and Ulrich 2017, Burkle *et al.* 2021, Dalsgaard *et al.* 2021, Rather *et al.* 2023). By correlating pollen grains with bee species, this study mapped pollination networks using R software of statistical analysis tool and highlighted species-specific floral preferences, offering valuable insights into plant-bee interactions.

Results and Discussion

This study highlights the crucial role of wild bees in maintaining plant genetic diversity and ecosystem stability in the IHR. A total of 16 bumblebee species were documented from diverse Himalayan localities, spanning Jammu and Kashmir, Ladakh, Himachal Pradesh, Uttarakhand, and Arunachal Pradesh (Table 1). These species were associated with eight-angiospermic families such as Asteraceae, Fabaceae, Lamiaceae, Balsaminaceae, Malvaceae, Amaranthaceae, Berberidaceae, and Solanaceae (Fig. 1). Asteraceae emerged as the most frequently visited family, linked to 12 species (75 %), including *Bombus alboplueralis*, *B. melanurus*, *B. rainai* and *B. haemorrhoidalis*. Other frequently utilized families included Balsaminaceae (4 species), Malvaceae (2 species), Solanaceae (2 species) and single associations with Fabaceae, Lamiaceae, Amaranthaceae and Berberidaceae. Pollen morphological traits exhibited distinct patterns among the plant families (Fig. 2). Unlike previous findings, species-specific foraging, SEM analysis revealed mixed foraging behaviour, where multiple species pollinate diverse plant families within a single trip. Species such as *B. tunicatus*, *B. longiceps*, and *B. difficillimus* were associated with multiple families, reflecting broader foraging adaptability. In contrast *B. semenovianus* and *B. sikkimi* exhibited strong floral specialization with Fabaceae and Balsaminaceae, respectively. Notably, *B. jacobsoni* exhibited adaptability to plant families such as Amaranthaceae and Berberidaceae, while *B. simillimus* foraged on Asteraceae and Lamiaceae families, respectively. Other species, including *B. keriensis*, *B. festivus*, *B. albopleuralis*, *B. semenovianus*, *B. waltoni*, and *B. eximus* contributed to genetic mixing across plant populations. Geographical records revealed species-specific distributions, with high altitude specialists like *B. semenovianus* occurring in Zaskar (Ladakh), while *B. eximus* and *B. sikkimi* were restricted to Arunachal Pradesh. Asteraceae pollen grains were predominantly echinate or spiny, enhancing adherence to bumblebee body hairs, while Balsaminaceae pollen grains displayed a reticulate and tricolpate structure. Lamiaceae pollen was hexacolpate, Malvaceae pollen grains were large with pronounced spines, and Fabaceae pollen was tricolpate with smooth to finely reticulate exine. The predominance of Asteraceae in bumblebee floral associations aligns with the previous Himalayan studies reporting this family as a key nectar and pollen source due to its extended blooming period, diverse morphology, and composite inflorescence (Goulson 2010, Saini *et al.* 2015, Burkle *et al.* 2021, Dalsgaard *et al.* 2021, Ollerton 2021, Williams *et al.* 2022, Rather *et al.* 2023). No

Table 1. Bumblebee pollinators and their preferred plant families in the Indian Himalayan Region.

Sl. No.	Bumblebee species	Plant families	Pollen grain characteristics	Geo coordinates with locality
1.	<i>B. semenovianus</i> Skorikov, 1914	Fabaceae	Tricolpate with smooth to finely reticulate exine patterns, indicating a significant role in pollinating legumes.	N 33° 33'43.12" E 076° 58'57.02" (Zaskar, Kargil, Ladakh)
2.	<i>B. similimus</i> Smith, 1852	Asteraceae, Lamiaceae	Spiny exine surface. Hexacolpate apertures, showing adaptability to different floral structures.	N34°32'13.27" E74°38'13.69" (Razdanpass, Bandipora, J&K)
3.	<i>B. tunicatus</i> Smith, 1853	Asteraceae, Balsaminaceae	Spiny exine; Reticulate and tricolpate, showing preference for both common and specialized floral structures.	N 31°25'24.92" E 78°15'59.11" (Sangla, Kinnaur, HP)
4.	<i>B. ferganicus</i> (Radoszkowski, 1893)	Balsaminaceae	Reticulate patterns, highlighting specialized pollination behavior.	N 33°40'08.13" E 76°37'20.04" (Hemis, Hamling, Kargil, Ladakh)
5.	<i>B. keriensis</i> Morawitz, 1886	Asteraceae	Echinate pollen morphology, indicating a strong preference for this diverse plant family.	N34°01'34.14" E74°22'03.89" (Gulmarg, Baramulla, J&K)
6.	<i>B. jacobsoni</i> Skorikov, 1912	Amaranthaceae, Berberidaceae	Spherical and smooth. Reticulate patterns, indicating versatility in pollination.	N 34°37'38.08" E 74°48'58.44" Gurez Valley, Dawar, Bandipora, J&K
7.	<i>B. festivus</i> Smith, 1861	Asteraceae, Malvaceae	Spiny exine; Large, spiny exine, showing the ability to handle diverse pollen types.	N 30°41'52.05" 79°35'32.16"E Ghangharya, Chamoli, Uttarakhand
8.	<i>B. albopleuralis</i> Friese, 1916	Asteraceae	Strong preference for Asteraceae family pollen.	N 34°03'28.53" E 74°23'16.74" Gulmarg WLS, Baramulla, J&K
9.	<i>B. eximius</i> Smith, 1852	Malvaceae	Known for pollinating plants with large, complex pollen grains.	N 28°14'34.58" E 95°55'22.66" Mayudiya pass Mehao WLS, Roing, Lower Dibang Valley, Arunachal Pradesh
10.	<i>B. melamurus</i> Lepelletier, 1835	Asteraceae	Emphasizes the importance of this species in pollinating the Asteraceae family.	N34°25'31.33" E75°44'47.19" Drass, Kargil, Ladakh
11.	<i>B. rainai</i> Williams, 2022	Asteraceae	Highlights its role in maintaining the genetic diversity of Asteraceae.	N 34°32'04.20" E74°38'14.51" Razdan pass, Gurez valley, Bandipora, J&K
12.	<i>B. longiceps</i> Smith, 1878	Asteraceae, Balsaminaceae	Adaptability to different floral resources, contributing to the pollination of diverse plant species.	N 31°56'37.48" E 78°05'20.76" Pin Valley National Park, Parvati Pall, Mudh, Spiti, HP
13.	<i>B. difficilimus</i> Skorikov, 1912	Asteraceae, Solanaceae	Spiny exine; Large and spiny pollen grains, indicating the capability to handle robust pollen.	N 30°45'40.04" E 79°51'05.81" Joshimath, Nanda devi biosphere reserve, Niti, Bugyal, Chamoli, Uttarakhand
14.	<i>B. minutus</i> Bingham, 1897	Asteraceae, Solanaceae	Similar pollination niche as <i>Bombus difficilimus</i> with a preference for structurally diverse pollen grains.	N30°20'36.27" 78°23'54.58"E Chamba, Uttarakhand
15.	<i>B. haemorrhoidalis</i> Smith, 1852	Asteraceae	Reinforces the species' importance in pollinating composite flowers.	N 27°30'17.47" E 92°06'17.11" Zemithang, Khobleteng, Tawang, Arunachal Pradesh
16.	<i>B. sikkimi</i> Friese, 1918	Balsaminaceae	Specialized role in pollinating with distinctive floral morphology.	27°30'8.86"N 92°06'13.44"E Sela pass, Near Army Camp, Tawang, Arunachal Pradesh

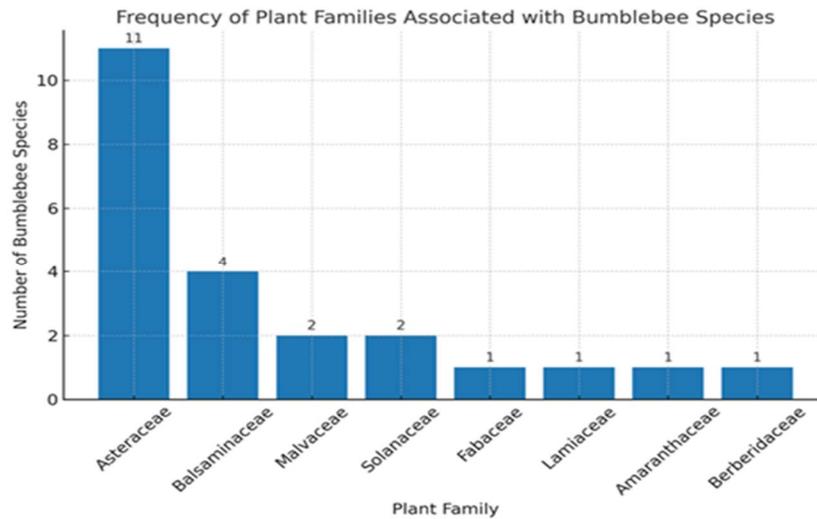
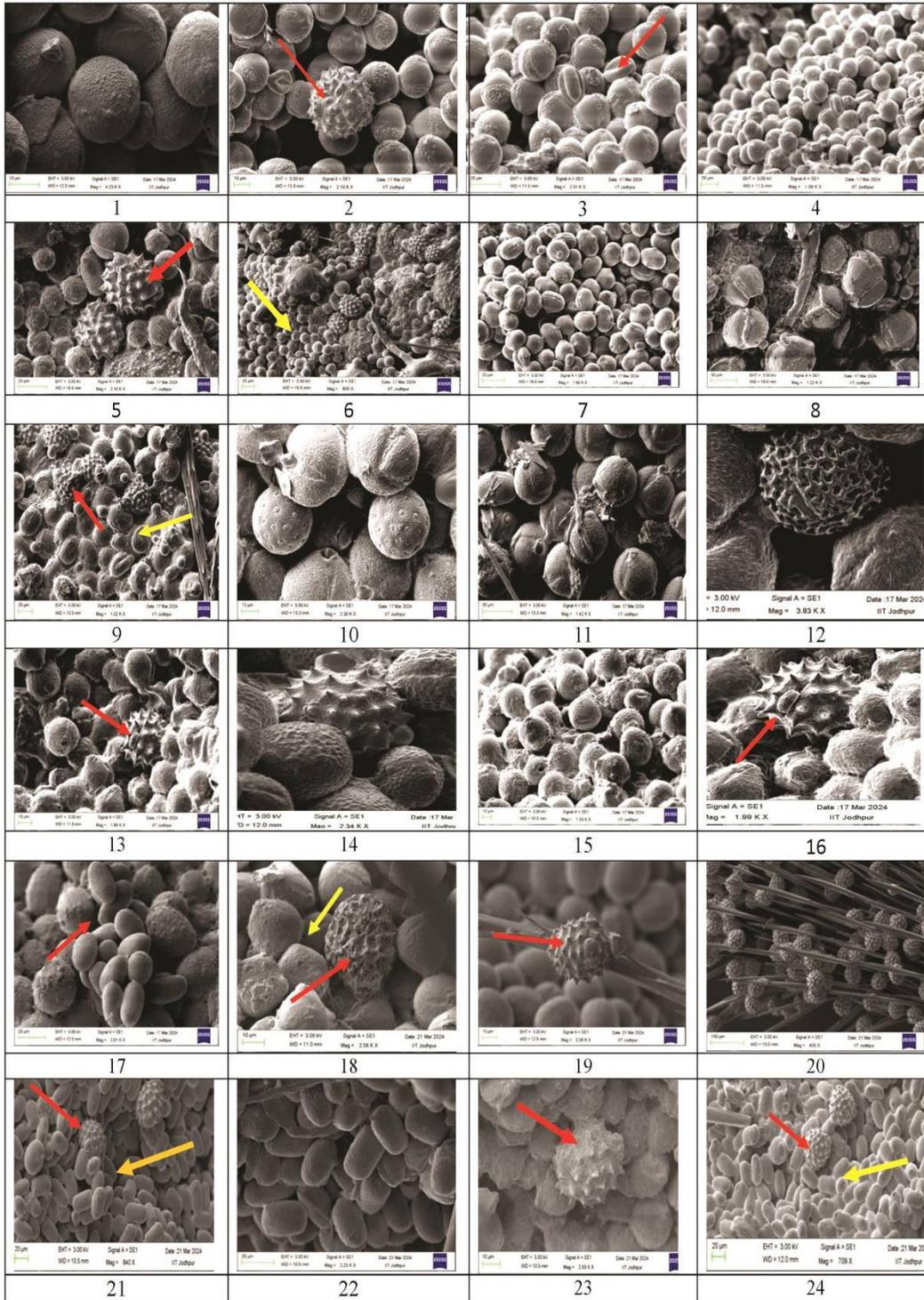


Fig. 1. Frequency of species associated with different plant families.

work has been carried out on linkage of Himalayan Bumblebees and their associated pollen resources through SEM Analysis. This study is the first attempt to identify the linkage between bumblebees and their associated flora, the bipartite interaction network demonstrate the relationship between the plant families and 16 *Bombus* species recorded during the study. The network highlights the diverse floral resources utilised by the bumblebee across various plant families (Fig. 3). These findings challenge traditional views on bumblebee foraging, highlighting their adaptability and ecological importance in mountain ecosystems. Mixed foraging enhances pollination efficiency, promotes genetic diversity, and strengthens ecosystem stability. It supports rare plant species and improves bee nutrition through dietary diversity. Himalayan wild bees facilitate genetic mixing, enhancing plant adaptability to environmental changes. Their ability to utilize diverse floral resources ensures stable pollination services, especially in high-altitude ecosystems. The decline of key pollinators could disrupt these networks, threatening plant genetic diversity and ecosystem stability. Pollen morphological diversity recorded not only confirms the pollination versatility of Himalayan bumblebees but also underscores the importance of maintaining floral diversity to sustain pollinator populations. In light of observed global pollinator declines (Potts *et al.* 2010), conserving these resource rich families such as Asteraceae and Balsaminaceae is critical for ecosystem resilience. Moreover, pollinators are crucial for maintaining these networks and preventing genetic diversity loss across Himalaya region.

The study highlights the critical role of wild bumblebees in sustaining plant-pollinator networks, maintaining genetic diversity and enhancing eco-system resilience in the Indian Himalayan Region using SEM analysis. Sixteen species across eight angiospermic families, reveals both specialized and generalist foraging strategies with Asteraceae emerging as the dominant floral resource. Variation in pollen morphology and species-specific distribution patterns highlights the adaptability of certain taxa and the vulnerability of high- altitudes specialists. SEM analysis revealed their diverse pollination networks, emphasizing the need to protect these pollinators for ecosystem health. These findings emphasize the need to conserve diverse floral resources-particularly dominant and seasonal families like Asteraceae and Balsaminaceae to safeguard pollination services and maintain ecological stability. Future research should focus on



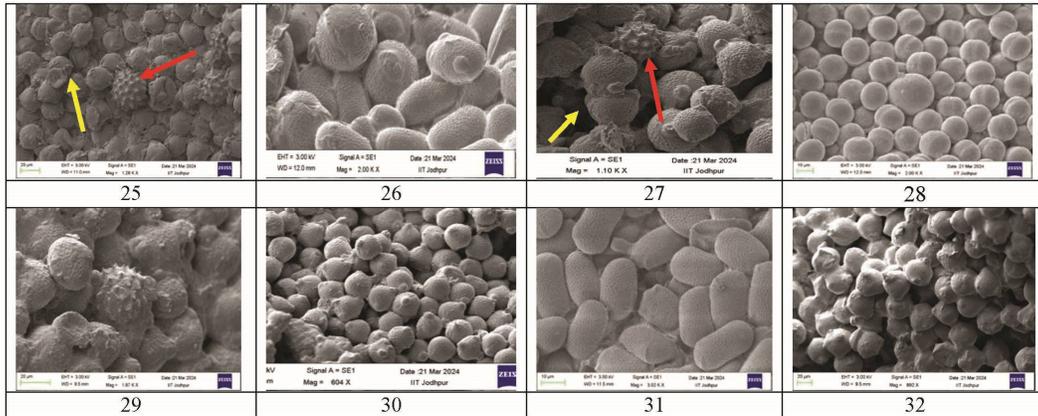


Fig. 2. SEM analysis of pollen grains- Linking plant families with different bumblebee species (Pollen identified on the hind leg). 1. Fabaceae: *B. semenovianus*; 2) Asteraceae: *B. simillimus*; 3) Rosaceae: *B. simillimus*; 4) Ranunculaceae: *B. simillimus*; 5) Asteraceae: *B. tunicatus*; 6) Solanaceae: *B. tunicatus*; 7). Apiaceae: *B. waltoni*; 8) Rosaceae: *B. waltoni*; 9) Asteraceae: *B. keriensis*; 10) Amaranthaceae: *B. jacobsoni*; 11) Solanaceae: *B. jacobsoni*; 12) Malvaceae: *B. jacobsoni*; 13) Asteraceae: *B. jacobsoni*; 14) Asteraceae: *B. albopleuralis*; 15) Solanaceae: *B. albopleuralis*; 16) Asteraceae: *B. festivus*; 17) Balsaminaceae: *B. ferganicus*; 18) Malvaceae: *B. eximius*; 19) Asteraceae: *B. simillimus*; 20) Asteraceae: *B. melanurus*; 21) Asteraceae: *B. tunicatus*; 22) Balsaminaceae: *B. tunicatus*; 23) Asteraceae: *B. rainai*; 24) Asteraceae: *B. longiceps*; 25) Asteraceae: *B. haemorrhoidalis*; 26) Balsaminaceae: *B. longiceps*; 27) Asteraceae: *B. difficillimus*; 28) Berberidaceae: *B. jacobsoni*; 29) Asteraceae: *B. miniatus*; 30) Solanaceae: *B. difficillimus*; 31) Balsaminaceae: *B. sikkimi* and 32) Solanaceae: *B. miniatus*.

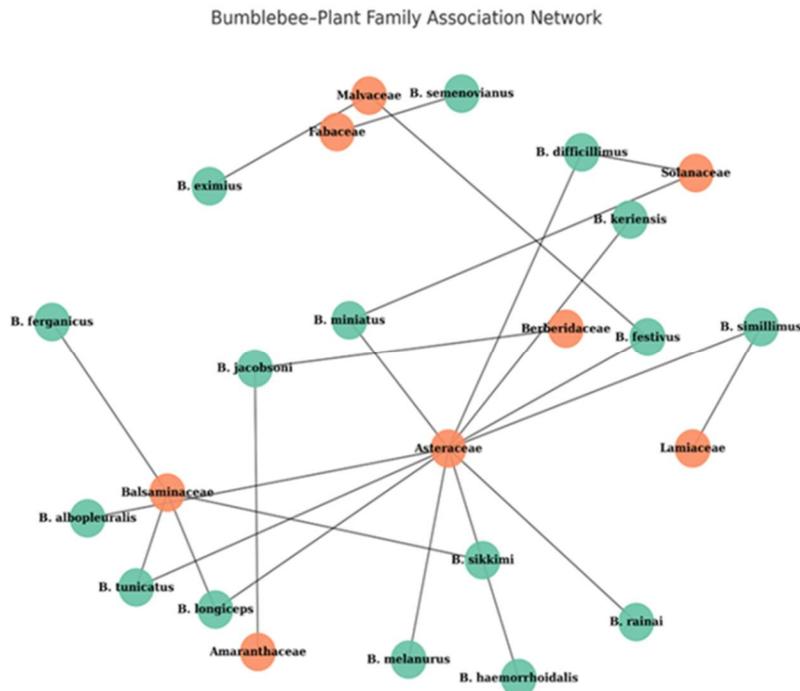


Fig. 3. Bumblebee–plant family bipartite network in the Indian Himalayan Region, showing species (green) linked to their associated plant families (orange).

long-term monitoring of plant-pollinator networking, climate change impacts, and advanced techniques to further understand pollination dynamics and support effective conservation strategies in the Indian Himalayan Region.

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