# Unveiling Biochemical Drivers of Bee Pollination in Short- Day Indian Onion (Allium cepa L.) Varieties

**Trupti R. Pandit**
ICAR-Directorate of Onion and Garlic
**S. A. Dwivedi**
Lovely Professional University
**Parakkattu S. Soumia**
ICAR-Directorate of Onion and Garlic
**Dhananjay V. Shirsat**
ICAR-Directorate of Onion and Garlic
**Chandrashekhar L. Pote**
ICAR-Directorate of Onion and Garlic
**Amol R. Pawar**
ICAR-Directorate of Onion and Garlic
**Mayur B. Patil**
ICAR-Directorate of Onion and Garlic
**Komal Gade**
ICAR-Directorate of Onion and Garlic
**Payal Mahadule**
ICAR-Directorate of Onion and Garlic
**A. Thangasamy**
ICAR-Directorate of Onion and Garlic
**Ram Dutta**
ICAR-Directorate of Onion and Garlic
**Vijay Mahajan**
ICAR-Directorate of Onion and Garlic
**Vadivelu Karuppaiah**
karuppaiahv2008@gmail.com

ICAR-Directorate of Onion and Garlic

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## Abstract

Pollination is crucial for onion (*Allium cepa*L.) seed production, and honey bees (*Apis mellifera*) are the major pollinators. The biochemical content of pollen and nectar has a substantial impact on pollinator foraging behavior and seed yield. However, there has been little investigation into biochemical traits of short-day Indian onion cultivars that influence bee visitation and pollination effectiveness. This study looks at the biochemical characteristics of pollen and nectar in short-day Indian onion varities to unveil their role in bee pollination. The study investigates nectar sugar composition, amino acid profiles, total phenols, and antioxidant properties, and their relation with bee pollination in onion. Field experiments were carried out to assess the floral visitors, while biochemical composition of nectar and pollen were also analyzed using LC-MS/MS, and spectrophotometry. The results revealed that, among the onion cultivars, 'Bhima Super' had best bee attracting biochemical traits, with high protein (391.8 mg/g), flavonoids (855.3 mg/g), and antioxidant activity (3.6 µg/g) in its pollen. Besides, the nectar of B. super was also rich in protein (83.6 mg/g), flavonoids (9.7 mg/g), and sugars (40.53 mg/g). In contrast, the onion cultivar 'Bhima Shweta' revealed the less amount of this biochemical content. Foraging trend of bees revealed that 'Bhima Super' had the highest foraging rate (14.151 visits/min) and foraging speed (6.703 flowers/min), correlating with its biochemical traits. Cultivar,'Bhima Shakti' also showed a positive correlation between nectar sugar (43.8%), antioxidants (r = 0.682), and bee activity, with its pollen contains rich in iron (0.520 µg/g), zinc (0.75 µg/g), and manganese (0.37 µg/g). These findings confirm strong correlations between pollen and nectar biochemistry and bee foraging. The study insights into pollen and nectar of short day India onion cultivars, providing new avenue for improving managed pollination strategies in onion seed production.

## Introduction

Onion (*Allium cepa* L.) is one of the widely cultivated vegetable crops. It is used in culinary applications, salads, soups, curries, and seasonings. They are high in antioxidants such as quercetin, vitamins (C, B6, folate), and prebiotic fibers, which help with digestion, immune function, and heart health (1). The bioactive compounds, such as quercetin and sulfur-containing substances, contribute to the therapeutic qualities. These include anti-inflammatory and immune-boosting effects, cardiovascular benefits like reducing blood pressure and cholesterol levels (2), antimicrobial activity against various pathogens, respiratory health support by acting as natural expectorants, and potential cancer-preventive properties, particularly for digestive tract cancers.

The quality seed production in onion is a major concern, considering its pollination mechanism (3). Moreover, successful pollination and subsequent seed development, which are in extricable linked to the biochemical characteristics of pollen and nectar, are primary responsible for attracting bees, seed yield and quality. Pollination in onions predominantly depends on cross-pollination, which mainly done by insect pollinators, particularly honey bees (4,5). Onion flowers are entomophilous; yet, their distinctive protandry mechanism, biochemical makeup, and floral architecture lessen pollinator attraction, frequently resulting in insufficient pollination and poor seed yield (6).

Cross-pollination is the principal environmental function provided by insect pollinators, essential for the sexual reproduction of flowering plants. Insect pollination is predicted to have an economic benefit ranging from $153 to $577 billion each year (7). Bees account about 92% of insect-mediated pollination, followed by flies, moths, and beetles (8). Approximately 87 of the 115 major food crops rely on animal pollination for quality and productivity (9).

The biochemical composition of pollen and nectar is crucial for attracting and sustaining pollinators. Pollen, which contains proteins and phenolic compounds, is an important dietary resource for bees, influencing their foraging behavior and colony development (10,11). Nectar, which contains sugars, amino acids, and trace elements, serve as an immediate energy source for pollinators and influence their visiting rates (12,13). The biochemical heterogeneity of onion cultivars can have a substantial impact on bee pollinators foraging preferences and efficiency (14,15). Recent studies emphasize the importance of understanding the relationship between floral biochemical traits and pollinator behavior in order to optimize onion seed production (16). The superficial nectaries of onion blossoms render nectar easily accessible to wide range of pollinators, facilitating a broad entomophilous pollination syndrome (17). Floral nectar is an important source of energy for pollinators and its volume, sugar concentration, and composition all have profoundly affecting pollinator foraging behavior (18). Bees prefer nectar sugar concentrations greater than 30% (w/w), with sucrose, glucose, and fructose being the major sugars found in flowers that are pollinated by bees (7).

Onion nectar contains a higher concentration of sugars, which are quite appealing to honey bees (18). The biochemical properties of nectar and pollen, such as pH, electrical conductivity (EC), moisture content, protein, antioxidants, and total sugars, have a significant influence bee preferences and behavior. Given the vital function of pollinators in onion cross-pollination and seed production, understanding the biochemical compositions of onion nectar and pollen is crucial for enhancing pollinator attractiveness and efficacy. The purpose of this study is to examine the relationship between nectar and pollen quality and honey bee visitation across the onion varieties and to ascertain their impact on pollinator choice (19).

Although there are few studies that have examined the role of honey bees (4,20), as well as impact of nectar composition on pollinator attraction in onion (20,21), not much work done with Indian short day onion varities. There are still **a significant gap in understanding how biologically active compounds in onion nectar (such as amino acids, phenolics, and secondary metabolites) affects pollinator choice and efficiency.** While the impact of sugar content has been well documented, the effect of **non-sugar compounds** in regulating pollinator behavior and seed set is still **unexplored.** Additionally, considering environmental changes, **the impact of short-term weather variability on nectar secretion, pollen viability, and pollinator foraging dynamics is still not fully understood.** Unveiling this gap is crucial because onion seed production could be vulnerable to **fluctuations in pollinator availability and floral resource dynamics,** particularly under stress (22,23).

The study aims to find varietal differences in nectar and pollen properties that promote pollination efficiency in onion and to ascertain relationships between biochemical characteristics and pollination and seed set in onion(24). The findings would provide new insights for developing cultivars-specific managed pollination strategies, breeding initiatives, and agricultural practices aimed at increasing onion seed yields and promoting sustainable crop production.

## MATERIALS AND METHODS

# Plant material and experimental site

Seven popular short-day onion Indian cultivars including 'Bhima Super', 'Bhima Shakti', 'Bhima Shweta', "Bhima Red", 'Bhima Dark Red', 'Bhima Shubhra', and 'Bhima Raj' were used for this study. These cultivars are available at the ICAR Directorate of Onion and Garlic Research (ICAR-DOGR), Pune, India (N 18840, E 73880, and 553.8 m above mean sea level). The soil at the study site is sandy loam, containing 32–35% clay, 40% sand, and 20% silt with a pH of 7.9. Experiment was laid under shade net cages; bulbs of each cultivar were planted with a randomized block design in 2023-24 in *Rabi* season. Recommended doses of potassium, phosphorus, and sulfur fertilizers were applied at the time of sowing, along half a dose of nitrogen at 30, 45, and 60 days after sowing (DAT) were used. Further recommended plant protection procedures were followed till flowering stage. The plots size was 10 m x 10 m, (100 sq. m), and Three replications were maintained, with eight rows and 200 bulbs planted in each plot.

# Foraging behavior

The observation on foraging behaviors, including foraging rate and speed of bee species (*Apis mellifera*) were recorded using standard procedure as described by (25). In brief, the foraging rate was quantified by counting the number of flowers visited by each forager visited per minute. While foraging speed were recorded by measuring the duration each species spent on a flower using a stopwatch. The time spent between arriving and leaving the flower was recorded time (sec) spent on foraging speed. The observations began after 25% bloom in the onion umbels, and data were collected made weekly during the crop's blooming period (26). All the observations were recorded at the following hour intervals: 8:00 hrs, 10:00 hrs, 12:00 hrs, 14:00 hrs, 16:00 hrs, and 18:00 hrs. A total of eight observations, at weekly intervals, were made throughout the bloom period. Bees choose flowers depending on their nectar and pollen content, preferring those with a balanced nutrient profile (27). Higher concentrations of certain sugars and amino acids can attract more *A. mellifera* and other pollinators. The chemical profile of Allium flowers may influence bee learning and memory, resulting in repeated visits to onion flowers (28).

## Collection of pollen grains, and nectar

The pollen grains and nectar from each cultivar were obtained during the peak blooms period. Pollen grains were collected using an *A. mellifera* pollen trap at the hive entrance. All collected pollen grains were gently extracted from the pollen trap at weekly intervals. Additionally, Pollen was also collected from bees visiting the onion flowers was gently removed using fine brush, and quantity was weighed using an electronic precision balance (29).

The nectar from onion flower of all cultivars were collected using two techniques: the capillary suck method, which used a micropipette for meticulous extraction to prevent contamination, and the centrifuge method, in which flowers were systematically collected and centrifuged to extract nectar as a described by Power et al.(30) and Swanson and Shuel (31).

## Estimation of biochemical parameters

The methanolic extract of the pollen collected from each treatments were used for the estimation of the biochemical parameters, however the nectar were used as it is. The all parameters were estimated in the three biological as well as technical replicates (32).

## pH and EC measurement

The pH of the pollen and nectar samples was determined by using the method by International Honey Commission. To each 10 g pollen or nectar samples, 90 ml of distilled water (D/W) was added in a 100 ml beaker. The mixture was stirred with a magnetic stirrer, and the Ph was measured using a Eutech pH 2700 Meter. Electrical conductivity (EC) of pollen and nectar samples was measured following procedure of International Honey Commission. A 10 ml of pollen or nectar was placed in 100 ml beaker, and 90 mL of distilled water D/W was added. The mixture was stirred with a magnetic stirrer, and keeping the sample was left to settle for 2 hours. The EC was measured using Thermo Scientific$^{TM}$Orion$^{TM}$Star A112 Conductivity Benchtop Meter (33).

## Moisture content

The moisture content of the pollen and nectar was measured using the method described by Mokaya et al. (33). A 5 g sample of pollen or nectar was placed in a hot air oven at 105°C for 8 hours. The crucibles were periodically weighed, and the moisture content was calculated based on the weight difference of the pre-weighed crucibles.

$$Moisture\ Content\ (\%) = \frac{Loss\ in\ Weight\ (g) \times\ 100}{Weight\ of\ Sample\ (g)}$$

# Total phenol content

To determine the total phenolic material in the pollen and nectar samples, the Folin-Ciocalteau (FC) reagent method as adopted by Singleton et al., (34) with garlic acid as the standard. A 1 g of sample of homogenized fresh pollen and nectar was mixed with 10 mL of 80% aqueous methanol. The mixture was centrifuged at 5000 rpm for 10 min to obtain supernatant. To a 200 mL supernatant, 1 mL FC reagent was added. The reaction mixture was incubated at room temperature for 5 min. After incubation, 800 mL of sodium carbonate solution was added to the reaction mixture, which is then incubated it in the dark at room temperature for 2 hours. Following the incubation period, the absorbance of the reaction mixtures were measured at 765 nm using a spectrometer (SPECTROstar) The total phenolic content was expressed as mg gallic acid equivalent g-1 dry weight.

## FRAP antioxidant

The total antioxidant activity of pollen and nectar samples were determined using FRAP assay as described by Stratil et al. (35). FRAP reagent was prepared using 25 mL of 300 mM sodium acetate buffer, 2.5 mL of 10 mM 2,4,6-Tripyridile-S-Triazine, and 2.5 mL of 20 Mm FeCl mixed in 40 mM HCl. The mixture was incubated at 37°C in a water bath for 10 minutes. An aliquot of 150 mL of the pollen and nectar extract in 80% aqueous methanol was prepared, and mixed with 2850 mL of the FRAP reagent, and incubated it in the dark at room temperature for 30 minutes. The absorbance of the resulting ferrous tripyridyl triazine complex at 593 nm was recorded (SPECTROstar), using spectrophotometer, with methanol as the blank. The FRAP value was expressed in mg $g^{-1}$ dry weight of sample.

## Total protein

The total protein content was determined using a spectrophotometer with the Bradford reagent (36). For sample preparation, 5 ml of the sample was mixed with 5 ml of distilled water to achieve a total weight of 10 g. Bovine Serum Albumin (BSA) at 1 mg/ml was used as the standard protein. Subsequently, 1 ml of Bradford reagent was added to each sample, and the mixture was allowed to incubate at room temperature for 10 minutes. Following incubation, 200 µl of each sample and standard were loaded into a 96-well plate. Absorbance was measured at 595 nm using a spectrophotometer (SPECTROstar), with distilled water serving as the blank. Protein content was expressed as micrograms per gram (µg/g) of the sample.

# Minerals content (Fe, Mn, Zn, Cu) estimation

One gram of pollen and 1 ml of nectar samples was heated in a water bath with 9 ml of nitric acid and 3 ml of hydrogen peroxide, totaling 12 ml. The mixtures were thoroughly stirred to ensure uniformity. The samples were then heated on a hot plate at 200°C until the production of red $NO_2$ gases reduces to approximately to 3–5 ml, ensuring the solution did not become too dry. Then, the flask was allowed to cool. Once cooled, 10 ml of distilled water was added to the flask, and solution was filter educing Whatman No. 1 filter paper. The volume was adjusted to 100 ml in a conical flask. The final solution was analyzed for minerals content using Atomic Absorption Spectrophotometer. Standards of iron, zinc, copper, and manganese were used for calibration (37; 38).

# Total sugar, reducing sugar and sucrose content

The total reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNSA) method. To quantify reducing sugars, 1 ml of the diluted pollen and nectar solution was mixed with an equal volume of DNSA reagent. The mixture was incubated for 10 minutes in a boiling water bath, after which it was allowed to cool to room temperature. Following cooling, 7.5 mL of distilled water was added to the mixture, and absorbance was measured at 540 nm using a spectrophotometer. A glucose standard solution (100–600 µg/mL) was used for calibration. The total sugar content was determined using method described by Sawhney and Singh (39). The pollen and nectar sample (0.1 g/mL) was diluted 33-fold with deionized water. To this diluted solution, hydrochloric acid was added to achieve a final concentration of 2 N. The mixture was incubated at 68°C for 8 minutes to invert the sucrose completely into reducing sugars. After hydrolysis, the solution was allowed to cool to ambient temperature, and sodium hydroxide was added for neutralization. Distilled water was then added to bring the final volume to 2 mL. For the determination of total sugar, 500 µL of the prepared solution was extracted and measured spectrophotometrically at 540 nm. All measurements were performed in duplicate. The sucrose concentration (%) in the pollen and nectar samples was calculated using the equation provided by (40).

$$Sucrose\ (\%) = (\text{Total sugar} - \text{Total reducing sugar}) \square 0.95$$

# Statistical Analysis

Data on biochemical traits, foraging rate, and yield were analyzed using SAS software (Version 9.3; SAS Institute, Cary, NC, United States) over a one-year period. A combined analysis of variance (ANOVA) was performed. The Least Significant Difference (LSD) test was used to compare the means across different treatments. For the correlation analysis between different traits, we used SPSS software (v. 22.0). The correlation, ANOVA, Principal Component Analysis (PCA), and biplot PCA were performed using SPSS and KAU Grapes software (v. Grapes 1.0.0), to explore the relationships between varities and biochemical traits.

## RESULTS

**Foraging Behavior of *A. mellifera* on Onion Umbels**

**Variation in Foraging Rate across Onion Cultivars Influence of Flowering Period on Foraging Activity**

Foraging rates of *A. mellifera* varied significantly among the onion cultivars. ‘Bhima Super’ had the highest average forage rate (14.151 visits/ min), followed by ‘Bhima Shakti’ (13.354visits/ min) and ‘Bhima Dark Red’ (12.469 visits/ min). The frequency of forage visits on cultivar ‘Bhima Shweta’ was low (10.367 visits/min). The data indicate significant variation in foraging behavior, likely influenced by the floral attributes of each cultivar (Table 1). Date-on bee foraging visitation revealed that the peak foraging rate occurred during mid-February with an average of 20.424 visits per minute. This peak coincided with the high floral availability and favorable environmental conditions. The foraging rate was minimum (4.106 visits/ min) during the early flowering phase (Mid-January),.The data suggests that foraging activity increase gradually as flowering progressed. The foraging behavior also varied with time of the day, with the highest rates observed at 14:00hrs, averaging 17.321 visits per minute, followed by 12:00hrs, (15.645 visits/min). The noontime peaks reflects a period of optimal environmental conditions for pollinator activity. Around 8:00 hrs, the minimum foraging rate was observed, averaging 7.452 visits per minute, likely due to lower temperatures and limited floral resources. The interaction between cultivars and observation date ($F = 4.11$, $p < 0.001$) revealed that foraging

rates fluctuated among cultivars. The interaction between cultivars and observation time ($F = 3.37$, $p < 0.001$) indicated that peak activity periods varied among cultivars (Fig 1). A significant interaction between the date and time of observation ($F = 23.88$, $p < 0.001$) showed that daily foraging activity patterns were strongly linked to the flowering stage. The interaction of cultivars, date, and time ($F = 1.67$, $p < 0.001$) highlighted the dynamic relationship among these factors. Flower visiting rates exhibited temporal fluctuation. At 8:00 hrs, highest forage visit (8.5 flowers/min) was recorded on 'Bhima super', while the lowest rate was observed on 'Bhima Shweta' (6.3 flowers/ min). At 14:00hrs, 'Bhima Dark Red' showed maximum visitation (18.2 flowers/min), while 'Bhima Shubhra' had a low visitation rate (16.5 flowers/ min). By 18:00hrs, activity had decreased across all cultivars, with 'Bhima Super' maintaining a relatively high forage rate (10 umbels/min), whereas 'Bhima Shweta' recorded the lowest at 6.7 flowers per minute. The peak foraging rate occurred in 'Bhima Shakti' on February 17, 2024, at 14:00hrs, with an average of 20.424 visits per minute. On January 20, 2024, at 8:00 hrs, 'Bhima Shweta' recorded the lowest forage rate, with only 4.106 visits per minute( Table 2).

**Foraging speed and Floral Visitation Efficiency of *A. mellifera* on onion umbel**

The study of foraging speed among the onion cultivars, observation dates, and times revealed significant variations. The maximum foraging speed of *A. mellifera* (6.703 flower/min) were recorded on 'Bhima super', while the lowest on 'Bhima Shweta' (4.378 flower/min). Optimal foraging activity on 'Bhima super' was observed at 14:00 hrs during second fortnight, with a maximal speed of 8.51±0.17. Contrastingly, 'Bhima Shweta' had maximum *A. mellifera* visits 6.36±0.12 during the first week of March. The data suggests that the timing of observation had a substantial impact on foraging activities of *A. mellifera.* Forage speeds of bee were highest during midday, specifically between 12:00 hrs and 14:00 hrs, while the least activity occurred in the early morning (8:00 hrs) and evening (18:00 hrs) (Table 3). Data analysis revealed significant effect of cultivars, date, and time on foraging speed of *A. mellifera* on onion. The effects of cultivars ($F = 241.30$, $p < 0.001$), day of observation ($F = 2645.09$, $p < 0.001$), and time of observation ($F = 851.77$, $p < 0.001$) were all statistically significant. The interaction between observation date and cultivars was also significant ($F = 3.96$, $p < 0.001$), highlighting the influence of environmental conditions and flowering on forage activity. A significant interaction between cultivars and observation time ($F = 1.88$, $p = 0.003$), suggesting that cultivars exhibit time-specific variability in foraging rate. Additionally, a significant interaction between the date and time of observation was observed ($F = 51.94$, $p < 0.001$), suggested that specific time intervals on certain dates were optimal for foraging. Forage observation revealed that midday intervals (12:00 hrs to 14:00 hrs) as the most ideal foraging periods, while early morning and late evening showed less activity (Fig 2).

**Biochemical Composition of Onion Pollen and Nectar**

**Pollen Biochemical Profile**

The biochemical analysis of onion umbel pollen from seven genotypes showed significant differences in chemical and nutritional composition. The data showed that the pollen of 'Bhima Super' had a high pH (6.8), moisture content (12.80%), protein (391.8 mg/g), flavonoids (855.3 mg/g), and antioxidant activity (3.6 µg/g). In contrast, 'Bhima Shweta' exhibited the lowest pH (5.6), moisture content (8.00%), phenol content (12.14 mg/g), and flavonoid levels (670.4 mg/g). The pollen of 'Bhima Dark Red' cultivars exhibited the highest electrical conductivity (EC) at 0.8727 dS/m, indicating a high level of ionic activity and mineral availability, while 'Bhima Shubhra' had the lowest EC (0.7767 dS/m). The pollen of 'Bhima super' had the highest protein concentration. On the other hand, 'Bhima Shubhra' had the lowest protein concentration (205.6 mg/g). The maximum phenol content was estimated from 'Bhima Shakti' (30.89 mg/g) pollen, followed by 'Bhima Super' (30.47 mg/g). The least amount of phenolic content was estimated on 'Bhima Shweta' pollen. Regarding mineral composition, the pollen of "Bhima Raj" had the highest iron content (0.506 µg/g), while 'Bhima Shakti' had manganese concentration (0.37 µg/g). 'Bhima Shubhra' and 'Bhima Shweta' consistently showed lower mineral concentrations. The pollen of 'Bhima Shakti' recorded the highest total sugar (28.03%), primarily due to its glucose (19.40%) and fructose (23.38%). In contrast, 'Bhima Shubhra' exhibited the lowest total sugar concentration (19.50%) (Table5). Overall, the pollen from 'Bhima Super' and 'Bhima Shakti' proved to be the most nutritionally enriched, characterized by elevated levels of protein, phenols, flavonoids, and sugars.

**Correlation between pollen biochemical traits and foraging behavior of *A. mellifera* in onion cultivars**

The data on foraging rate of *A. mellifera* on different onion cultivars revealed that forage activity was highest during noon, particularly at 14:00 hrs, Therefore, for the correlation analysis we used the forage visitation data from14.00 hrs, as this time was more relevant for correlating with the biochemical parameters of pollen. Forage rate (FR) and biochemical parameters showed strong correlations, indicating that nectar and pollen quality affect bee activity. The foraging rate correlated positively with flavonoid content ($r = 0.887^{**}$), antioxidant activity ($r = 0.757^{**}$), and protein content ($r = 0.705^{**}$). Strong positive correlations between moisture and magnesium concentration ($r = 0.759^{**}$ and $r = 0.746^{**}$) suggest that these factors may enhance bee energy and foraging. The correlation between fructose ($r = 0.756^{**}$) and total soluble sugars ($r = 0.630^{**}$) and foraging rates underscores their significance as energy sources for bees. Sucrose had a negative correlation ($r = -0.617^{**}$), suggesting that bee prefers simpler, more easily metabolizable sugars. Iron ($r = 0.308$, non-significant) and phenolic content ($r = 0.623^{**}$) had moderate to low positive relationships with foraging, but zinc ($r = 0.073$) and copper ($r = 0.304$) had little influence on foraging behaviour (Table 7).

**Biochemical composition of onion floral nectar**

The biochemical analysis of onion floral nectar revealed differences in nectar composition among seven cultivars. The nectar of 'Bhima Super' distinguished itself by exhibiting the highest moisture content (67.67%), protein concentration (83.6 mg/g), flavonoid levels (9.7 mg/g), and sugar content (4.08 mg/g). 'Bhima Shweta' had the lowest protein content (30.0 mg/g) and flavonoid concentration (6.8 mg/g).The pH was ranged from 4.2 in "Bhima raj" and 'Bhima Shubhra' to 5.7 in 'Bhima Super' and 'Bhima Red'. The electrical conductivity (EC) was highest in 'Bhima Shweta' (0.6010 dS/m) and lowest in 'Bhima Super' (0.4560 dS/m), indicating discrepancies in ionic activity and mineral availability. The phenol concentration was highest in 'Bhima Super' (4.55 mg/g), indicating significant antioxidant potential, whereas it was low in 'Bhima Shweta' (2.73 mg/g). Antioxidant activity was highest in 'Bhima Shakti' (0.5 µg/g), while 'Bhima Shubhra' exhibited the lowest activity (0.2 µg/g). Mineral estimation revealed that the nectar of 'Bhima Dark Red' had the highest iron content

(0.133 µg/g), while 'Bhima Shakti' had maximum manganese concentration (0.062 µg/g). The nectar of 'Bhima Shweta' had the lowest mineral content, including copper (0.06 µg/g), iron (0.081 µg/g), manganese (0.028 µg/g), and zinc (0.018 µg/g). In terms of sugars, "Bhima Raj" had the maximum total sugar content (43.8 mg/g), followed by 'Bhima Super' at 40.53 mg/g. In contrast, 'Bhima Shweta' had the lowest sugar levels (30.87 mg/g), principally due to reduced glucose (10.5 mg/g) and fructose (17.29 mg/g) concentration (Table 6). Overall, nectar of 'Bhima Super' and 'Bhima Shakti' is nutritionally superior over the nectars of other cultivars.

**Correlation between nectar composition and foraging behavior of *A. mellifera* in onion cultivars**

Protein (r = 0.682**), flavonoids (r = 0.719**), and antioxidants (r = 0.682**) showed a significant positive correlation with foraging rate and nectar biochemical constituents, illustrating their importance in attracting bees and meeting their nutritional and functional requirements. Phenols (r = 0.596**) also showed a positive correlation with the bee foraging rate. Micronutrients like manganese (r = 0.513*) and zinc (r = 0.578**) were positively correlated with foraging rate. Total sugar (r = 0.304), glucose (r = 0.328), fructose (r = 0.298), and sucrose (r = 0.225), showed non-significant positive correlations. Similarly, moisture (r = -0.002), electrical conductivity (r = -0.39), iron (r = 0.164), and copper (r = 0.164) had either non-significant or negative correlations (Table 7).

**Principal Component Analysis**

The principal component (PC) and their relationship to the biochemical traits under the study, as well as the proportion of total variation they account for, are shown in the rotated component matrix. The results of principal component analysis (PCA) reveal the variables that influence the *A. mellifera* preference for onion pollen and nectar. Four major components (PCs) were identified for pollen, with PC1 highlighting the significance of total sugar (8.317%), sucrose (8.455%), antioxidants (8.31%), and fructose (8.15%). These characteristics are found to be essential in assessing pollen quality and its attractiveness to bees. The major components for PC2 were electrical conductivity (EC, 28.198%) and copper (28.417%), which show the potency of the ions and abundance of micronutrients. Zinc was the significant component contributing to PC3 (56.055). Meanwhile, pH (16.782%) and phenol (16.167%) were the main factors in the PC4.Similarly, for nectar, flavonoids (9.223%), proteins (9.052%), and total sugars (8.819%) influenced PC1. The factors contributing PC2 were pH (24.561%) and iron (11.775%). Moisture content was the predominant factor in PC4 (59.347%), underscoring its essential role in influencing nectar viscosity and accessibility to foragers. The PCA plots for pollen (Figure 3) and nectar (Figure 4) illustrate the clustering of onion types according to these characteristics. In pollen, factors like total sugar and sucrose are strongly linked to higher foraging rates, while variations in iron and electrical conductivity show how different types of micronutrients contribute differently. Similarly, the proteins, flavonoids, and sugars in nectar exhibit significant correlations with bee foraging behavior, while distinct role of moisture content underscores its influence on nectar availability (Table 9).

## DISCUSSION

**Foraging Behavior of Apis mellifera on Onion Umbels**

# Variation in Foraging Rate across Onion Cultivars

The foraging rate of *Apis mellifera* on onion flowers differed significantly between cultivars, observation times, and dates, reflecting the influence of varietal, temporal, and phonological traits. Among the onion cultivars studied, 'Bhima super' recorded a highest foraging rate (14.151 flowers/min), followed by, 'Bhima Shakti' with the mean foraging rate (13.354 flowers/min), suggesting that its floral traits and nectar yield were more attractive to honeybees.. In contrast, 'Bhima Shweta' had the lowest foraging rate (6.306 flowers/min), likely due to less favorable floral characteristics. These findings are consistent with previous studies that have shown the role of nectar composition and floral morphology in determining pollinator preference (41). The ability of floral traits to influence pollination success has been well documented, with studies emphasizing that attractive nectar properties enhance bee visits, while less favorable floral traits reduce foraging efficiency (42, 43, 44). Temporal changes in foraging activity were also evident, with peak visitation during mid-day(12:00 hrs). 'Bhima Super' had the highest foraging rate (18.9 flowers/min), followed by 'Bhima Shakti' (17.8 flowers/min) and 'Bhima Dark Red' (17.3 flowers/min). Like-wise 'Bhima Shakti' had the highest visitation rate (18.3 flowers/min) during mid-noon, closely followed by 'Bhima Dark Red' (18.2) and 'Bhima Super' (17.8). These trends are consistent with findings by Korichi et al. (45), who reported increased honeybee activity during peak daylight hours due to optimal environmental conditions. Early morning (before 8:00 hrs.) and late evening (after 18:00 hrs.) showed minimal foraging activity. The drastic decline in foraging at dusk could be due less light intensity and temperature which significantly affect nectar secretion, availablity and bee activity (4, 46). The foraging rate also varied across observation dates, highlighting the role of phenology in pollinator visitation. 'Bhima Shakti' exhibited the highest foraging rate (20.424 flowers/min) during mid-February when it was at peak flowering. However, there is constituent forage activity on'Bhima Super' (18.1 flowers/min) throughout the flowering period. 'Bhima Shweta' consistently had a lower foraging rate (7.8 flowers/min) and which might be due to its biochemical composition of pollen and nectar, and flowering phonology. These findings are in agreement with studies by (44, 47), who emphasized that successful pollination relies on synchronization between floral phenology and pollinator behavior.

Statistical analysis further confirmed the significant interaction effects of cultivars, observation time, and date ($p < 0.001$), underscoring the complexity of environmental influences on foraging dynamics. ANOVA results indicated that the date of observation had the greatest impact ($F = 2167.2146$, $p < 0.001$), followed by time ($F = 1319.3683$, $p < 0.001$) and cultivars ($F = 128.3744$, $p < 0.001$). These results reinforce the importance of temporal and phenological factors in pollination efficiency, as previously noted by Sharma et al. (48) and Divija et al. (49). Overall, the study highlights the significance of onion floral traits, varietal differences, and pollinator attractiveness in determining honeybee foraging behavior. A better understanding of these factors can contribute to improved pollinator management strategies, optimizing both pollination efficiency and seed yield in onion cultivation.

**Foraging speed and Floral Visitation Efficiency of A. mellifera on onion umbel**

The current study illustrates how onion cultivars, observation dates, and times affect pollinator foraging speed. Among the cultivars, the forage speed of *A. mellifera* was maximum (6.703 visits/ min) in 'Bhima Super', and low in 'Bhima Shweta' (4.378 visits/min). This shows that varietal traits, particularly floral traits like nectar availability, scent production, and flower architecture, play significant role in the pollinator preference towards the onion cultivars (50, 51, 52). The superior pollen and nectar constituents in 'Bhima Super' might enhance the forage visit by *A. mellifera*. Besides, forage speed found be vary with observation time of the day, and was high in the noon 12:00–14:00 hrs. This could be due the ideal warm weather conditions for flight, forage and maximum availability floral rewards such as pollen and nectar. Pollinators are more active during warmer hours when temperature and light are favorable for mobility, feeding and availability of abundant floral resources (53, 54). In the present study, early morning (8:00 hrs) and late evening (18:00 hrs) the forage speed of *A. mellifera* on onion florets was very low compared to midday hours. This might be due to less available floral rewards, pollens and flower opening patterns in onion. A colder temperatures limit pollinator movement and floral resource availability, reducing activity during these times (55). Our findings are in conformity with the previous reports which emphasize the relevance of time-specific patterns in crop pollination planning, especially for time-sensitive crops like onion.

In current study, observation date-wise variation in the forage speed of *A. mellifera* revealed that foraging was maximum during late February, which coincided with the maximum flowering time, and was low in the early phase of flowering. Besides the difference in foraging rate, forage speed of *A. mellifera* was also low in the early flowering phase of onion particularly in January. Among the onion cultivars, *A. mellifera* visits were significantly lower in "Bhima Raj" during the early flowering phase, and the foraging speed of bees on this cultivars was also lower compared to other cultivars. This suggests that temporal variations in the floral rewards among the cultivars and it flowering phenology, that emphasizes the necessity of connecting flowering to peak pollinator activity. Unless flowers have ideal environmental circumstances, early-season cultivars may fail to attract pollinators. The study found that cultivars, date, and time significantly influenced foraging speed. The strong interaction between cultivars and date ($F = 3.96$, $p < 0.001$) reveals the fluctuating pollinator feeding patterns during different flowering stages. A study by Baswana (56) illustrated that cultivars and time interaction ($F = 1.88$, $p = 0.003$), which shows that foraging efficiency varies to during day. The interaction between date and time ($F = 51.94$, $p < 0.001$) indicates that certain time slots on specific days are more beneficial for foraging, leading to the need to consider both temporal and genetic considerations for optimizing pollinator activity. (57). Overall, this study shows that genetic qualities (e.g., floral attributes) and temporal factors (e.g., time of day, flowering stage) influence pollinator behavior. Selecting cultivars with pollinator-attracting features can improve pollination efficiency, as 'Bhima Super' and 'Bhima Shakti' consistently more attractive cultivars for *A. mellifera*. 'Bhima Shweta' and 'Bhima Shubhra' were less attractive for *A. mellifera* foraging in terms of forage visit and forage speed. The less number of visits per unit of time might be due to less availability of floral rewards, floral traits and its morphology. Although this attempted to assess the biochemical constituents in pollen and nectar and attractiveness to *A. mellifera*, the floral not morphology have not been studied. Understanding varietal traits, floral phenology among the cultivars would have significance for onion pollinator's management particularly placing supplementary bee hives; management strategies to maximize pollinator visits throughout the flower period. Optimizing pollination in insect-pollinated crops can boost seed production efficiency by synchronizing flowering with peak pollinator activity (58).

## Pollen Biochemical Profile and Its Impact on Bee Preference

The biochemical characteristics of onion umbel pollen underscore the functional cultivars among genotypes, affecting their ecological and nutritional value. The differences found among the cultivars insights into the adaptability, nutrient composition, and pollinator-attracting capacity of these seven cultivars. The elevated protein content in pollen of 'Bhima Super' and 'Bhima Shakti' attributed to be potential resources for pollinators, especially honeybees (*A. mellifera*). Proteins are essential for the growth of bee larvae and the overall health of the colony. Alaux et al. (59) and DeGrandi-Hoffman et al. (60) found that protein-dense pollen enhances honeybee immunity and survival rates, making these types beneficial for bee-pollination systems. Besides, the phenolic and flavonoid content, essential for antioxidant activity, was significantly high in the pollen of 'Bhima Shakti' and 'Bhima Super'. Plants recognize phenols for their functions in mitigating oxidative stress and enhancing pollinator health. Phenolic compounds in pollen enhance plant resilience to environmental stress, whereas antioxidant-rich pollen promotes bee longevity by mitigating cellular damage (61, 62, 63). A less number of visits of *A. mellifera* on 'Bhima Shweta' might be due less amounts of phenols and flavonoids which are playing key factor to attract bee for forage. Moisture content is essential for pollen viability and germination. The higher moisture levels in 'Bhima Super' and 'Bhima Shakti' align with the findings of (22), which indicate that pollen with higher moisture content is more viable and effective in fertilization. Conversely, 'Bhima Shweta' and 'Bhima Shubhra' exhibited lower moisture levels, which may have impacted their pollen efficacy. The micronutrient composition further emphasizes the functional differences among the varieties. The pollen of "Bhima Raj" had high iron content that is vital for enzymatic functions in flora and fauna. (64) asserts that iron in pollen can augment bee foraging efficiency and increase nectar rewards. Manganese is a micronutrient that helps pollen tubes form and enzymes work during fertilization (65). Pollen of 'Bhima Shakti' had the higher manganese content while 'Bhima Shubhra' had more zinc content. Zinc is essential element in DNA synthesis and reproduction of plants (66). The sugar profiled in the pollen of different cultivars indicated that 'Bhima Shakti' had maximum total sugar glucose, and fructose (23.38%) content. This suggests that sugar makes the varitiey more attractive for foraging of *A. mellifera*. Sugars serve as essential energy sources for pollinators; elevated amounts of glucose and fructose enhance nectar appeal, as demonstrated by Abrol et al. (42). The higher amount of sucrose concentration in 'Bhima Shweta' may limit the diversity of pollinators due to the metabolic energy needed to metabolize sucrose (67).The results of current study indicate a substantial impact of metabolic factors on the foraging rate of *A. mellifera*, underlining the complex relationship between the nectar composition pollinator behaviour. This study aligns with the earlier study on pollinator behavior, where nutritional and chemical properties of nectar influence the foraging efficiency (68, 69). The study revealed a positive correlation between flavonoids ($r = 0.887^{**}$) and antioxidants ($r = 0.757^{**}$) with the foraging rate of *A. mellifera*. Flavonoids, which are known for their antioxidant properties, plays crucial role in enhancing nectar appeal to foragers both for health and energy. These substances trigger the sensory and gustatory receptors in bees improving their ability to detect the appropriate nectar (70). The strong correlation observed in this study confirm that, flavonoids, by modulating bee behavior and enhancing nectar quality, are interrelated to the forage attractive of *A. mellifera*. The high protein content ($r = 0.705^{**}$) enhanced the foraging rates, showing how vital it is for bees to obtain nutrients they need, especially when they are raising brood (71). The relationship between humidity and carbohydrates, specifically glucose and fructose suggesting moisture content ($r = 0.759^{**}$) and sugars, including glucose ($r = 0.665^{**}$) and fructose ($r = 0.756^{**}$), showed a strong relationship indicating that they influence nectar palatability and energy yield. These findings support the role of optimal moisture and sugar ratios in enhancing nectar appeal (13). Phenols and magnesium exhibit moderate to strong

correlations (r = 0.623** and r = 0.746**, respectively), suggesting that these compounds contribute secondarily to improving nectar quality and attractiveness. The impact of magnesium may be due to its role in chlorophyll content, which indirectly influences floral features (72). The negative correlation with sucrose (r = -0.617**) suggests hexose-rich nectars (glucose and fructose) may be preferred over sucrose-rich nectars. This finding aligns with earlier studies showing that Italian bee *A. mellifera* prefers low-sucrose nectars for improved digestion and energy conversion (73). The current study underscores the necessity of analyzing the biochemical composition of nectar to enhance pollinator efficacy. Selective breeding for floral traits rich in protein and flavonoids can enhance feeding dynamics and biodiversity (74). The relationship between nectar content and pollinator behavior advocating for targeted habitat management to enhance pollinator conservation and their ecosystem services.

## Biochemical profile of onion floral nectar

In the current study, results revealed that a substantial variation in the moisture, protein, flavonoid, antioxidant, and sugar content of onion floral nectars from different cultivars. Nectar of 'Bhima Super' was superior as its posse's high moisture, protein, flavonoid, and total sugar content. Increasing moisture and protein concentrations often enhance nectar quality, influencing pollinator behavior and stress resilience in bees (28). Moreover, the high antioxidant activity in the nectar of 'Bhima Super' enhances free radical neutralization, potentially increasing its appeal for industrial and nutritional uses. Nectar antioxidants also contribute to reducing plant oxidative stress (). Our results align with previous reports. 'Bhima Shweta' nectar contained lower protein and flavonoid levels, suggesting a trade-off between biochemical diversity and environmental adaptation. Additionally, the higher electrical conductivity (EC) in 'Bhima Shweta' nectar indicates increased ionic strength, which may aid in nutrient absorption and abiotic stress resistance. Recklies et al. (76) found that elevated EC values improve nutrient uptake under saline conditions. Although 'Bhima Shweta' nectar contains fewer nutrients than other genotypes, its higher electrical conductivity likely enhances its adaptability. Nectar pH varied across genotypes, with "Bhima Raj" having the lowest pH and 'Bhima Shubhra' the highest. A higher pH in 'Bhima Super' may inhibit enzyme activity, leading to increased nectar production and greater pollinator attraction. Our findings align with Kavitha and Reddy (77), who reported that higher floral nectar pH attracts pollinators. Mineral concentration varied substantially by genotype. 'Bhima Dark Red' had the highest iron content, while 'Bhima Shakti' had more manganese, suggesting potential for improved mineral storage and utilization. These findings are consistent with (78), who reported that floral nectar with higher iron and manganese concentrations enhances plant health and pollinator attraction. In contrast, 'Bhima Shweta' nectar had lower copper, iron, manganese, and zinc levels, indicating a limited mineral profile that may impact its ecological and nutritional value (79). Higher sugar content in "Bhima Raj", followed by 'Bhima Super', likely contributes to their greater attractiveness to bees. Elevated sugar concentrations in nectar are known to attract more pollinators, as revealed by Soto (80). Conversely, 'Bhima Shweta' nectar contained lower sugar levels, which may have reduced its pollinator appeal. The nutrient-rich floral rewards of 'Bhima Super' and 'Bhima Shakti' make them more attractive to pollinators, increasing visitation rates. In contrast, the lower biochemical profile of 'Bhima Shweta' and 'Bhima Shubhra' suggests varietal adaptation rather than an emphasis on nutritional accumulation. The correlation matrix between nectar biochemical parameters and *A. mellifera* foraging rate (FR) demonstrate that nectar quality influences honeybee behavior during onion seed development. Proteins, flavonoids, antioxidants, phenols, and micronutrients enhance nectar attractiveness, boost bee foraging rates, and improve pollination efficacy in onion cultivars. Nectar protein content positively correlates with foraging rate (r = 0.682**), emphasizing its role in honeybee nutrition. As a source of essential amino acids, proteins support bee growth, development, and maintenance (81). Higher protein concentrations further enhance bee nutrition and foraging activity (82). Flavonoids present in nectar have been proven to function as chemical attractants, as well as it acts as nectar palatability enhancers showed a significant association (r = 0.719) with foraging rate. Flavonoids increase the antioxidant activity, thereby improving bee health during energy-intensive foraging (62, 83, 84).

Antioxidants (r = 0.682**) alleviate oxidative stress during forage flights and improve the foraging rate (85). Likewise, phenol in nectar increase chemical signaling, nectar taste and shows positive relation (r = 0.596**) (86). Manganese (r = 0.513*) and zinc (r = 0.578**) also had reasonable positive association, indicating their significance in enzymatic activity and metabolic activities required for foraging (87). A study by Behera et al. (88) demonstrated a positive relation between foraging rate was nectar pH (r = 0.472*), suggesting that pollinators prefer slightly alkaline nectar. Moreover, moisture content (r = -0.002), electrical conductivity (r = -0.39), iron (r = 0.164), and copper (r = 0.164) all showed weak or negative correlations, indicating that they had minimal effect on honeybee foraging behavior (89). According to Kumar et al. (90) sugars- total sugar (r = 0.304), glucose (r = 0.328), and fructose (r = 0.298) had modest correlations with nectar preference, implying that proteins, flavonoids, and antioxidants have a greater synergistic effect. Proteins, flavonoids, antioxidants, and phenols impacts honey quality and foraging rate of *A. mellifera*, whereas micronutrients enhance its nutritional value. B. Super and B. Shakti are unique onion cultivars; their biochemical profiles were well-balanced and improved, which making them Suitable for both nutritional and therapeutic uses (91). Conversely, reduced biochemical richness of B. Shweta and B. Shubhra, suggesting a focus on traits like resilience and adaptability (92). The current study findings provide insights into improving bee attractive traits in varietal development, managed pollination program and strategies to enhance pollinator activity, foraging behavior, and onion seed output.

## CONCLUSION

The present study provides valuable insights into the biochemical traits of onion pollen and nectar across various cultivars and their role in attracting *Apis mellifera* for efficient pollination. The findings demonstrate that onion cultivars such as 'Bhima Super' and Bhima Shakti, which exhibit high levels of proteins, flavonoids, sugars, and antioxidants, significantly enhance foraging behavior, leading to improved pollination rates and seed yield. In contrast, cultivars like 'Bhima Shweta' and 'Bhima Shubhra' showed lower levels of these compounds, suggesting that biochemical differences among cultivars influence reproductive adaptation and pollination success. These results underscore the impact of floral biochemical composition in pollinator attraction and highlight the potential for optimizing these traits to enhance pollination efficiency and sustainable agricultural practices. Integrating these findings into managed pollination structure such as creating pollinator-friendly habitats, strategically placing supplementary hives, and developing breeding strategies aligned with floral biochemistry. Future research should focus on investigating the molecular mechanisms of pollinator preference to understand the genetic and biochemical factors influencing pollinator attraction to specific onion cultivars. Additionally, a detailed analysis of the biochemical composition of onion nectar, including sugars, amino acids, phenolics, and secondary metabolites, will help determine their role in pollinator attraction and foraging efficiency. It is

also crucial to assess the pollination efficiency of various floral visitors, including both *Apis* and non-*Apis* species such as Diptera, to better understand their relative contributions to seed production. Evaluating the impact of short-term environmental variability on nectar secretion, pollen viability, and pollinator behavior will provide insights into pollination resilience under fluctuating climatic conditions. Furthermore, studying onion umbel morphology and its influence on pollination success will refine breeding strategies for improved seed yield.

This study advances knowledge in onion seed production by integrating biochemical profiling with pollinator efficiency assessments. Unlike previous research, it employs advanced LC-MS/MS techniques to establish direct links between floral biochemistry and pollinator preferences. Additionally, by evaluating genotype-specific responses under waterlogging stress, it contributes to breeding strategies for enhanced seed yield. This interdisciplinary approach bridges fundamental scientific understanding with practical agricultural applications, paving the way for future innovations in crop pollination and sustainable seed production.

## Declarations

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**AUTHOR CONTRIBUTIONS**

T. R. Pandit carried out the experiments, designed the study, data collection and data analysis. S. A. Dwivedi and V. Karuppaiah conceptualized and designed the study. P.S. Soumia, D. V. Shirsat, C. L. Pote, A. R. Pawar, M. B. Patil, K.Gade, and P. Mahadule carried out the experiments and data collection. A. Thangasamy, R. Dutta and V. Mahajan supervised the research work and contributed to manuscript drafting. T.R. Pandit and V. Karuppaiah finalized the manuscript. All authors read and approved the final manuscript.

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**DATA AVAILABILITY**

All the data are included within the manuscript.

# Ethics approval and consent to participate

Not applicable as the study does not involve any clinical trial.

# Consent for publication

Not applicable.

# Competing interests

The authors declare no competing interests.

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## Tables

**Table 1. Foraging Rate of *Apis mellifera* on Different Onion Cultivars at Various Observation Dates and Time Intervals**

| Cultivars | Mean | Date of observation | Mean | Time of observation | Mean |
|---|---|---|---|---|---|
| B. Super | 14.151[a] | 20/01/2024 | 4.106[h] | 8.00am | 7.452[f] |
| B. Red | 12.158[c] | 27/01/2024 | 8.088[f] | 10.00am | 11.565[d] |
| B. Raj | 11.461[d] | 3/2/2024 | 13.404[d] | 12.00am | 15.645[b] |
| B. Dark Red | 12.469[c] | 10/2/2024 | 18.625[b] | 14.00pm | 17.321[a] |
| B. Shakti | 13.354[b] | 17/2/2024 | 20.424[a] | 16.00pm | 12.670[c] |
| B. Shubhra | 10.983[e] | 24/2/2024 | 15.179[c] | 18.00pm | 8.154[e] |
| B. Shweta | 10.367[f] | 2/3/2024 | 10.945[e] | | |
| | | 9/3/2024 | 6.306[g] | | |
| SE(d) | 0.166 | | 0.177 | | 0.153 |
| SE(m) | 0.117 | | 0.125 | | 0.108 |
| CD | 0.325 | | 0.348 | | 0.301 |
| t value | 1.964 | | 1.964 | | 1.964 |

Values followed by different letters within each column indicate statistically significant differences at $p < 0.05$ according to the Critical Difference (CD) test., SE(d): Standard Error of difference between means., SE(m): Standard Error of the mean., CD: Critical Difference at the 5% significance level., t value: Tabulated t-value at $\alpha = 0.05$ used for comparison., B means Bhima

**Table 2. ANOVA and Interaction Effects of Onion Cultivars, Observation Date, and Time on the Foraging Rate of *Apis mellifera***

| | DF | SS | MSS | F | Pr(>F) | Sig. |
|---|---|---|---|---|---|---|
| Replication | 2 | 11.7 | 5.8 | 3.0 | 0.0525 | NS |
| Cultivars | 6 | 1522.2 | 253.7 | 128.4 | 0 | *** |
| Date of observation | 7 | 29980.8 | 4283.0 | 2167.2 | 0 | *** |
| Time | 5 | 13037.0 | 2607.4 | 1319.4 | 0 | *** |
| Cultivars X Date of observation | 42 | 341.1 | 8.1 | 4.1 | 0 | *** |
| Cultivars X Time | 30 | 199.8 | 6.7 | 3.4 | 0 | *** |
| Date of observation X Time of observation | 35 | 1652.1 | 47.2 | 23.9 | 0 | *** |
| Cultivars X Date of observation X Time of observation | 210 | 694.2 | 3.3 | 1.7 | 0 | *** |
| Error | 670 | 1324.1 | 2.0 | NA | NA | NA |

DF: Degrees of freedom; SS: Sum of squares; MSS: Mean sum of squares; F: F-value; Pr(>F): Probability value indicating significance level; Sig.: Significance level., *** indicates highly significant differences at $p < 0.001$; NS indicates non-significant.

**Table 3: Foraging Speed of *Apis mellifera* on Different Onion Cultivars across Observation Dates and Time Intervals**

| Cultivars | Mean | Date of observation | Mean | Time of observation | Mean |
|---|---|---|---|---|---|
| B. Super | 6.703[a] | 20/01/2024 | 1.669[h] | 8.00am | 4.215[e] |
| B. Red | 5.463[d] | 27/01/2024 | 2.518[g] | 10.00am | 6.008[c] |
| B. Raj | 5.178[e] | 3/2/2024 | 4.396[e] | 12.00am | 6.712[b] |
| B. Dark Red | 5.813[c] | 10/2/2024 | 7.03[c] | 14.00pm | 7.562[a] |
| B. Shakti | 6.421[b] | 17/2/2024 | 9.136[b] | 16.00pm | 4.862[d] |
| B. Shubhra | 4.765[f] | 24/2/2024 | 9.871[a] | 18.00pm | 3.829[f] |
| B. Shweta | 4.378[g] | 2/3/2024 | 5.904[d] | | |
| | | 9/3/2024 | 3.728[f] | | |
| SE(d) | 0.077 | | 0.082 | | 0.071 |
| SE(m) | 0.054 | | 0.058 | | 0.05 |
| CD | 0.151 | | 0.162 | | 0.14 |
| t value | 1.964 | | 1.964 | | 1.964 |

Values followed by different letters within each column indicate statistically significant differences at $p < 0.05$ according to the Critical Difference (CD) test., SE(d): Standard Error of difference between means., SE(m): Standard Error of the mean., CD: Critical Difference at the 5% significance level., t value: Tabulated t-value at $\alpha = 0.05$ used for comparison., B means Bhima

**Table 4: ANOVA and Interaction Effects of Onion Cultivars, Observation Date, and Time on the Foraging Speed of *Apis mellifera***

| | DF | SS | MSS | F | Pr(>F) | Sig. |
|---|---|---|---|---|---|---|
| Replication | 2 | 0.4 | 0.2 | 0.5 | 0.6321 | NS |
| Cultivars | 6 | 618.2 | 103.0 | 241.3 | 0 | *** |
| Date of observation | 7 | 7905.8 | 1129.4 | 2645.1 | 0 | *** |
| Time | 5 | 1818.4 | 363.7 | 851.8 | 0 | *** |
| Cultivars X Date of observation | 42 | 71.0 | 1.7 | 4.0 | 0 | *** |
| Cultivars X Time | 30 | 24.2 | 0.8 | 1.9 | 0.0031 | ** |
| Date of observation X Time of observation | 35 | 776.2 | 22.2 | 51.9 | 0 | *** |
| Cultivars X Date of observation X Time of observation | 210 | 75.2 | 0.4 | 0.8 | 0.9362 | NS |
| Error | 670 | 286.1 | 0.4 | NA | NA | NA |

DF: Degrees of freedom; SS: Sum of squares; MSS: Mean sum of squares; F: F-value; Pr(>F): Probability value indicating significance level; Sig.: Significance level., *** indicates highly significant differences at $p < 0.001$; NS indicates non-significant.

**Table 5: Biochemical Composition of Pollen from Different Onion Umbel Cultivars**

| Treatments | pH | EC (dS/m) | Moisture (%) | Protein (mg/1 g) | Phenol (mg GAE/1 g) | Flavonoid (mg QE/1 g) | Antioxidants (mg AA/1 g ) | Copper (mg/g or ppm) | Iron (mg/g or ppm) | Manganese (mg/g or ppm) | Zinc (mg/g or ppm) | Total Sugar (%) | Gluc (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T1 - 'BHIMA SUPER' | 6.80 | 0.79 | 12.80 | 391.80 | 30.47 | 855.30 | 3.60 | 0.10 | 0.32 | 0.28 | 0.58 | 26.30 | 17.3 |
| T2 - 'BHIMA RED' | 6.20 | 0.83 | 8.20 | 339.40 | 24.23 | 732.20 | 2.60 | 0.08 | 0.25 | 0.30 | 0.73 | 22.77 | 16.3 |
| T3 - "BHIMA RAJ" | 6.30 | 0.81 | 9.10 | 345.00 | 25.33 | 708.90 | 2.00 | 0.13 | 0.51 | 0.28 | 0.58 | 22.47 | 16.1 |
| T4 - 'BHIMA DARK RED' | 6.00 | 0.87 | 9.50 | 371.30 | 21.15 | 797.20 | 2.30 | 0.13 | 0.38 | 0.30 | 0.65 | 24.80 | 17.1 |
| T5 'BHIMA SHAKTI' | 6.70 | 0.87 | 12.00 | 377.70 | 30.89 | 826.20 | 3.60 | 0.10 | 0.52 | 0.37 | 0.75 | 28.03 | 19.4 |
| T6 - 'BHIMA SHUBHRA' | 5.90 | 0.78 | 8.90 | 205.60 | 22.83 | 709.50 | 1.70 | 0.09 | 0.17 | 0.26 | 0.66 | 19.50 | 15.2 |
| T7 - 'BHIMA SHWETA' | 5.60 | 0.78 | 8.00 | 249.10 | 12.14 | 670.40 | 1.50 | 0.08 | 0.18 | 0.16 | 0.53 | 20.57 | 13.4 |
| GM | 6.20 | 0.82 | 9.79 | 325.70 | 23.86 | 757.10 | 2.50 | 0.10 | 0.33 | 0.28 | 0.64 | 23.49 | 16.4 |
| SE(+) | 0.20 | 0.02 | 0.42 | 10.60 | 1.45 | 8.00 | 0.10 | 0.01 | 0.02 | 0.02 | 0.03 | 1.01 | 0.55 |
| CD @5% | 0.60 | 0.05 | 1.30 | 32.60 | 4.46 | 24.80 | 0.40 | 0.03 | 0.07 | 0.07 | 0.10 | 3.10 | 1.69 |
| CV (%) | 5.60 | 3.55 | 7.48 | 5.60 | 10.51 | 1.80 | 10.20 | 15.97 | 12.28 | 14.83 | 9.15 | 7.42 | 5.80 |

Potential of Hydrogen – Ph, Electrical Conductivity – EC, Moisture – Moist, Protein – Prot, Flavonoid – Fla, Antioxidant – Antiox, Phenol – Pheno, Iron – Fe, Copper – Cu, Manganese – Mn, Zinc – Zn, Total Sugar - Tot Sug, Glucose – Glu, Fructose – Fru, Sucrose – Suc, mg- microgram, g- gram, ppm- parts per million, %- percentage

**Table 6: Biochemical Composition of Nectar from Different Onion Umbel Cultivars**

| Treatments | pH | EC (dS/m) | Moisture (%) | Protein (mg/1 g) | Phenol (mg GAE/1 g) | Flavonoid (mg QE/1 g) | Antioxidants (mg AA/1 g ) | Copper (mg/g or ppm) | Iron (mg/g or ppm) | Manganese (mg/g or ppm) | Zinc (mg/g or ppm) | Total Sugar (%) | Gluc (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T1 - 'BHIMA SUPER' | 5.70 | 0.46 | 65.67 | 83.60 | 4.55 | 9.70 | 0.40 | 0.09 | 0.10 | 0.03 | 0.04 | 40.53 | 15.4 |
| T2 - 'BHIMA RED' | 5.70 | 0.52 | 49.67 | 41.00 | 3.96 | 8.20 | 0.30 | 0.08 | 0.08 | 0.04 | 0.03 | 35.00 | 11.2 |
| T3 - "BHIMA RAJ" | 4.20 | 0.51 | 54.67 | 79.20 | 3.43 | 8.10 | 0.30 | 0.07 | 0.13 | 0.04 | 0.02 | 36.53 | 12.0 |
| T4 - 'BHIMA DARK RED' | 5.30 | 0.56 | 56.00 | 74.00 | 3.43 | 9.00 | 0.30 | 0.06 | 0.13 | 0.05 | 0.03 | 38.13 | 12.9 |
| T5 'BHIMA SHAKTI' | 5.50 | 0.59 | 60.00 | 87.00 | 2.97 | 9.50 | 0.50 | 0.07 | 0.10 | 0.06 | 0.04 | 43.80 | 14.8 |
| T6 - 'BHIMA SHUBHRA' | 4.20 | 0.58 | 61.67 | 45.70 | 2.91 | 6.90 | 0.20 | 0.06 | 0.09 | 0.03 | 0.02 | 31.30 | 10.6 |
| T7 - 'BHIMA SHWETA' | 5.40 | 0.60 | 67.67 | 30.00 | 2.73 | 6.80 | 0.30 | 0.07 | 0.08 | 0.03 | 0.02 | 30.87 | 10.5 |
| GM | 5.10 | 0.55 | 59.33 | 62.90 | 3.42 | 8.30 | 0.30 | 0.07 | 0.10 | 0.04 | 0.03 | 36.60 | 12.5 |
| SE(+) | 0.30 | 0.02 | 0.52 | 3.40 | 0.18 | 0.50 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 1.97 | 1.05 |
| CD @5% | 0.80 | 0.07 | 1.60 | 10.40 | 0.54 | 1.40 | 0.10 | 0.01 | 0.03 | 0.01 | 0.01 | 6.06 | 3.24 |
| CV (%) | 8.70 | 6.85 | 1.52 | 9.30 | 8.94 | 9.70 | 18.00 | 10.97 | 15.97 | 13.49 | 22.80 | 9.30 | 14.5 |

Potential of Hydrogen – Ph, Electrical Conductivity – EC, Moisture – Moist, Protein – Prot, Flavonoid – Fla, Antioxidant – Antiox, Phenol – Pheno, Iron – Fe, Copper – Cu, Manganese – Mn, Zinc – Zn, Total Sugar - Tot Sug, Glucose – Glu, Fructose – Fru, Sucrose – Suc, mg- microgram, g- gram, ppm- parts per million, %- percentage

**Table 7: Pearson's Correlation Matrix Between Foraging Rate and Biochemical Traits of Onion Umbel Pollen (Upper Diagonal) and Nectar (Lower Diagonal)**

| Parameters | FR | pH | EC | Moist | Prot | Fla | Antiox | Pheno | Fe | Cu | Mn | Zn | Tot Sug | G |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FR | 1 | .514* | 0.335 | .759** | .705** | .887** | .757** | .623** | 0.308 | 0.304 | .746** | 0.073 | .630** | .66 |
| pH | .472* | 1 | 0.231 | .600** | .570** | .646** | .732** | .815** | .476* | .505* | .681** | 0.083 | 0.401 | .63 |
| EC | -0.39 | -0.113 | 1 | 0.199 | 0.379 | 0.267 | 0.343 | 0.318 | .517* | -0.16 | 0.433 | 0.193 | 0.153 | .65 |
| Moist | -0.002 | 0.052 | 0.109 | 1 | .584** | .833** | .839** | .683** | .445* | 0.299 | .729** | -0.09 | .638** | .62 |
| Prot | .682** | 0.045 | -0.302 | -0.088 | 1 | .766** | .723** | .556** | .687** | 0.377 | .793** | -0.23 | .623** | .62 |
| Fla | .719** | 0.2 | -0.432 | -0.103 | .701** | 1 | .868** | .677** | .456* | 0.26 | .865** | 0.036 | .691** | .71 |
| Antiox | .682** | .503* | -0.26 | 0.096 | .642** | .653** | 1 | .771** | .518* | 0.357 | .851** | 0.073 | .672** | .76 |
| Pheno | .596** | 0.306 | -.663** | -0.223 | 0.342 | .543* | 0.276 | 1 | .541* | 0.247 | .781** | 0.267 | .529* | .81 |
| Fe | 0.164 | -0.275 | -0.32 | -0.254 | .599** | 0.393 | 0.215 | 0.09 | 1 | -0.1 | .618** | -0.201 | .476* | .68 |
| Cu | 0.164 | -0.275 | -0.32 | -0.254 | .599** | 0.393 | 0.215 | 0.09 | 1.000** | 1 | 0.141 | -0.081 | 0.196 | 0.0 |
| Mn | .513* | 0.19 | 0.016 | -.482* | .573** | .465* | 0.421 | 0.163 | 0.125 | 0.125 | 1 | 0.003 | .694** | .86 |
| Zn | .578** | 0.398 | -0.274 | -0.129 | .583** | .705** | .571** | 0.355 | 0.253 | 0.253 | 0.405 | 1 | 0.151 | 0.2 |
| Tot Sug | 0.304 | -0.046 | -0.229 | -0.281 | .699** | 0.331 | 0.275 | .457* | .568** | .568** | 0.372 | 0.236 | 1 | .63 |
| Glu | 0.328 | -0.016 | -0.376 | -0.043 | .527* | 0.283 | 0.393 | .508* | 0.429 | 0.429 | 0.07 | 0.323 | .582** | 1 |
| Fru | 0.298 | 0.153 | -0.096 | -0.09 | 0.424 | 0.323 | 0.174 | 0.329 | 0.031 | 0.031 | 0.4 | 0.19 | .481* | 0.2 |
| Suc | 0.225 | -0.151 | -.454* | -0.063 | .477* | 0.383 | 0.28 | .447* | .596** | .596** | 0.048 | 0.375 | .605** | .67 |

*. Correlation is significant at the 0.05 level (2-tailed).snippi

**. Correlation is significant at the 0.01 level (2-tailed).

Potential of Hydrogen – Ph, Electrical Conductivity – EC, Moisture – Moist, Protein – Prot, Flavonoid – Fla, Antioxidant – Antiox, Phenol – Pheno, Iron – Fe, Copper – Cu, Manganese – Mn, Zinc – Zn, Total Sugar - Tot Sug, Glucose – Glu, Fructose – Fru, Sucrose - Suc

**Table 8: Descriptive Statistics of Foraging Rate in Relation to Pollen and Nectar Composition**

| Descriptive statistics | Pollen | | Nectar | |
|---|---|---|---|---|
| Parameter | Mean | Std. Deviation | Mean | Std. Deviation |
| FR | 17.3238 | 1.42123 | 17.3238 | 1.42123 |
| PH | 6.2095 | 0.51469 | 5.1300 | .72355 |
| EC | 0.8184 | 0.04917 | .5451 | .06306 |
| MOIST | 9.7859 | 1.87004 | 59.3334 | 6.08605 |
| PROTEIN | 32.5667 | 6.88726 | 62.9110 | 22.76031 |
| FLAVINOID | 757.1143 | 66.87984 | 8.3018 | 1.29585 |
| AO | 2.4571 | 0.82254 | .3218 | .10654 |
| PHENOL | 23.8667 | 6.64736 | 3.4248 | .65841 |
| IRON | 0.3314 | 0.14217 | .1023 | .02389 |
| COPPER | 0.0724 | 0.01261 | .1023 | .02389 |
| MG | 0.0432 | 0.01288 | .2777 | .06857 |
| ZN | 0.6406 | 0.09027 | .0271 | .00971 |
| TS | 37.7257 | 4.98837 | 36.5953 | 5.68225 |
| GL | 16.4262 | 2.01946 | 12.5286 | 2.42849 |
| FRUC | 20.0067 | 2.61336 | 20.8757 | 5.66538 |
| SUCR | 1.2914 | 0.3261 | 3.3558 | .75589 |

Potential of Hydrogen – Ph, Electrical Conductivity – EC, Moisture – Moist, Protein – Prot, Flavonoid – Fla, Antioxidant – Antiox, Phenol – Pheno, Iron – Fe, Copper – Cu, Manganese – Mn, Zinc – Zn, Total Sugar - Tot Sug, Glucose – Glu, Fructose – Fru, Sucrose - Suc

**Table 9: Rotated Component Matrix of Pollen and Nectar Biochemical Traits**

| % contribution of variables on PCs | POLLEN | | | | NECTAR | | | |
|---|---|---|---|---|---|---|---|---|
| **Variables** | **PC1** | **PC2** | **PC3** | **PC4** | **PC1** | **PC2** | **PC3** | **PC4** |
| **FR** | 7.031 | 2.005 | 2.203 | 13.368 | 6.833 | 7.76 | 0 | 0.111 |
| **pH** | 7.421 | 1.254 | 0.096 | 16.782 | 0.654 | 24.561 | 0.879 | 0.036 |
| **EC** | 2.746 | 28.198 | 5.247 | 0.388 | 5.815 | 0.412 | 20.427 | 4.95 |
| **Moisture** | 6.712 | 4.902 | 0.047 | 2.797 | 0.586 | 1.77 | 6.108 | 59.347 |
| **Protein** | 6.904 | 0.01 | 7.42 | 0.514 | 9.052 | 0.105 | 4.242 | 5.016 |
| **Flavonoid** | 7.428 | 1.079 | 0.613 | 16.874 | 9.223 | 4.579 | 1.322 | 0.08 |
| **Antioxidant** | 8.31 | 1.722 | 0.083 | 0.35 | 5.847 | 9.868 | 2.239 | 5.669 |
| **Phenol** | 6.852 | 0.003 | 5.792 | 16.167 | 5.397 | 0.989 | 17.413 | 9.826 |
| **Iron** | 4.148 | 10.445 | 15.202 | 12.278 | 4.57 | 11.775 | 7.77 | 1.057 |
| **Copper** | 1.8 | 28.417 | 0.184 | 11.851 | 4.57 | 11.775 | 7.77 | 1.057 |
| **Manganese** | 7.895 | 1.897 | 2.067 | 4.322 | 4.187 | 2.002 | 17.342 | 10.084 |
| **Zinc** | 0.01 | 11.434 | 56.055 | 1.492 | 6.612 | 11.576 | 3.092 | 0.04 |
| **Total. Sugar** | 8.317 | 1.588 | 1.454 | 0.052 | 8.819 | 5.498 | 0.145 | 0.049 |
| **Glucose** | 7.822 | 5.078 | 0.161 | 0.794 | 8.851 | 3.255 | 4.008 | 2.031 |
| **Fructose** | 8.15 | 0.023 | 3.119 | 1.893 | 9.49 | 2.055 | 3.604 | 0.317 |
| **Sucrose** | 8.455 | 1.945 | 0.257 | 0.078 | 9.493 | 2.018 | 3.639 | 0.33 |

FR- Foraging rate, **pH** – Potential of hydrogen, EC- Electrical Conductivity , PC1- Pinciple componenet 1, PC2- Principle componenet 2, PC3- Principle componenet 3, PC4- Principle componenet 4

## Figures

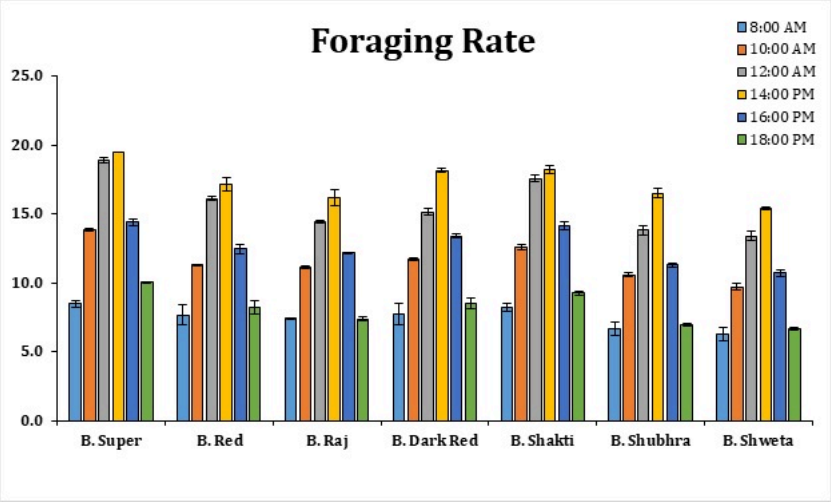


**Figure 1**

Foraging rate of *Apis mellifera* from seven different onion cultivars of onion seed production with six dates of observation

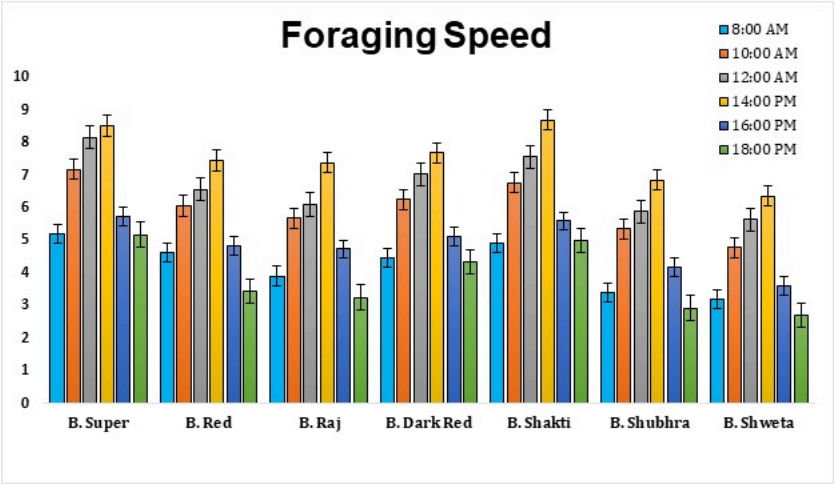


**Figure 2**

Foraging speed of *Apis mellifera* from seven different onion cultivars of onion seed production with six dates of observation

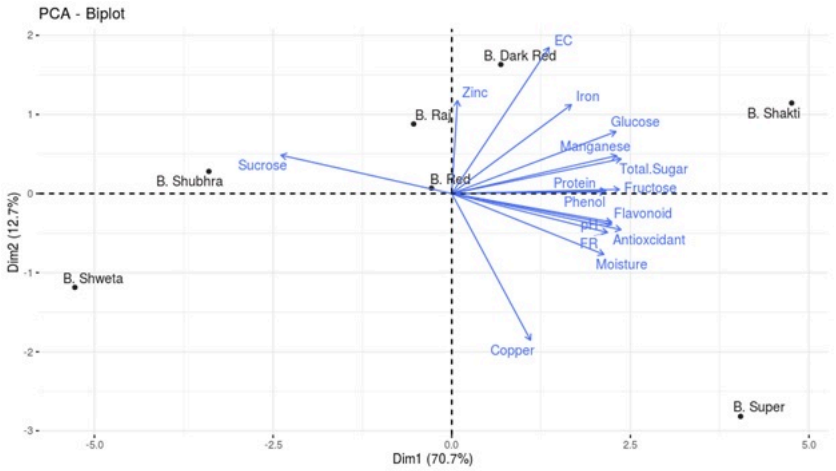


**Figure 3**

Principal component biplot presenting grouping of 7 onion cultivars Pollen and distribution of biochemical traits for foraging rate of *Apis mellifera*

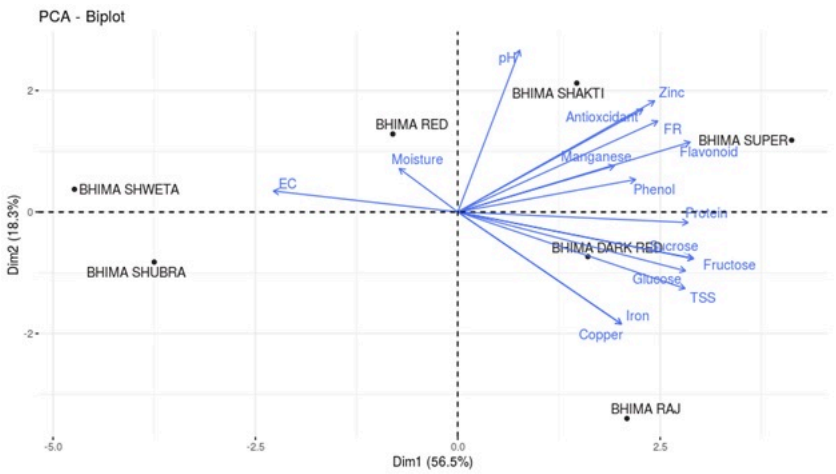


**Figure 4**

Principal component biplot presenting grouping of 7 onion cultivars Nectar and distribution of biochemical traits for foraging rate of *Apis mellifera*