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Impact of Flower Position and Pollinator Diversity on Yield Parameters of Pomegranate (*Punica granatum* L.)

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Abstract

The present study investigated the flower position characteristics of Pomegranate (variety: Bhagwa) pollinator diversity and their effect on fruit yield. The study covered floral biology, fruit attributes related to size, flower vigor, stigma receptivity, and aril development. The presence of both staminate (male) and hermaphrodite (bisexual) flowers in pomegranate allows it to be self- and cross-pollinated. The pomegranate inflorescence is a cyme. Flowers can appear solitary on spurs and in pairs or clusters terminally. The study revealed that the weight of pomegranate fruit resulting from the solitary flower was higher (351 g) with a greater number of arils (743 per fruit) than the fruit from the terminal flower (306.67 g and 620 arils) and lateral flower (221 g and 352 arils). Studies on modes of pollination provide evidence that qualitative and quantitative traits of fruits were improved by open and *Apis cerana* pollination. A total of 19 species of flower visitors were recorded. *Apis dorsata* was the primary flower visitor, followed by *A. florea* and *A. cerana*. The yield per plant under open conditions was 24.93 kg, which was on par with bee-pollinated (22.76 kg) and significantly higher than the control (15.36 kg).

Introduction

Pomegranate (*Punica granatum* L., Punicaceae) is an important fruit crop in tropical and subtropical regions. It is an economically important fruit crop cultivated for its wholesome fruit and juice. In recent years, it has become an essential export-oriented crop in India, where it is cultivated over an area of 1.20 lakh ha, with a yearly output of 7.5 lakh tons and a productivity of about 6.60 tons per ha. The state of Maharashtra is the largest producer in India. Flowering occurs about one month after bud break on newly developed branches of the same year, generally on spurs or short branches. Flowers can be solitary, in pairs, or in clusters. Usually, the solitary flowers appear on spurs along the branches, while the clusters are terminal (Varun et al., 2020). Some earlier studies (Wetzstein et al., 2013) have shown that flower size characteristics and ovule development can be quite variable and are related to flower type and position. Single and terminal flowers within a cluster were larger than lateral flowers. In addition, lateral flowers exhibited a high frequency of flowers with poor ovule development, sufficient to impact fruiting in that flower type negatively. However, there are hardly any such studies on Indian cultivars. Thus, the description of floral position characteristics and how they vary with fruit sizes in the variety 'Bhagwa' was the focus of this study.

Pomegranate is andro-monoecious, where staminate and hermaphrodite flowers develop on the same plant. Hermaphrodite (vase shape) and staminate flowers (bell shape) are easily recognizable. Flowers have an attractive red calyx and corolla with about 200–350 anthers per staminate flower and hermaphrodite flowers with 400–1,000 ovules (Derin & Eti, 2001). The stigma of the hermaphrodite flower is at the height of anthers or slightly above them (Wetzstein et al., 2013). This position allows for self-pollination as well as pollination by insects. A study has shown that fruit sets increase with open and cross-pollination (Derin & Eti, 2001).

Though the primary pollinators are bees, it was reported that wind could also accomplish it. Several studies on emasculation and pollination of different cultivars of pomegranate in India, Turkmenistan, and Tunisia have shown that the fruit is produced by self-pollination naturally (Nalawadi et al., 1973; Levin, 2006).

Nevertheless, the fruit set varies by self-pollination among different pomegranate cultivars. A greater percentage of fruit set was observed by hand pollination and pollination under natural conditions, i.e., open pollination. However, there is a lack of information on supplementary pollination using *Apis cerana* in pomegranate (Nalawadi et al., 1973). Due to the need to fulfill export market standards, the fruit has to be of a specific size, which is related to pollination success and, consequently, to seed number (Derin & Eti, 2001). In addition, sometimes problems may appear in fertilization and fruit set between different flower types of a cultivar or between flower types of different cultivars (Gozlekci & Kaynak, 1998)

The present study aims to understand the effects of pollination methods and flower position on fruit set in a popular cultivated pomegranate, Bhagwa.

Materials and Methods

Study area

The present study was conducted at three locations: a) University of Agricultural Sciences, GKVK Campus, Bangalore, India (12.97° N – 77.59° E; 924 m.a.s.l.), b) Melur village of Shidlaghatta Taluk, Chikkaballapur district, Karnataka (13.20° N – 77.49° E, 903.44 m.a.s.l c) Devanahalli, Bangalore Rural district, Karnataka, India (13.17° N – 77.45° E, 910.61 m.a.s.l) from 2019-2022. These three locations come under the eastern dry zone of Karnataka, India, which is dominant with red soil and hot, humid, semiarid climatic conditions with annual rainfall of 680- 890 mm. Major crops like Ragi (Finger millet), maize, pulses, mulberry, groundnut, fruits, vegetables, and flowers crops enhance the diversity of pollinators in this zone.

The observations were made on the 'Bhagwa' variety of pomegranate by following the recommended package of agronomical practices.

Pollinators' species diversity and richness were studied in all three locations in 2019. In contrast, other parameters like floral biology in 2020, foraging activity and fruit yield based on flower position in 2021, and modes of pollination in 2022 were recorded in Melur village of Shidlaghatta Taluk, Chikkaballapur district.

1. Species richness, diversity, and abundance of flower visitors

Species richness, diversity, and abundance of flower visitors on pomegranate were carried out in all three locations during April-June 2019. The flower visitors of pomegranates were observed at 10:00h, 13:00h, and 16:00h for three days in each location. Data was collected on species using the visual count method from 3 different locations *viz*., University of Agricultural Sciences GKVK Campus Bangalore, Melur village of Shidlaghatta Taluk, Chikkaballapur district, Karnataka and Devanahalli, Bangalore Rural district, Karnataka, India. The data was used to work out the Shannon-Weiner diversity, H' index, which is a measure of diversity that combines species richness (the number of species in a given area) and their relative abundance.

$$\text{Shannon-Weiner Diversity Index } H' = -\Sigma (\ln pi \times pi)$$

Where pi is the proportion of the i^{th} species of pollinator, ln is the natural log with base e.

The species richness of flower visitors in the study area was calculated by the number of species recorded during the study period.

Floral Biology

Flower development stages were recorded in the early-to-mid season blooming period of the crop from April to June 2020 in Melur village of Shidlaghatta taluk (13° 20' N – 77° 49' E) at 903.44 m.a.s.l.

a. Flower structure

Ten newly opened staminate and hermaphrodite flowers were collected from selected plants within the same orchard block. Flowers (n = 10) were immediately brought to the laboratory, individual flowers numbered, and observations were recorded on the number of sepals, petals, stamens, pollen grains per anther/flower and ovules; length of sepal, petals, stamens, pistil, flower, stigma, style, and stylopodium; length from the base of flowers to the sepal notch; length from the base of flowers to the tip of sepals; length of the stigma and style; and stigma diameter. The basal portion of flowers containing the ovary and ovules was dissected for later evaluations of ovule numbers.

b. Anthesis, anther dehiscence, and flower longevity

Two sets of 50 flower buds, one for hermaphrodite and another for staminate flowers, were bagged one day before the observation. The flower blooming time was observed from 06:00 to 18:00h the following day. The number of opened flowers from both types was noted at hourly intervals. The time of opening of a maximum number of flowers was recorded.

For anther dehiscence, 50 flower buds from each hermaphrodite and staminate flower were selected, and the time of anther dehiscence was observed in each flower from 06:00 to 18:00h of the day. The anther dehiscence was observed by pollen being sprinkled or sprayed as yellow dust around the anthers at every one-hour interval.

Ten flowers from each type were tagged to record the longevity of individual flowers and observed for the length of time that a flower remained open to be functional and then closed.

c. Qualitative and quantitative estimation of nectar

The total quantity of nectar produced in a flower was measured by closing a set of flowers using brown papers before it opened to prevent flower visitors. Nectar was collected from 5 flowers three times a day (09:00, 13:00, and 16:00h) on both staminate and hermaphrodite flowers using a micro-syringe, and the quantity was measured in micro lt.

The nectar quality was determined using a hand refractometer, and TSS was recorded. Sugar concentration in nectar was measured thrice daily (09:00, 13:00, and 16:00h) on staminate and hermaphrodite flowers.

d. Stigma receptivity

The receptivity of the stigma was recorded by visual observation of the stigmatic surfacer. Flowers 0, 1, 2, 3, and 4 days old were selected for the stigma receptivity test. One drop of three percent hydrogen peroxide (H_2O_2) solution was placed on the stigmatic surface. The production of bubbles with fumes in contact with the stigmata indicates receptivity (Kearns & Inouye, 1993).

e. Pollen viability and germination

The viability of the pollen grains from groups of flowers of different ages, like one, two- and three-day-old flowers, was tested at 09:00h, 13:00h, and 17:00h using a two percent acetocarmine solution. Pollen grain germination test was done by dusting pollen grains with agar + sucrose method using 15% sucrose and 25 ppm boric acid with 1% agar (Engin & Hepaksoy, 2003). The pollen germination was recorded 24 hours after pollen plating.

f. Scanning Electron Microscopy (SEM) Analysis

Flowers were collected from mature pomegranate trees before anthesis with cotton tissue bags to avoid contamination from the other pollen sources. The stamens were rubbed out of the flowers through fine screens and dried for 6 hours to allow pollen release. Pollen was collected in small vials. Before SEM examination, the samples were dried at room temperature for 4-6 hours. The study was conducted in the Department of Plant Pathology, Indian Institute of Horticulture Research, Bangalore. For the SEM study, samples were mounted directly on metallic stubs using double-sided adhesive tape and coated with gold in a sputtering chamber. The prepared samples were observed with a scanning electron microscope (SEM) at 15 kV (Arzani et al., 2005) and photographed at 3,000x for the whole grain.

Foraging activity

The foraging activity of bee species on pomegranate was recorded in Melur village of Shidlagatta Taluk, Chikkaballapura district, in 2021. Flowers were selected randomly from selected plants in the pomegranate field. Ten flowers were selected every hour, and each flower was observed for five minutes. Observations were recorded by counting the pollinator visits of three major bee species, *Apis dorsata*, *Apis florea,* and *Apis cerana,* for five minutes per flower, starting from 09:00h to 18:00h for every hour in a day for five days.

Fruit yield based on flower position

Fruit yield based on flower position with open pollination was conducted in Melur village of Shidlagatta Taluk, Chikkaballapura district, in 2021. Under open pollination conditions, randomly selected tagged flowers were made to assess the effects of flower position on subsequent fruit size and yield. Studies were made using fruits produced from single-flower, terminal flowers within a flower cluster, and lateral flowers within a flower cluster that had opened on the same day (Plate 1). Ten flowers for each type were tagged, and fruit yield for each flower was assessed later. The fruit set from each tagged flower was harvested at maturity, corresponding to standard commercial maturity criteria and harvest time. Quantitative and qualitative yield parameters such as fruit weight (g), moisture content in the fruit (%), Ash (g) rind thickness (cm), diameter of the fruit (cm), test weight (g), and total number of seeds/ fruits were measured.

Modes of pollination

A study to assess the effect of different modes of pollination in Pomegranate yield having in its experimental design the open pollination, bee pollination, and control treatments was conducted in a 10 m x 10 m plot (10 Plants) per treatment in Melur village of Shidlagatta Taluk, Chikkaballapura district, in 2022. Under open pollination, plants were kept open for pollinators to access. A similar plant set was prevented from pollinator visitation by erecting nylon nets as control. However, another set of plants was caged in a 40-mesh nylon net with a nucleus bee hive of *A. cerana* to evaluate bee pollination. The observations were recorded on fruit weight, diameter, rind thickness, test weight of seeds, the total number of arils/fruits, moisture content, Ash, yield per plant, and yield per hectare.

Statistical analysis

The collected data were statistically analyzed by using the statistical software SPSS version 24. Data collected on species diversity were worked out using the Shannon-Wiener index. Fruit yield parameters were analyzed using Analysis of Variance (ANOVA) and compared using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

Results

Floral biology

Pomegranate produces large, showy flowers with delicate petals that can be vivid orange-red to red. Two types of flowers, staminate and hermaphrodite, were observed for floral characteristics (Table 1). The number of petals and sepals is

six. The number of stamens in staminate and hermaphrodite flowers was 221.3 ± 75.31 and 190.7 ± 27.20, respectively, and are composed of yellow anthers each containing 62500 ± 4949.74 and 53000 ± 7071.06 pollen grains, attached to long red filaments. The number of pollen grains per staminate flower was 138.31 ± 10.95 lakhs, whereas 109.94 ± 26.03 lakhs per hermaphrodite flower. The red calyx is thick and leathery, fused at the base, and terminates in triangular lobes that persist and form the characteristic crown of the mature fruit. In contrast to the large number of stamens, hermaphrodite flowers contain a single elongate pistil of length 2.79 ± 0.14 cm terminated by a stigma and with an inferior ovary containing 1403 ± 149.34 ovules.

Table 1. Floral characteristics of pomegranate flower.

Parameters	Staminate Flowers (n = 10)	Hermaphrodite Flowers (n = 10)
No. of Sepals	6	6
No. of Petals	6	6
No. of stamens	221.3 ± 75.31	190.7 ± 27.20
Pollen grains/anther	62500 ± 4949.74	53000 ± 7071.06
Pollen grains/flower (in lakhs)	138.31 ± 10.95	109.94 ± 26.03
No. of ovules	-	1403 ± 149.34
Length of sepal (cm)	2.34 ± 0.32	3.55 ± 0.60
Length of petal (cm)	2.38 ± 0.17	2.45 ± 0.37
Length of stamens (cm)	0.72 ± 0.09	0.64 ± 0.17
Length of pistil (cm)	1.19 ± 0.29	2.79 ± 0.14
Length of flower (cm)	3.71 ± 0.24	4.89 ± 0.57
Base to sepal Notch (cm)	1.51 ± 0.60	2.68 ± 0.44
Base to tip of sepal (cm)	2.47 ± 0.46	3.8 ± 0.86
Stigma + Style length (cm)	-	1.39 ± 0.19
Stigma diameter (cm)	-	0.15 ± 0.05
Stigma+Style+ Stylopodium (cm)	-	1.37 ± 0.16

Time of anthesis and dehiscence

Anthesis of both staminate and hermaphrodite flowers commences around 08:00h. It continued till 16:00h in staminate flowers while the hermaphrodite flowers continued anthesis till 17:00h (Fig 1) with peak anthesis between 12:00 to 13:00 h in staminate (n = 14) and 13:00 to 14:00 h in hermaphrodite flower (n = 12). The time of anther dehiscence varied from 08:00 to 14:00h in staminate flowers, and 08:00 to 15:00h in hermaphrodite flowers (Fig 2). Anther dehiscence was maximum between 11:00 to 12:00 h in both types of flowers.

Flower longevity

The hermaphrodite flower remained open and functional for up to four days, whereas the staminate flower lasted for three days (Fig 3).

Nectar quantity and TSS content in nectar

Both staminate and hermaphrodite flowers offered nectar to flower visitors, and the nectar was available throughout the day. The quantity of nectar recorded between 09:00 to 10:00h in the staminate flower (12.50 µL) was slightly higher than that in the hermaphrodite flower (8.90 µL), and it declined with time by evening hours. Total soluble sugar content ranged from 27 - 31% in staminate flowers, whereas in hermaphrodite flowers, it was 25 - 30% throughout the day (Table 2).

Stigma receptivity

Stigma was receptive from the day before anthesis until the fourth day after anthesis (Table 3).

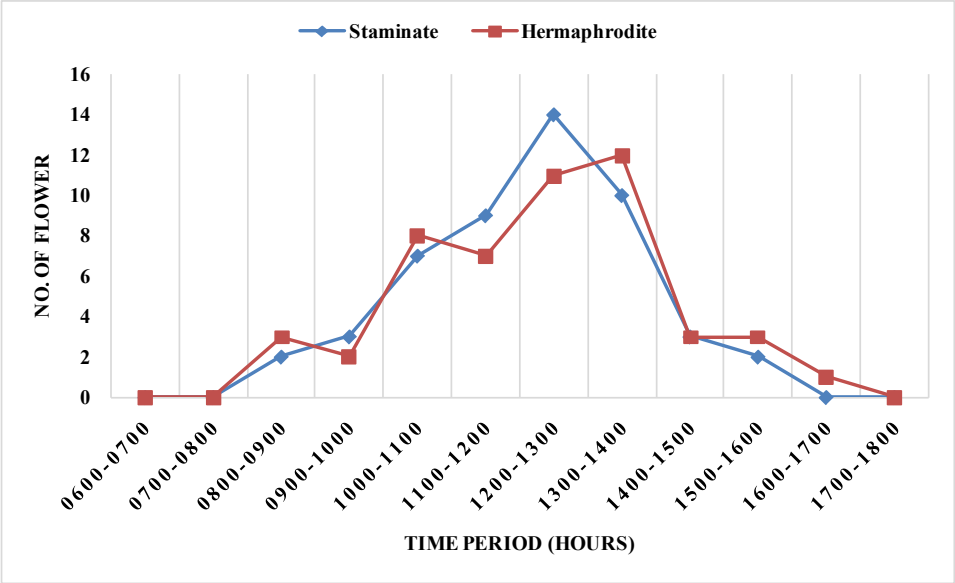

Fig 1. Time and rate of anthesis in pomegranate flower.

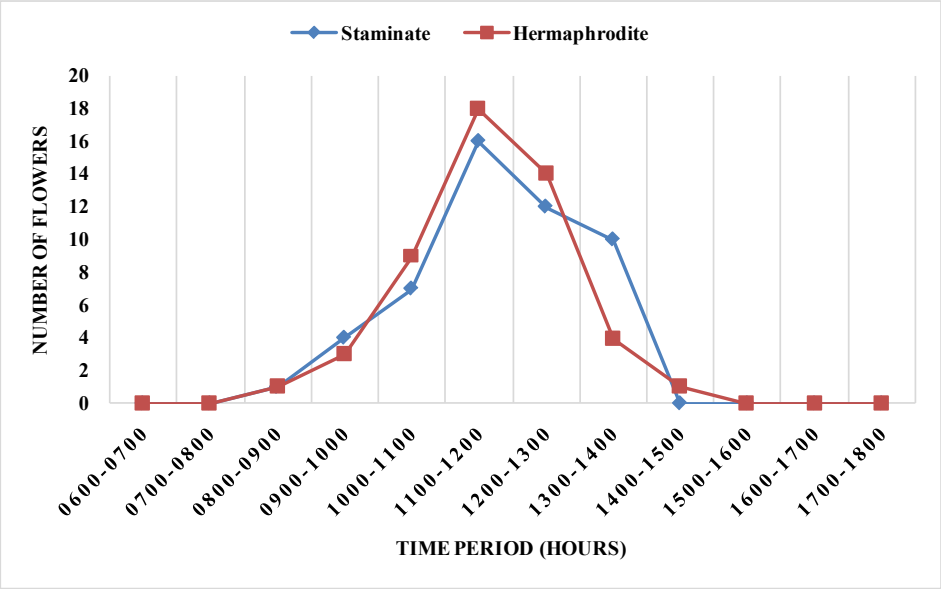

Fig 2. Time and rate of anther dehiscence in pomegranate flower.

Pollen viability and pollen tube germination

In staminate flowers, maximum pollen viability (95.13%) was observed on the day of anther dehiscence, followed by 93.22 % on day 2 and 73.03% on day 3. In hermaphrodite flowers, the pollen viability was low on the first day (64.81%). However, 95.94% of pollen grains were viable on the second day, followed by 70.21% on day 3. The maximum pollen viability was observed in the morning hours of the day in both types of flowers (Fig 4; Plate 3)

The length of the pollen tube (Plate 4) was 620.27 µm (n = 20) in pollen grains from the staminate flower, while it was relatively shorter in the case of those from the hermaphrodite flower (577.64 µm) (n = 20).

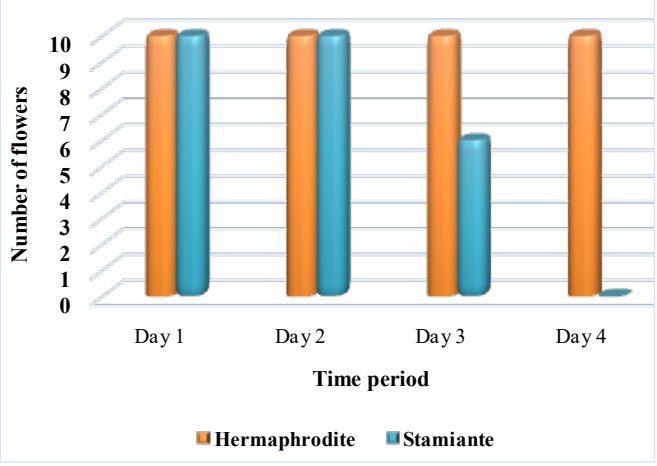

Fig 3. Longevity of pomegranate flower.

Table 2. Nectar quantity and TSS content in nectar.

Time period	Nectar quantity (µl)		TSS (%)	
	Staminate	Hermaphrodite	Staminate	Hermaphrodite
9.00-10.00	12.50	8.90	27.00	25.00
13.00-14.00	4.00	2.90	27.00	27.00
16.00-17.00	2.20	3.10	31.00	30.00
Total	**18.70**	**14.90**	-	-

Table 3. Stigma receptivity test.

Days	Showing the fumes when hydrogen peroxide dropped on the stigma at different times			
	1000h	1200h	1400h	1600h
0	√	√	√	√
1	√	√	√	√
2	√	√	√	√
3	√	√	√	√
4	√	√	√	√

Photomicrographs of pollen grains were made from freshly prepared slides using Biovis image plus Digital Imaging System (Plate 5). The pollen morphology of the studied pomegranate cultivar was prolate (elliptical).

Species richness, diversity, and relative abundance of flower visitors

We recorded 19 species of five insect orders visiting the flowers of pomegranate (Fig 5). The majority of them were

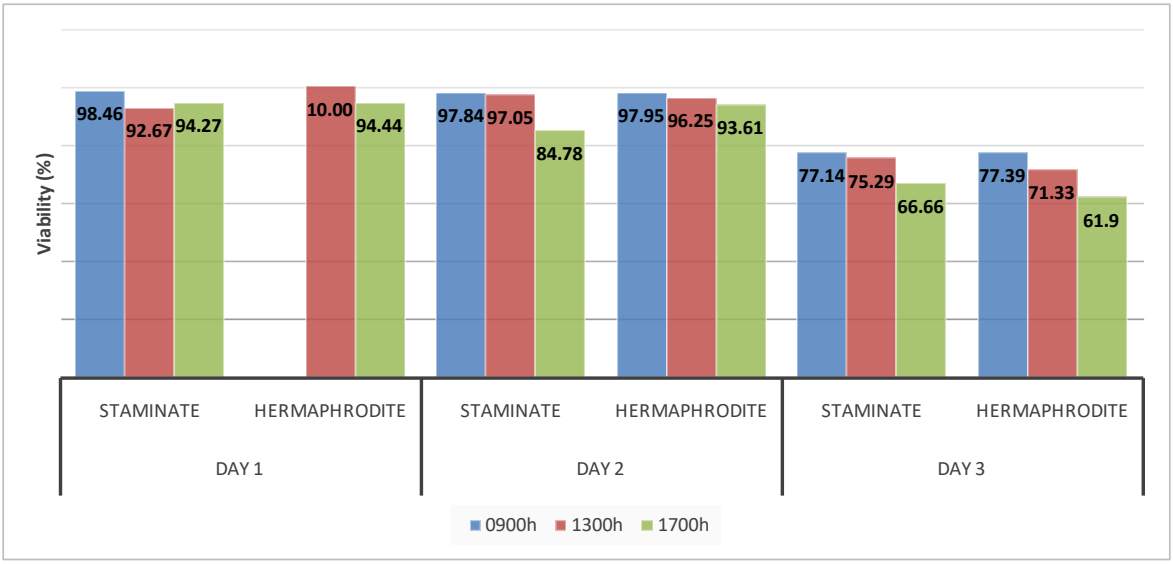

Fig 4. Pollen viability in Pomegranate flower.

hymenopterans (63%), of which most were bees. In terms of numbers, *Apis dorsata* Fab. was the highest (74 bees/10 min/5 plants), followed by *Apis florea* Fab. (67 bees) and *Apis cerana* Fab. (27 bees) (Table 4). Among non-*Apis* bee species, *Tetragonula iridipennis* Smith, *Amegilla zonata* (Linnaeus), *Ceratina binghami* Cockerell, *Xylocopa fenestrata* Fab., *Thyreus* sp. *Megachile lanata* Fab., *Hoplonomia westwoodi* (Gribodo), and *Lasioglossum* sp. were common visitors of pomegranate flowers. Among the non-bee hymenopterans, Formicidae (*Formica* spp.) were found to visit a large proportion of crops. In addition, four species of lepidopterans, one species each of the orders Thysanoptera, Odonata, and Coleoptera, were observed as flower visitors in pomegranate. (Plate 6). Diversity of flower visitors was found to be higher in Devanahalli (H' = 2.243), followed by Melur Shidlagatta (H' = 2.127) and GKVK Bangalore (H' = 1.868) (Table 5).

Foraging behavior of bees

The peak activity of *A. dorsata* was observed between 10:00-11:00h (3.00 bees/plant/5 min) and the second peak at 17:00h. The frequency of visitation was 3.00 bees/plant/5 min. At the same time, it ranged from 0.33-1.00 bees/plant/5 min in the case of *A.cerana,* with the peak activity at 13:00h (Fig 6).

Table 4. List of flower visitors in pomegranate at different locations during the study.

Sl. No	Species names	Family	Order
1	*Apis cerana* Fab.	Apidae	Hymenoptera
2	*Apis dorsata* Fab.		
3	*Apis florea* Fab.		
4	*Tetragonula iridipennis* Smith		
5	*Amegilla zonata* (Linnaeus)		
6	*Ceratina binghami* Cockerell		
7	*Xylocopa fenestrata* Fab.		
8	*Thyreus* sp.		
9	*Megachile lanata* Fab.	Megachilidae	
10	*Hoplonomia westwoodi* (Gribodo)	Halictidae	
11	*Lasioglossum* sp.		
12	*Formica* spp.	Formicidae	
13	*Scirtothrips dorsalis* Hood	Thripidae	Thysanoptera
14	*Pieris brassicae* (Linnaeus)	Pieridae	Lepidoptera
15	*Eurema* sp		
16	*Cepora Nerissa* (Fabricius)		
17	*Acraea terpsicore* Fab.	Nymphalidae	
18	*Coccinella* sp.	Coccinellidae	Coleoptera
19	*Anax indicus* Lieftinck	Libellulidae	Odonata

Table 5. Diversity index of floral visitors in Pomegranate at different locations.

Locations	Total number of floral visitors (10 min/5 plants)	Species richness (S)	Shannon Weiner diversity index (H')
GKVK, Bangalore	63	10	1.86893
Devanahalli	136	13	2.24334
Melur, Shidlaghatta	242	19	2.12776

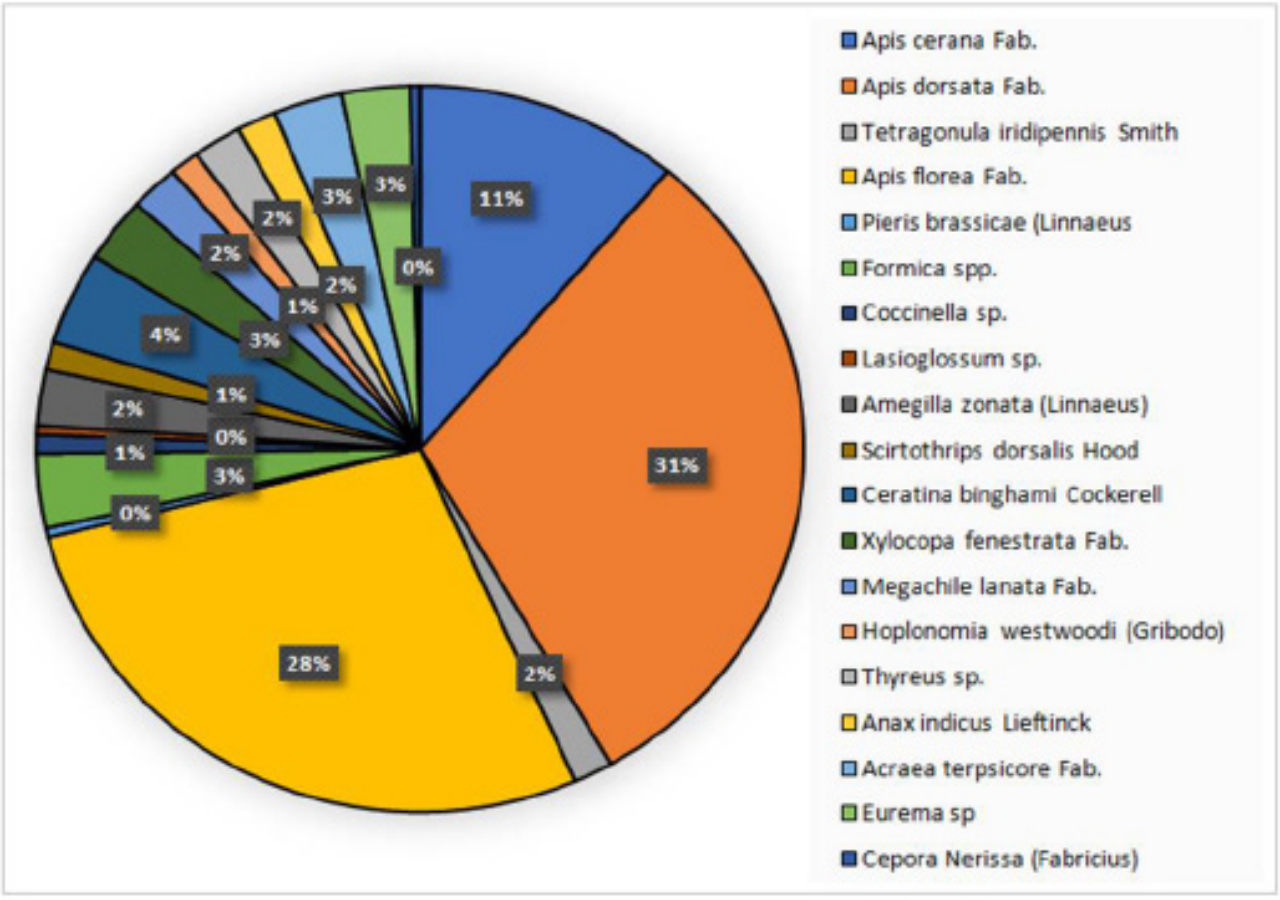

Fig 5. Relative abundance of flower visitors in pomegranate**.**

The activity of *A. florea* foragers commenced after 1000h with a lower frequency of visitation (3.00 bees/plant/5 min). The highest frequency of visitation by *A. florea* was recorded between 13:00-14:00h (4.67 bees/plant/5 min), followed by *A. dorsata* in pomegranate variety Bhagwa (Fig 6).

Fruit yield based on flower position

The quantitative attributes of fruit exhibited a wide range of variations concerning the position of the flower (Table 6). Fruit weight was found to be high in those developed from single flowers (351 g) followed by terminal flowers (306.67 g), and the least weight was recorded in fruits from lateral flowers (221 g). Further, the diameter of the fruit was directly correlated with the fruit weight and was higher in the fruits from single flowers (8.97 cm), followed by fruits from terminal and lateral flowers. The number of seeds in the fruit showed significant differences and linear correlation with fruit weight. A greater number of seeds was recorded in fruits from single flowers (743 seeds), followed by terminal (620 seeds) and lateral flowers (352 seeds). In contrast, there was no significant difference in moisture content (78.96–79.47%), ash content (0.06 g), and rind thickness (0.42 cm) of the fruit among the fruits obtained from different positioned flowers.

Modes of pollination

Compared to self-pollination, better quantitative and qualitative traits were observed in fruits by open and bee pollination (Table 7). Average fruit weight (311.67 g, $P = 0.0004$), rind thickness (0.42 cm, $P = 0.025$), fruit diameter (8 cm, $P = 0.003$), test weight (31.46 g, $P = 0.008$), and arils per fruit (620, $P = 0.00000003$) were higher in open-pollinated conditions. Fruits obtained from bee (*A. cerana*) pollination were heavier (284.5 g, $P < 0.05$), larger (7.87 cm, $P < 0.05$), test weight (29.83 g, $P < 0.05$), and aril per fruit (485.33 $P < 0.05$) than those from self-pollinated plants.

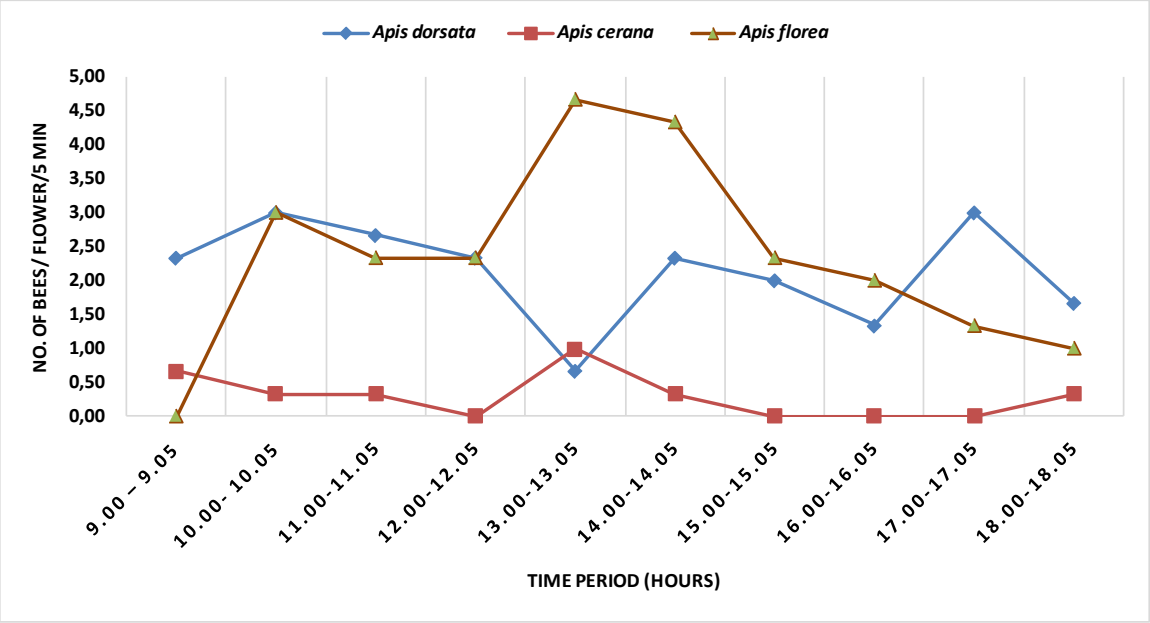

Fig 6. Foraging activity of bee species on pomegranate flower.

There was no significant difference in fruits' moisture and ash content among different pollination modes. (*P > 0.05)*. The total yield per plant was maximum in open-pollinated conditions (24.93 kg), which was on par with bee-pollinated (22.76 kg). Self-pollination showed a lower yield per plant (15.36 kg).

Discussion

The pomegranate plant characteristically produces two types of flowers – hermaphrodite and functionally male flower types (Shulman et al., 1984; Wetzstein et al., 2011a). Bisexual flowers can appear solitary, in pairs, or clusters.

Table 6. Yield attributes of pomegranate fruit based on flower position.

Parameter	Fruit weight (g)	Moisture content (%)	Ash (g)	Rind thickness (cm)	Diameter (cm)	Total number of arils/fruit
F1	351.00[a]	79.39	0.06	0.40	8.97[a]	743.00[a]
F2	306.67[ab]	78.96	0.06	0.42	8.00[b]	620.00[b]
F3	221.00[b]	79.47	0.06	0.40	7.70[b]	352.00[c]
P- value	0.0098*	0.3283	0.2294	0.6232	0.0290*	0.00000095*
SEM ±	19.94	0.24	0.002	0.012	0.25	16.99
CD (0.05)	68.99	0.82	0.0056	0.04	0.88	58.81
CV (%)	11.79	0.52	4.77	5.24	5.36	5.15

F1 - Single flower F2 - Terminal flower within a flower cluster F3 - Lateral flowers within a flower cluster.
*p value < 0.05, samples are significantly different. Treatment means followed by the same alphabets are statistically on par with each other.

Table 7. Effect of modes of pollination on yield parameters of Pomegranate.

Parameter	Fruit weight (g)	Moisture content (%)	Ash (g)	Rind thickness (cm)	Diameter (cm)	Test Weight (g)	Total number of arils/fruit	Yield per plant (kg)	Yield per hectare (tons)
Open pollination	311.67[a]	78.77	0.06	0.42[a]	8.00[a]	31.46[a]	620.00[a]	24.93[a]	20.77[a]
Caged (Control)	192.03[b]	78.67	0.05	0.30[b]	7.27[b]	28.07[b]	312.00[c]	15.36[b]	12.80[b]
Caged with *Apis cerana* (Bee pollination)	284.50[ab]	79.00	0.05	0.37[ab]	7.87[a]	29.83[ab]	485.33[b]	22.76[ab]	18.96[ab]
P-value	0.0004*	0.084	0.3583	0.025*	0.003*	0.0080*	0.00000003*	0.0004*	0.0004*
SEM ±	10.19	0.33	0.0025	0.02	0.09	0.49	7.60	0.81	0.68
CD (0.05)	35.25	1.14	0.0089	0.075	0.32	1.70	26.31	2.82	2.35

*p-value < 0.05, samples are significantly differed. Treatment means followed by the same alphabets are statistically on par with each other.

In most cases, the solitary flowers appear on spurs along the branches, while the clusters are terminal (Varun et al., 2020). The three bisexual flower types show marked differences in floral characteristics evaluated in earlier studies (Wetzstein et al., 2011). The current study attempted to verify the influence of these flower positions on fruit production and fruit size and the ultimate impact on the yield of Pomegranate in the Bhagawa variety.

The flowers of the variety Bhagawa have six sepals and six petals. The multiple long stamens (0.72 cm) are inserted into the calyx walls in a circle and frequently have 190 to 221 stamens per flower. The hermaphrodite flower is significantly longer and wider than the male flower. This results in a much larger ovary and a longer pistil. The length of the hermaphrodite flower in the variety 'Beautiful' was more (3.6 cm) than the male flower (2.7 cm) (Wetzstein et al., 2011), and similar observations were recorded with the variety Bhagawa in the present study. Both male and hermaphrodite types of pomegranate flowers remained open and functional for three to four days. The length of time a flower is functional may be a critical determinant of male and female reproductive success (Steinacher & Wagner, 2010).

The time of anthesis depends on several environmental factors and the variety grown. In variety 'Ganesh' peak period of anthesis 10:00h to 12:00h (Babu et al., 2009a), the peak anthesis period was observed between 12:00h to 13:00h for variety Bhagwa in the present study. Interestingly, the same variety showed a peak period of anthesis between 10:00h and 12:00h, as reported by (Babu et al., 2009 b), indicating that the same cultivars' floral biology varies according to environmental factors. The optimum temperature for opening flower buds varies between cultivars. It is stated that the final function of the anther that causes the release of pollen grains is that endothecum tissue is responsible for the splitting of the anther. Anther dehiscence will begin a few hours before the anthesis or much later, depending on the cultivar. This is done between 7 and 15 hours later, or it can take 24 hours (Gur, 1986). Anther dehiscence was seen immediately after the anthesis in Bangalore condition. Humidity and elevated temperature can somewhat accelerate dehiscence (Josan et al., 1979).

Anthesis and stigma receptivity in Pomegranate were studied by Josan et al. (1979), who reported that the time taken by flowers to complete anthesis was 3 to 5 hours. The stigma became receptive one day before anthesis and remained receptive up to the second day after anthesis. The pollen grains showed viability in both types of flowers for all three days. However, the viability of pollen was absent in hermaphrodites during morning hours on its 1st day of opening later once it gained its viability, and the same existed till the close of the flower (Sharma et al., 2022). Bhalachandra (2014) recorded nectar and pollen-providing bee flora in the Western ghat area of Nasik, Maharashtra. Nectaries in the flower are located between the stamens and the ovary base (Fahan, 1976). About 18.70 µL of nectar is produced in pomegranate flowers, with TSS content ranging from 25 to 30% throughout the day. Hence, the visiting pollinators get a reward for pollen and nectar together, facilitating higher visits from bees.

A total of nineteen flower visitors were recorded on the Pomegranate flowers, with the highest contribution from honey bees and other hymenopterans, as Derin and Eti (2001) and Chavhan et al. (2023) reported. Among different species of bee pollinators, *A. dorsata* was the most dominant with the highest relative abundance (31%), followed by *A. florea* (28%), *A. cerana* (11%), and *Tetragonula iridipennis* (2%), were discovered to be the primary pollinators in pomegranate while, lepidopterans were frequent visits but are poor pollinators. *P. granatum* produces more pollen than nectar, and the pollen plants are essential for bee colonies, especially during colony build-up, when bees need large amounts of protein for brood rearing. Therefore, bees are found to be efficient pollinators. The present study follows Partap (1997) where, in addition to the honey bees (*Apis cerana* and *A. mellifera*), Pomegranate is also visited by other insects like butterflies, moths, and beetles, which are not the major pollinator species.

The effect of the flower position on yield parameters in three hermaphrodite flower types (single, terminal, lateral) was evaluated. Fruit obtained from a single flower exhibited significantly higher mean fruit weight than the other two types. Hence, the position of the flower makes a significant impact on the yield of pomegranate. Single and terminal flowers within a cluster were larger than lateral flowers. In addition, lateral flowers exhibited a high frequency of flowers with poor ovule development sufficient to negatively impact fruiting in that flower type (Wetzstein et al., 2011). Flower size and position had marked effects on the size of the set fruit. Flower type and position strongly affected flower quality. Almost half of the lateral flowers had abortive ovules on dissection and were thus incapable of setting fruit (Wetzstein et al., 2013). Flower size and position have been shown to influence production in other fruit crops. In apples, king flowers (also the terminal flower in a cluster) consistently have higher fruit sets than lateral flowers, which has been suggested to occur because king flowers form earlier, are larger, and have better vascular connections than lateral flowers (Dennis, 1986). King flowers are reported to have greater sink strength and greater numbers of cells (Westwood et al., 1967). Early flowers were proposed to have advantages in embryo development, sink establishment, and resource capture. In pomegranate, lateral flower development is delayed compared with terminal flowers. Flower position may potentially be a factor influencing fruit weight and ultimate yield in pomegranate. Therefore, a recommendation in the cultivation practices of Pomegranate must include the retention of single and terminal flowers rather than lateral flowers to get a higher yield.

The present study also attempted to evaluate different pollination modes for a single flower type (based on position) in Pomegranate. Previous studies by Karimi and Mirdehghan (2015) clearly indicate that open and supplementary pollination improved fruit and arils' quantitative traits (weight, diameter, and length). Several other studies showed that insect pollinators,

including honey bees, significantly benefit the fruit set and quality of pomegranate yields (Derin & Eti, 2001; Tao et al., 2010). Similar findings were obtained in the present study, which provides evidence that open and supplementary pollination is necessary for increasing the quantity and quality potential of fruits in commercial pomegranate production.

Conclusion

In pomegranates, flower size and position can indicate flower quality. Single larger flowers had significantly higher fruit set, greater final fruit weight, and more arils per fruit in larger commercial size categories. Thus, flower quality is an important issue in pomegranate, and cultural and environmental factors that influence flower size and vigor may directly affect fruit production and yield. Hymenopteran insects are the most important pollinators of pomegranate. *A. dorsata*, *A. florea,* and *A. cerana* were common visitors of pomegranate flowers. They spend effective time on the flower to collect floral rewards and contribute to pollination. The visits of pollinators, honeybees in particular, can improve the qualitative and quantitative characteristics of fruit, depending on the variety and conditions at the site. The crop yields due to various pollinators' activity in open field conditions increased 38.38% over control. More studies and experiments are needed to determine the economic value of pollinator services to pomegranate crops.

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Authors' Contribution

VKT: Conceptualization, supervision, and investigation.
NHL: Investigation and data curation.
RTN: Formal analysis and writing.
NT: Bee specimen identification and investigation.
All authors have read and approved the manuscript.

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APPENDIX

Plate 1. Pomegranate flower types. a) Terminal flower. b) Lateral flower. c) Single flower.

Plate 2. Drop of hydrogen peroxide on the stigma surface.

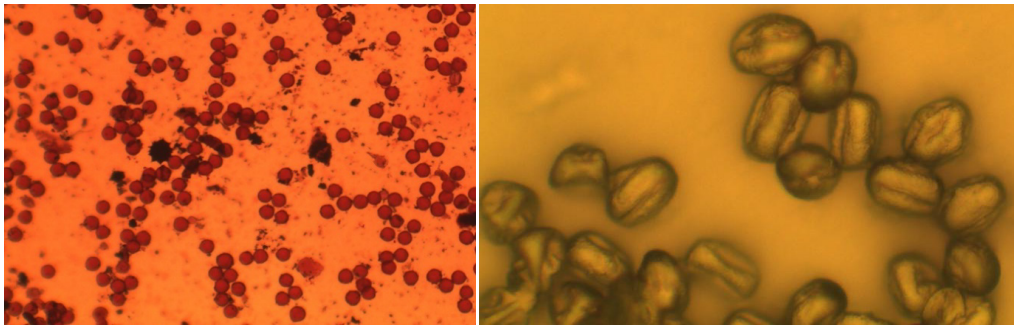


Plate 3. Pollen viability test.

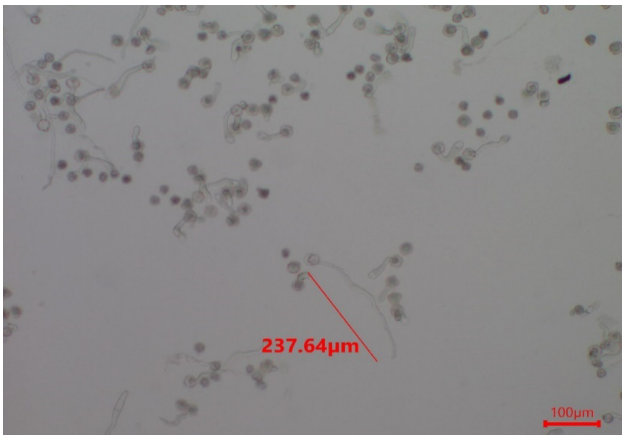
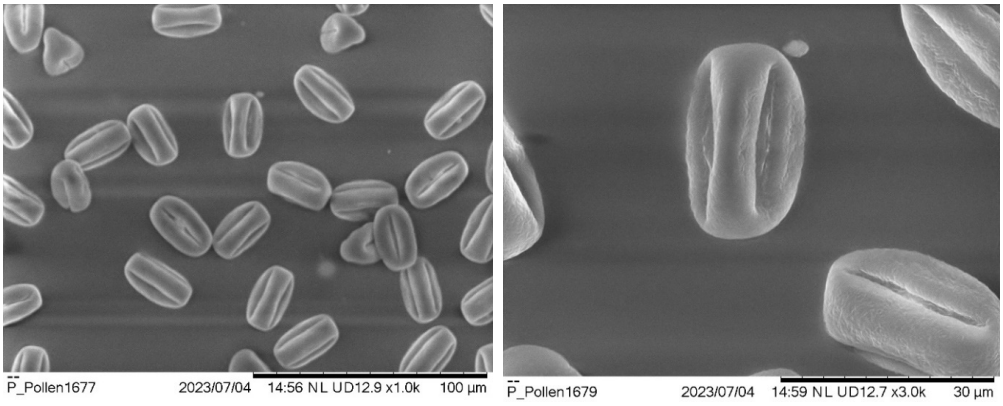


Plate 4: Pollen tube germination.



a. Pollen grains (1000x) **b. Whole grain shape (3000x)**

Plate 5. Scanning Electron Microscopy images of Pomegranate pollen.

a. *Apis dorsata* Fab. **b. *Apis cerana* Fab.**

Plate 7. Foraging activity of bees.

Apis dorsata **Fab.** *Apis cerana* **Fab.** *Apis florea* **Fab.**

Tetragonula iridipennis **Smith** *Amegilla zonata* **(Linnaeus)** *Ceratina binghami* **Cockerell**

Xylocopa fenestrata **Fab.** *Thyreus* **sp.** *Megachile lanata* **Fab.**

Hoplonomia westwoodi **(Gribodo)** *Lasioglossum* **sp.** *Formica* **spp.**

Pieris brassicae **(Linnaeus)** *Eurema* **sp**

Achraea terpiscore **Fab.** *Cepora Nerissa* **(Fabricius)**

Plate 6. Diversity of flower visitors on pomegranate.