

# Floral Phenology and Pollination Ecology of *Punica granatum* L. in Kathmandu, Nepal

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## Abstract

The phenological and ecological study on *Punica granatum* L., a cultivated and wild species found in outer Himalayan ranges and warm inner valleys (alt. 700 - 2700m), was carried out during April and May of 2006 and 2007 in Kathmandu Valley. The study covered blooming time, size of flower, its correlation and interaction with the visitors and pollinators. The prime pollinator was *Apis cerena* along with *A. mellifera*. Normal range of the length of a full blooming flower (mature flower) was 4.1 to 4.7 cm (in bagged flower) and 3.8 to 4.9 cm (in open flower). The fruiting rate was higher in case of the open flowers than the bagged one. Visitor's/pollinator's flower visit rate (visits/time) was found higher (most effective) in morning with sunny weather (766 times out of 1365). Similarly, the least effective time was dawn and dusk with cloudy and rainy weather (2 times each out of 1365).

**Key words:** floral phenology, visitors/pollinators

## Introduction

### Introduction to the species

*Punica granatum* L. is a member of the family Punicaceae. It is a glabrous shrub or small tree of 5-10m height. Branchlets are often spine-tipped, bark is smooth, dark grey, leaves are 2 - 8cm long, entire, lanceolate to broadly oblanceolate, opposite, shining, narrowed to a short petiole. Flowers on the tip of axillary shoots, with crinkle petals, blooms between late spring and early summer (May-June). Calyx 2 - 3 cm long, tubular with 5 - 7 triangular fleshy lobes. Petals 5 - 7, bright red, rarely white or yellowish (Partap 1997, Lama *et al.* 2001, Joshi & Joshi 2001). Stamens numerous. Pollen grains  $22.6 \pm 1.04 \mu\text{m}$  long and  $21.6 \pm 1.9 \mu\text{m}$  broad, yellow in colour, spherical in shape, and tri-colporate with a smooth exine. Fruits are round or ovoid, 5 cm in diameter, at first greenish then brownish, orange to scarlet, with a semi-woody pericarp, interior with separating membranous wall containing numerous seeds. Seeds have edible fleshy red, pink or whitish external layer (Partap 1997, WHO 1999, Bista *et al.* 2001, Lama *et al.* 2001, Joshi & Joshi 2001).



**Fig.1.** A *Punica granatum* tree on its natural habitat

It grows wild on both shores of the Mediterranean belt, Arabia, Persia, Bengal, China, Japan, Central and Western Asia. There are pocket areas in the Hind Kush Himalayan (HKH) region, where its pure wild forests exist in hot, dry valley areas. This species has been

introduced into the East and West Indies. It is cultivated in all countries, where the climate is warm including Asia especially the eastern Himalayas and southern Europe (Kochhar 1998, <http://www.herbdatanz.com/pomegranate.htm>).

In Nepal, a single species of *Punica* (*Punica granatum* L.) has been reported (Bista *et al.* 2001). In Nepal, both cultivated and wild forms grow in open and dry slopes of warm valleys and outer hills ranging from 700 to 2700m (Lama *et al.* 2001, Joshi & Joshi 2001, Chaudhary 1998, DMP 1970). It is locally known as Anar and Darim.

### Phytochemicals and Uses

The root and bark contain tannin (20-22%) and alkaloids (0.5-1%). The seeds contain steroidal oestrogen. The fruit pulp contains protein, carbohydrate, fat, fibre, minerals, oxalic acid and vitamins A, B and C. In early times Greeks and Romans used *Punica* for tanning leathers, which is obtained from root, bark, stem, leaves and fruit rind (Kochhar 1998, Joshi & Joshi 2001). Root, stem bark, rind, Fruit juice, powder of whole fruit and seeds are used as medicine. It is very useful in tapeworm infections, diarrhoea and dysentery. Besides, it is used in leucorrhoea, as a gargle agent in sore of throat, cardiac disorders, leprosy and stomachic problems. The juice is used as tonic, refrigerant, anti-inflammatory and anti-prostate cancer (DMP 1970, WHO 1999, Lama *et al.* 2001, Joshi & Joshi 2001, <http://www.herbdatanz.com/pomegranate.htm>). The juice is highly nutritious for the patients so it is an expensive fruit in Nepal.

The present article throws light on floral phenology and ecology influencing visitors and pollinators, which has not been previously studied.

### Floral phenology and Pollination ecology

The time and duration of budding, flowering, wilting and fruiting of any plant is essential aspect in the study of pollination as they provide vivid knowledge on the activity of the visitor (Kearns & Inouye 1993). The role of corolla, which is mainly related to attract pollinators, depends upon its structure, color and appearance. In some flowers a spot of different color on the corolla directs the insect to the interior of the flower, guiding the pollinators toward their destination. For instance, *Pedicularis dendrothauma* has a corolla with a pinkish spot (Macior 1990, Adhikari 2003).

The length of stamen and gynoecium also play an active role in pollen transfer. Besides these, flowers have other floral cues like nectar, sugar, odour and oil to attract the insects, which help in pollination. In some species, insect visit is directly related with plant height (Kearns & Inouye 1993) and also varies with size of the inflorescence (Thomson 1988). So the floral phenological study is an integral part of the pollination studies.

Both plants and pollinators are affected by environmental variables (Kearns & Inouye 1993). Flower development and opening, nectar secretion, anther dehiscence and seed development are all dependent on ambient temperature. Similarly, air temperature affects the activity of flower-visiting insects. Bees, the most important pollinators, can't go outside their hive in cold weather (Adhikari 2003, Adhikari 2004). Some evolutionary co-adaptation can be seen between many flowers and their pollinators (Macior 1990 & 1984).

### Conservation and Pollination

Pollination systems are under increasing threat mainly from anthropogenic sources, including fragmentation and alteration of habitat, changes in land use pattern, modern agri practices, move towards monocultures, use of chemicals such as pesticides, and invasions of alien species (Kearns *et al.* 1998, Adhikari 2004). In many places of the world, the 'pollination crisis' is evident in declines of honeybees and native bees and in damage to webs of plant-pollinator interactions. The inevitable and obligate role of pollination makes it clear that the conservation of pollination systems is an important priority for all (Kearns *et al.* 1998, Adhikari 2003). The declining apple production in the Hind Kush-Himalayas (HKH) region is due to the loss of local /wild pollinators and consequently due to the inadequate crop pollination (Partap & Partap 2001, Ahmad *et al.* 2002, Adhikari 2003).

### Historical review of pollination study

The science of anthecology began around 200 years ago. Since then, Kolreuter (1761), Muller (1881), Knuth (1898-1905), Robertson (1929), Miyamoto (1962), and others have accumulated prodigious records of pollinator diversity on flowers. Less than 50 years, however, have elapsed since Kugler and other pollination ecologists, following studies of insect

behavior by Frisch and his colleagues, began critical studies on pollination dynamics (Macior 1971).

The studies on the pollination ecology started with the works on *Pedicularis* in Europe (Knuth 1898-1905), North America (Macior 1982, 1983, 1986a, 1986b, 1986c) and Japan (Macior 1988). They have demonstrated close correspondence of floral function and pollinator behavior. Recently, such studies have been carried out in Asia (Macior 1990). For example, Deyrup & Menges (1997) studied on *Dicerandra frutescens* (Lamiaceae), Proctor, Yeo & Lack (1996) studied on insect visitors, Adams (1982) studied on *Pedicularis* pollination, Boyle & Menges (2001) studied on *Hypericum cumulicola* (Hypericaceae), and Paulus & Gack (1998) studied on the pollination of *Ophrys* (Orchidaceae). Kearns & Inouye (1993) published a book on 'Techniques for Pollination Biologists'. O'Neill (1997) and Dafni (1984), has significant contribution on the field. Similarly Jones & Little (1983) also published book named "Hand book of experimental pollination biology" (Adhikari 2003).

The pollination ecology of *Pedicularis* species in Asia include the contributions of Macior (1990) in Kasmir Himalaya, Macior (1995), and Macior & Sood (1991) in Himachal Pradesh (India), Macior & Tang (1997)

in China, Tang *et al.* (1998), and Macior *et al.* (2001) in China (Adhikari 2003).

Since then till now, many authors have thrown light on pollination biology. However, Nepal has remained virgin in this area except a few of the works done by the author.

### Study Area

Study area included different places of Kathmandu valley, Central Nepal. The Kathmandu valley lies between 27°34' – 27°46" N Latitude and 85°10' – 85°52' E longitude with its unique physiography (altitude ranging between 1350 – 2765 msl) covering an area of 650 sq km. The valley is drained by the rivers Bagmati and Bishnumati and their tributaries. The cool subtropical to temperate climate and surrounding ranges (Shivapuri 1910m, Nagarjoun - 2500m, Phulchowki - 2765m, and Chandragiri- 2220m), which abound with scenic natural beauty (ever green oak laurel forest, which contain different religious areas and picnic spots of botanical interest (Adhikari 1988). *Punica granatum* has been cultivated in many places inside and outside valley.

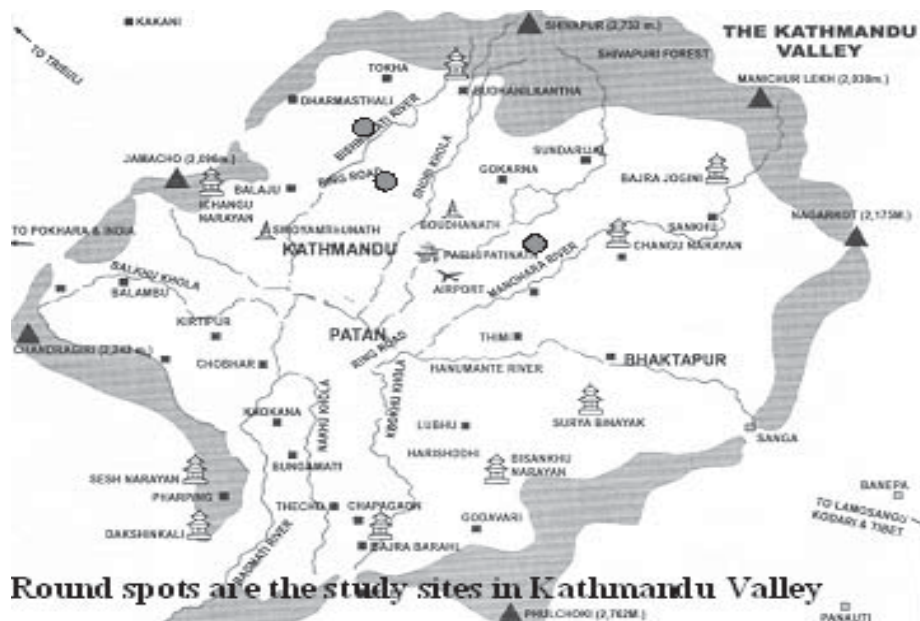


Fig.2. Study area

## Methodology

The flowering phenological record of *Punica* along with visit of insects was studied throughout the study period. The photography was also done.

### Floral phenology

For the study of floral phenology, flowers were classified into different developmental classes/ conditions viz.

- 1 = Tight, unopened bud,
- 2 = developing bud/opening bud,
- 3 = open flower,
- 4 = mature flower,
- 5 = flower (corolla) wilting,
- 6 = corolla fallen off, fruit developing,
- 7 = Shade off / died out.

All together 20 days were spent for the study of flower development. Flower conditions were recorded in every five days. The measurement of each flower size/ length of corolla tube was taken at a five-day interval, for a total of five times. In addition, flowers were sectioned in order to observe the construction, arrangement and origin of all parts. A time table of morning, day and evening including dawn and dusk was prepared to watch the visit of pollinators in different flowers.

### Pollination ecology

For the study of pollination ecology of *Punica granatum*, observation of pollinators' activities along with the collection and preservation of pollinators were done in April – May (the main blooming period) of 2006 and 2007. Altogether 77 hours of patient watching was carried out. While watching the visitors or pollinators, the insects' behavior on the flowers, number and species of visitors, their frequency of visit during different day times and weather conditions, date, no. of flowers on plant, whether the flower was shaded or sunlit during the observation as well as their visiting period were observed and recorded. Weather conditions were also recorded at regular time intervals. Visitors / pollinators were collected from the *Punica* flowers. The insect visitors were trapped and preserved for identification. Extensive non-timed flower observations for their development and visitors were made over 3 yrs (2005, 2006 & 2007)

### Bagging Studies

Some 15 healthy and unopened flowers (buds) were bagged in order to exclude the visitors/pollinators. The

conditions of all buds and flowers were studied on the same day or interval as that of the flowers with no bagging (5 times in each five days of interval).

## Results and discussion

Main blooming period of *P. granatum* flower was found 20 days ie. a bud of *P. granatum* normally takes 20 days for its complete maturation (Table 1 & 2). A young bud (condition - 1) measures up to 0.9 cm (in bagged condition) and up to 1cm (in open condition), which is not a significant different. The bagged flowers were seen to mature first than the open flowers (Table 1 & 2). It may be due to certain increase in temperature in the bagged flowers as compared to the open flowers.



Fig.3. *Punica* flowers at different developmental conditions

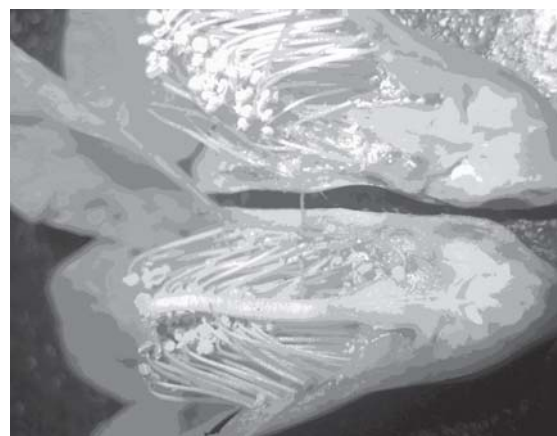


Fig. 4. Internal structure of *Punica* flower

The normal range of the length of a full blooming flower (mature flower) was found to be 4.1 to 4.7cm in bagged flower, while 3.8 to 4.9cm in open flower (Table 1 & 2). This small difference in length may not be due

to the bagging of flower. Fruiting is found higher in open flower (12/15) than in bagged flower (8/15). Fruiting in bagged flower may be either due to some error while bagging the flowers or due to some other pollinators present internally within the flowers.

**Table 1.** Floral phenological changes (in bagged flower)

Fl. no.	Time-6.15 AM		6.15 AM		6.15 AM		6.15 AM		6.15 AM	
	Date-20 - 4- 2005		25 - 04 - 2005		30 - 04 -2005		05 -05 -2005		10 - 05 -2005	
	Flower size(cm)	Flower condition	Flower size (cm)	Flower condition	Flower size (cm)	Flower condition	Flower size (cm)	Flower condition	Flower size(cm)	Flower condition
1	1.8	2	2.3	3	4.2	4	-	7	-	7
2	2.2	2	2.9	3	4.3	4	-	7	-	7
3	2.8	2	4.0	3	-	7	-	7	-	7
4	1.5	2	1.6	2	3.2	3	-	7	-	7
5	2.7	2	3.2	3	4.1	4	-	7	-	7
6	1.5	2	1.7	2	2.4	2	3.8	3	-	7
7	2.6	2	2.8	2	3.7	3	-	7	-	7
8	1.5	2	1.9	2	3.4	3	4.7	4	-	7
9	2.2	2	3.2	3	4.4	4	-	7	-	7
10	3.0	2	4.3	3	-	7	-	7	-	7
11	2.4	2	-	7	-	7	—	7	-	7
12	0.7	1	0.9	1	1.7	2	-	7	-	7
13	0.4	1	0.7	1	1.2	2	3.0	3	-	7
14	0.9	1	1.9	2	3.4	3	-	7	-	7
15	0.5	1	0.5	1	0.9	1	1.3	2	-	7

Note: - 1=Tight, unopened bud, 2=developing bud/opening bud, 3= open flower, 4=mature flowers, 5=flower (corolla) wilting, 6=corolla fallen off, fruit developing, 7=Shade off/died out

**Table 2.** Floral phenological changes (in open/unbagged flower)

Fl. no.	Time-7.15 AM		7.15 AM		7.15 AM		7.15 AM		7.15 AM	
	Date-20 - 4- 2005		25 - 04 - 2005		30 - 04 -2005		05 -05 -2005		10 - 05 -2005	
	Flower size(cm)	Flower condition	Flower size (cm)	Flower condition	Flower size (cm)	Flower condition	Flower size (cm)	Flower condition	Flower size(cm)	Flower condition
1	2.1	2	2.9	3	4.3	4	-	6	-	6
2	2.2	2	2.9	3	4.6	4	-	6	-	6
3	1.9	2	2.3	2	3.9	4	-	5	-	7
4	1.0	1	1.7	2	3.2	3	4.3	4	-	6
5	1.8	2	2.2	2	3.4	3	4.9	4	-	5
6	0.2	1	0.9	1	1.4	2	2.7	3	3.1	4
7	0.7	1	1.7	2	2.8	3	4.2	4	-	7
8	2.2	2	3.1	3	4.7	4	-	7	-	7
9	0.4	1	0.7	1	1.0	1	1.9	2	2.4	3
10	2.0	2	3.4	3	4.7	4	-	5	-	6
11	0.4	1	0.6	1	0.9	1	1.3	2	2.5	3
12	2.2	2	3.9	3	4.4	7	-	7	-	7
13	1.7	2	1.8	2	2.2	2	3.4	3	4.0	4
14	1.9	2	2.5	2	3.8	4	-	6	-	7
15	2.5	2	3.8	2	4.9	3	6.5	4	-	6

The result received after watching the pollinators or visitors in selected flowers are tabulated below. A great attempt was made to watch all flowers from dawn to dusk and from rainy to sunny days.

**Table 3.** Hours spent on different days for pollination studies and the visitors' frequency of visit

Time of day/periods	Hours spent for study	No. of visitors/pollinators	Relative percentage of hours spent	Average Visitor /hr	Date
Before or around Dawn (5.30-6.00)	3	16	3.89	5.33	20 <sup>th</sup> , 25 <sup>th</sup> , & 28 <sup>th</sup> , of April and 4 <sup>th</sup> , 6 <sup>th</sup> & 9 <sup>th</sup> of May 2007
Morning (6.00-12 AM)	36	980	46.75	27.22	21 <sup>st</sup> , 25 <sup>th</sup> , 30, th of April and 4 <sup>th</sup> 7 <sup>th</sup> & 10 <sup>th</sup> of May 2007
Afternoon (12-3PM)	18	196	23.38	10.88	20 <sup>th</sup> , 25 <sup>th</sup> & 29 <sup>th</sup> of April & 2 <sup>nd</sup> , 5 <sup>th</sup> & 10 <sup>th</sup> of May 2007
Late noon /early evening (3-6.30)	14	148	18.18	10.57	24 <sup>th</sup> , 26 <sup>th</sup> & 30 <sup>th</sup> of April and 8 <sup>th</sup> of May 2007
Evening/Dusk (6.30-7.30)	6	25	7.79	4.16	20 <sup>th</sup> & 25 <sup>th</sup> of April and 1 <sup>st</sup> , 4 <sup>th</sup> , 5 <sup>th</sup> and 9 <sup>th</sup> of May 2007
Total	77 hrs	1365	100	-	-

(Note-The term 'visitors' includes both the 'pollinators' and 'visitors'. Time spent by visitors on Punica flowers was generally 10 seconds to 5 minutes)

**Table 4.** Hours spent on different weather conditions for pollination studies and the visitors' frequency of visit

Weather condition	Hours spent for study	No. of visitors/pollinators	Relative percentage of hours spent	Average Visitor/hr	Remarks
Clear, no sun, no rain, no cloud/fog	8	115	10.39	14.37	Among 1365, 1008 are <i>A. cerana</i> , 278= <i>A. mellifera</i> , 40= <i>Bombus</i> sp., 10= <i>Helina</i> sp., 7= <i>Meliscaeva</i> sp., 6 = <i>Vespula</i> sp., 7= <i>Formica</i> sp., 5 = <i>Fannia</i> sp., & Unidentified sp=4,
Clear-sun	48	943	62.33	19.65	
Partly cloudy-sun	16	286	20.78	17.87	
Cloudy/ Foggy -rain	5	21	6.49	4.2	
Total	77 hrs	1365	100	-	

(Note: The term 'visitors' includes both the 'pollinators' and 'visitors'. Time spent by visitors on Punica flowers was generally 10 seconds to 5 minutes)

**Table 5.** List of Visitors/ Pollinators and their frequency of visit to Punica flowers

S N.	Visitors/Pollinators	No. of visits per watch hour	Relative % of all visits	Type of Interaction
1	<i>Apis cerana</i> (fam. Apidae : Honey Bee) Indigenous bee of Nepal	13.09	73.85	Primary Pollinator
2	<i>Apis mellifera</i> (fam. Apidae : Honey Bee)	3.61	20.37	Major Pollinator
3	<i>Helina</i> sp., (fam.–Muscidae:Dark fly)	0.13	0.73	Possible Pollen robber
4	<i>Meliscaeva</i> sp.,(fam. – Syrphidae:Hover fly)	0.09	0.51	Pollen/ nectar robber
5	<i>Bombus</i> spp (Apidae:Bumblebee)	0.52	2.93	Possible pollinator
6	<i>Fannia</i> sp.,(fam.– Fanniidae:Small Dark grey fly)	0.06	0.37	Nectar and/or pollen robber
7	Unidentified sp.,(fam.–Sepsidae: Small ant like fly)	0.05	0.29	—
8	<i>Formica</i> sp.( fam-Formicidae: Ant)	0.09	0.51	Causal visitor
9	<i>Vespula</i> sp.(Vespidae:Wasp)	0.08	0.44	Bee killer/eater

The present studies have proved that the visit rate of pollinators is higher in the sunny period (19.65 visits per hour) or on the flowers which are exposed to sun rather than in shade (Table -4). The visit rate (visit/hr) were found to be increased with increase in flower

density (in flower/m<sup>2</sup>) indicating the positively density-dependent visit rate to different flowers. Similar observations were found in *Dicerandra frutescens* by Deyrup & Menges (1997) and in *Hypericum cumulicola* by Boyle & Menges (2001).



**Fig.5.** *Apis cerena* moving towards *Punica* flower



**Fig.6.** *Apis cerena* collecting pollen from *Punica* flower

Cloudy and or foggy rain period has least number of visit rates (4.2/hr). However, visit rate is more in partly cloudy - sun (17.87/hr) and in clear weather condition (14.37/hr). The microclimates have an important effect even in the shade (Adhikari 2001, Kearns & Inouye 1993, Deyrup & Menges 1997). In the morning time (27.22/hr) with sunny days (19.67 visits per hour) are the most preferred time and weather (56.11% of total visits) =for the pollinators (table-3, 4 & 6). Even the sunlit flowers were more visited by visitors than the shaded flowers. The duration of the longest pollinator visit decreases as the morning progresses. It is probably due to the depletion of pollen supplies (Boyle & Menges 2001) and foraging become less profitable. Also in later days the visit rates were quite low. The dawn (5.33/hr) and dusk (4.16/hr) with cloudy- foggy

rain (1.5% each of total visits) are the least preferred time for the pollinators (table -3, 4 & 6). The dawn and Dusk with sun (0.15% and 0.29% of total visits respectively) have shown a very low visit rate even in a favorable weather condition (sunny weather)(table-6). It is probably due to a very limited sunny time in the dawn (normally before sun rise) and dusk (normally after sun set) periods. Although some flower visitors (birds, mammals, and some insects) are homoeothermic or capable of endothermic temperature regulation, others (e.g. many Diptera, solitary bees) are dependent on solar radiation to achieve the body temperature required for flight(Adhikari 2003), which is strongly supported by this study as bees are more active during the sunny periods.

**Table 6.** A two way contingency table for the visitors' activities at different weather conditions and time periods

Time	Weather conditions				Total no. of visitors (77 hrs)
	Clear, no sun, no rain, no cloud/fog (8 hrs)	Clear-sun (48 hrs)	Partly cloudy -sun (16 hrs)	Cloudy/ Foggy -rain (5 hrs)	
Before or around Dawn (5.30-6.00) (3hrs)	7	2	5	2	16
Morning (6.00-12 AM) (36 hrs)	64	766	142	8	980
Afternoon (12-3PM) (18hrs)	27	84	81	4	196
Late noon /early evening (3-6.30) 14 (hrs)	10	87	46	5	148
Evening/Dusk (6.30-7.30) 6 (hrs)	7	4	12	2	25
<b>Total no of visitors (77 hrs)</b>	<b>115</b>	<b>943</b>	<b>286</b>	<b>21</b>	<b>1365</b>

*P. granatum* is adapted for bee pollination rather than fly pollination. *Apis cerena*, which made 13.09 visits per hour, is the main/prime pollinator (includes 73.85% of total visits) of *Punica* (Table-5). Assuming 12 hr of visitation, each flower would be visited by about 157 *Apis cerena*, so pollinator limitation for seed production in *P. granatum* is unlikely. *A. mellifera* is also a major pollinator of *Punica* (20.37%). The lower value of *A. mellifera* is may be due to the less number of *A. mellifera* in the vicinity of study area. The bumble bees (*Bombus* sp.) are the other important pollinators or may be the pollen robbers having 2.93% of all visits (table-5). Some other insects (having less than 3% of all visits and having less than 0.1 visits per hour) like *Helina* sp., *Meliscaeva* sp., *Fannia* sp., *Formica* sp. and *Vespula* sp. have also been reported as a visitor of the *Punica* flower (table-5). However they are not the true pollinators and probably most of them are pollen/nectar robbers. According to Partap (1997), in addition to the aforementioned insects, *P. granatum* is also visited by insects like butterflies, moths, beetles and hornets. However, they were not reported during this study.

Though, it is a good source of both pollen and nectar for the bees and insects, *P. granatum* is mainly visited for pollen. Pollen plants like *P. granatum* are important in beekeeping, especially at the time of colony build-up when bees need large amounts of protein for brood rearing. Pollen is the sole source of proteins, lipids, minerals, and vitamins needed to feed the brood and immature adult bees.

A more detailed study is needed using the latest technologies and methods for the further exploration especially regarding the co-evolution of *Punica granatum* with its prime pollinators.

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