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Studies on the role of honey bees pollination in pomegranate (*Punica granatum* L.)

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ABSTRACT

This study investigated the foraging activity of honey bees and other pollinators during the flowering period of pomegranate (*Punica granatum*). The highest foraging activity of honey bees was recorded under open conditions during the IV SMW, particularly between 12:00 to 14:00 hrs. Thirteen insect pollinator species were observed, with ants, butterflies, and beetles being the most abundant. Open pollination significantly outperformed other methods, including protected nylon net with bee cages, in promoting higher fruit set (65.60 fruits per plant), fruit length (81.16 mm), fruit diameter (78.09 mm), and fruit weight (280.40 g). Additionally, open pollinated plants showed the highest number of arils (601.40) and total soluble solids (16.92°Brix).

Introduction

Pomegranate (*Punica granatum* L.), a tropical and subtropical fruit from the Punicaceae family, with a long tradition. According to Soriano *et al.* (2011), it likely originated in Iran and northern India. Xhuveli (2012) reports that wild pomegranate plants are sometimes cultivated in gardens for their fruit or aesthetic value. According to reports, pomegranates are produced all over the world between latitudes 41° N and 42° S. India ranks first in area (2,57,000 ha), production (3097.72 MT) and productivity 12.01 MT/ha of pomegranate. Maharashtra state is leading state in country growing first in area 137.85(000) ha), production (1554.25 (000) MT and productivity 11.28 MT/ha (Anonymous, 2023) Maharashtra is major producer of pomegranate fruit. It has emerged as a significant export crop in India during the last decade (Chandra and Jadhav, 2008).

In north India, there are two blossoming seasons, whereas Nalawadi *et al.* (1973) reported three in western India. There are three distinct flowering seasons in subtropical central and western India: ambe bahar (January-February), mrig bahar (June-July), and hast bahar (September-October). Growers like ambe bahar because of its significant results due to abundant blossoming in comparison to other flowering seasons (Prasanna Kumar, 1998). Flowering was seen in Karnataka lasting 80-87 days between June and August (Nalawadi *et al.*, 1973). Only one flowering season occurred in Punjab, from April to June (Josan *et al.*, 1979).

Pollination is the most important factor in increasing crop productivity in which there will be transfer of pollen from male to female reproductive structure for effective fruit setting. Wind, water and animals including insects are involved in pollination of flowering plants. Insects specially honey bees play an important role in cross pollination. Both

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self and cross pollination noticed in pomegranate, however cross pollination is favoured for increased fruit yield. Hand pollination yielded a higher proportion of fruit set than pollination under natural circumstances (Josan *et al.*, 1979; Bavale, 1978).

Previous scientific research has shown that self pollination can also result in significant crop yields (Martinez *et al.*, 2009; Patil and Pastagia, 2016), despite some research suggesting that insect pollinators, such as honey bees, are beneficial in improving pomegranate crop yield and fruit quality (Vazifeshenas *et al.*, 2015). This raises concerns about whether pollinators are necessary for pomegranates or if self-pollination is adequate. However, there is currently very limited knowledge on pomegranate cross-pollination and its impact on pomegranate fruit, especially in Phule Bhagwa Super variety. The study hypothesizes that integrating honey bees will increase pomegranate fruit quality and quantity along with studies on different insect pollinators. Hence, the current research was conducted to comprehend the impacts of various pollination modes on pomegranate fruit, especially in the western dry zones.

Material and Methods

The present investigation was carried out during flowering time of pomegranate under All India Co-ordinated Research Project on Arid Zone Fruit at Mahatma Phule Krishi Vidyapeeth, Rahuri. A standard package of practices was followed in the cultivation and management of the crop. The study was conducted on pomegranate cultivar Phule Bhagwa Super comprising three different treatments viz., (T₁) Open pollination to all insect pollinators, (T₂) Protected covered insect cage house without honey bees, and (T₃) Protected covered insect cage house with honey bees (1 hives of *Trigona irridipenis*). The experiment was laid out in randomized block design with seven replications.

The foraging activity of honey bees and other insects were recorded on randomly selected five flowers of pomegranate from 08:00 hrs to 16:00 hrs at two hourly interval for five minutes at weekly interval and expressed as the mean number of foragers per 5 flowers per 5 minute. The data were recorded on diversity of insect pollinators, number of fruit set/ plant, fruit length, fruit diameter, fruit weight, number of arils/ fruit and total soluble solids. The collected data were analyzed statistically.

Results and Discussion

Insect foragers diversity on pomegranate

Thirteen species of insect pollinators were recorded foraging

during the flowering period of pomegranate (*Punica granatum*). The study on the diversity of insect pollinators in pomegranate, honey bees were found to be the most dominant pollinator as documented below in Table 1. The results were in confirmation with the observation recorded by Chavhan *et al.* (2023) who studied on floral visitors of pomegranate recorded in Bhagwa variety revealed 20 species of insects.

Table 1. Insect pollinator diversity on pomegranate flower

Order	Family	Species
Hymenoptera	Apidae	<i>Apis cerana indica</i>
		<i>Apis mellifera</i>
		<i>Apis dorsata</i>
		<i>Apis florea</i>
		<i>Trigona irridipenis</i>
Lepidoptera	Formicidae	<i>Camponotus spp.</i>
	Pieridae	<i>Phoebis sennae</i>
	Lycaenidae	<i>Strymon melinus</i>
	Nymphalidae	<i>Vanessa cardui</i>
	Papilionidae	<i>Papilio demolius</i>
		<i>Pachliopta aristolochiae</i>
Diptera	Muscidae	<i>Musa domestica</i>
Hemiptera	Pentatomidae	<i>Nezara viridula</i>

Foraging activity of honey bees and other pollinator under open condition in pomegranate

Foraging activity of honey bees were observed throughout the flowering period of pomegranate (Table 2) from I to VII SMW i.e. from first week February to fourth week of March, 2023. The mean foraging activity increased from I week to IV week (first week of February to fourth week of February) as availability of flower to honey bee increased for pollen and nectar collection and then decreased from V SMW. Highest mean foraging activity recorded in IV SMW with 2.58 mean number of honey bees / 5 flower / 5 minute as pomegranate tree were in full flowering stage.

The foraging activity of honey bees was recorded at two hour interval in a pomegranate flowers from morning (08:00 hrs) till to evening (16:00 hrs) once in a week till to fruit setting of pomegranate during the year 2023 and mean foraging activity was calculated. Higher bee visitation was observed at 12:00 to 14:00 hrs interval with 1.90 mean numbers of honey bees / 5 flower / 5 minute which was followed by at 10:00 to 12:00 hrs interval with 1.71 mean numbers of honey bees / 5 flower / 5 minute.

The data recorded on foraging activity of other pollinators on pomegranate (Table 3) revealed that the ants were found

large in number (1.29 ants/ 5 flower/ 5 minute) as compared to other pollinators and as compared with different time duration, maximum number of other pollinators visited during 10:12 hrs interval with (1.22 other pollinators/ 5 flower/ 5 minute) and on the other side the lowest number of other pollinators recorded at 14:16 hrs interval (0.20 other pollinators/ 5 flower/ 5 minute).

Foraging activity of honey bees (*Trigona irridipenis*) under caged condition in pomegranate

It is evident from Table 5 that the foraging activities of honey bees under caged conditions in pomegranate were observed from 08:00 hrs to 16:00 hrs at two-hour intervals. These observations were conducted weekly to monitor the bees' activity patterns. The mean foraging activity was observed maximum during IV SMW after 10 percent flowering with 1.92 mean numbers of honey bees/ 5 flower/ 5 minute. Thereafter gradually decreased from 0.50 mean numbers of honey bees/ 5 flower/ 5 minute in V and VI SMW, respectively. Similarly, the (*Trigona irridipenis*) started visiting the pomegranate plot early at 08:00 hrs and it gradually increased and peak activity was observed at 12:00 hrs to 14:00 hrs interval with an average of 1.61 mean numbers of honey bees/ 5 flower/ 5 minute thereafter, decreased gradually from 14.00 hrs.

Effect of different mode of pollinations on quantitative and qualitative parameters

Quantitative parameters such as number of fruit set per plant, fruit length, fruit diameter, fruit weight, number of arils per fruit, and total soluble solids (TSS) differed significantly among different modes of pollination in pomegranate (Table 6).

The number of fruit set per plant was found significantly highest (65.60 fruits per plant) in case of open pollination

treatment and found superior to remaining treatment. Whereas, lowest number of fruit set per plant (29.20 fruits per plant) recorded in caged house without honey bees. The present findings are in conformity with the observation of (Chavhan, 2023; Karimi and Mirdehghan, 2015; Wetzstein *et al.*, 2011)

The fruit length was recorded significantly highest in the open pollinated plants (81.16 mm) followed by covered caged house with honey bees pollinated plants (76.02 mm). However, covered caged house without honey bees treatment (65.76 mm) recorded lower fruit length.

The fruit diameter showed significant difference between the treatment. It was comparatively highest in case of open pollinated plants (78.09 mm) which was at par with covered caged house with honey bees pollinated plants (75.88 mm). The least fruit diameter was recorded in covered caged house without honey bees treatment (64.89 mm).

As regards the open pollinated plants recorded significantly highest fruit weight of (280.40 g). On the other side covered caged house plants (170.60 g) which registered the significantly least fruit weight. Significantly higher fruit set and fruits weight of pomegranate was reported in case of bee pollination, when compared to the self-pollinated plants i.e. not observed in closed condition. Therefore, these results are also found in agreement with the findings reported by Derin and Eti 2001 and Tao *et al.* 2010.

Significantly highest number of arils per fruit was recorded in case of open pollinated plants (601.40). However, covered caged house plants without honey bees recorded significantly lower number of arils per fruit (260.20).

The maximum total soluble solid (TSS) content of 16.92°Brix was observed in the open pollination plot. In contrast, plants grown in covered caged houses without honey bees showed a significantly lower number of arils per fruit, recording only 260.20 arils. TSS detects soluble solids in a liquid and impacts taste, making it an important produce quality indicator (Hadiwijaya *et al.*, 2020; Bexiga, 2017). Karkar and Ghetya (2022) reported that TSS was found to be higher in the fruits of open-pollinated plants.

Table 2. Foraging activity of honey bees under open condition in pomegranate

Time	Number of honey bees/ 5 flower/ 5 minute				Total	Average
	08:10 hr	10:12 hr	12:14 hr	14:16 hr		
Week no. 1	0.66	0.66	0.33	0.00	1.65	0.41
Week no. 2	0.33	0.33	2.00	1.00	3.66	0.92
Week no. 3	0.66	2.33	2.66	3.33	8.98	2.25
Week no. 4	1.00	3.33	3.33	2.66	10.32	2.58
Week no. 5	2.00	3.36	3.33	1.33	10.02	2.51
Week no. 6	1.33	1.66	1.33	1.00	5.32	1.33
Week no. 7	0.00	0.33	0.33	0.00	0.66	0.17
Total	5.98	12.00	13.31	9.32		
Average	0.85	1.71	1.90	1.33		

Table 3. Quantitative and qualitative parameter of pomegranate

Treatment	Number of fruit set/ plant	Fruit length (mm)	Fruit diame- ter (mm)	Fruit weight (g)	Number of arils/ fruit	TSS (°Brix)
Open pollination (T ₁)	65.60	81.16	78.09	280.40	601.40	16.92
Caged house without hon- ey bees (T ₂)	29.20	65.76	64.89	170.60	260.20	15.38
Caged house with honey bees (T ₃)	52.20	76.02	75.88	258.20	560.40	15.82
SEm±	0.99	1.15	0.99	2.08	1.99	0.33
CD at 5%	3.05	3.55	3.07	6.42	6.14	1.02

Table 4. Foraging activity of other pollinators on pomegranate under open condition

Time	Number of other pollinators/ 5 flowers/ 5 minute				Total	Average
	House fly	Beetle	Butterfly	Ants		
08:10 hr	0.52	0.22	0.78	0.66	2.18	0.54
10:12 hr	1.32	0.66	0.58	2.33	4.89	1.22
12:14 hr	0.92	0.52	0.33	1.66	3.43	0.85
14:16 hr	0.30	0.00	0.00	0.52	0.82	0.20
Total	3.06	1.4	1.69	5.17		
Average	0.76	0.35	0.42	1.29		

Table 5. Foraging activity of honey bees (*Trigona irridipenis*) under caged condition in pomegranate

Time	Number of honey bees/ 5 flower/ 5 minute				Total	Average
	08:10 hr	10:12 hr	12:14 hr	14:16 hr		
Week no. 1	0.33	0.33	2.33	1.33	4.32	1.08
Week no. 2	0.33	1.33	2.33	1.66	5.65	1.41
Week no. 3	1.33	2.66	2.00	1.33	7.32	1.83
Week no. 4	2.00	3.33	1.33	1.00	7.66	1.92
Week no. 5	0.33	1.00	0.66	0.00	1.99	0.50
Week no. 6	0.33	1.00	0.66	0.00	1.99	0.50
Total	4.65	9.65	9.31	5.32		
Average	0.78	1.61	1.55	0.89		

Table 6. Quantitative and qualitative parameter of pomegranate

Treatment	Number of fruit set/ plant	Fruit length (mm)	Fruit diameter (mm)	Fruit weight (g)	Number of arils/ fruit	TSS (°Brix)
Open pollination (T ₁)	65.60	81.16	78.09	280.40	601.40	16.92
Caged house without honey bees (T ₂)	29.20	65.76	64.89	170.60	260.20	15.38
Caged house with honey bees (T ₃)	52.20	76.02	75.88	258.20	560.40	15.82
SEm±	0.99	1.15	0.99	2.08	1.99	0.33
CD at 5%	3.05	3.55	3.07	6.42	6.14	1.02



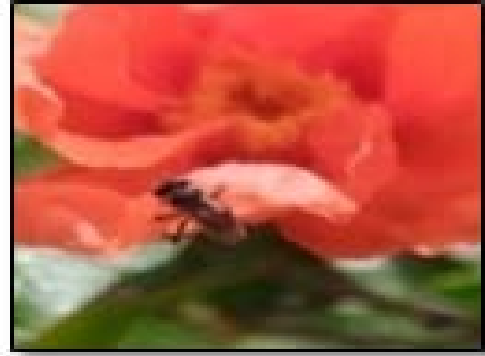
Apis mellifera



Apis dorsata



Apis cerena indica



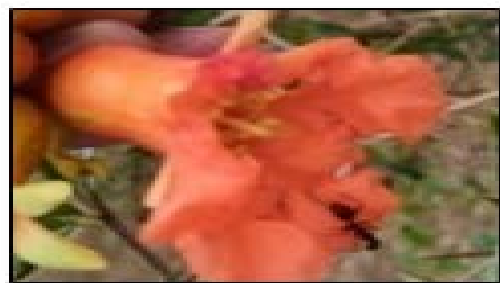
Trigona irridipennis



Apis florea



Pachliopta aristolochiae



Camponotus spp.

Conclusion

In conclusion, a total of 13 species of insect pollinators were observed during the flowering period of pomegranate, with honey bees being the most dominant pollinators. Peak

foraging activity of honey bees occurred during the IV SMW, with the highest foraging activity observed between 12:00 to 14:00 hrs under open conditions and 10:00 to 12:00 hrs in caged conditions. Ants were also found to be significant

in number. Open pollination, followed by protected nylon net with bee cages, proved to be the most effective for maximizing both quantitative and qualitative characteristics of pomegranate, such as fruit set, size, weight, and TSS.

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Conflict of Interest

The authors have no conflict of interest.

Data Sharing

All relevant data are within the manuscript.

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