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Pollination biology of *Albizia lebbek* (L.) Benth. (Fabaceae: Mimosoideae) with reference to insect floral visitors



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ABSTRACT

Indian siris, *Albizia lebbek* (L.) Benth. (Fabaceae: Mimosoideae) has significant importance to human beings for its multipurpose use. Insects play a crucial role in the pollination biology of flowering plants. In the current study, we studied the pollination biology of *A. lebbek* with special reference to insect floral visitors. The effectiveness of floral visitors was investigated in term of visitation frequency, visitation rate and pollen load during 2012 and 2013. In the second experiment, effect of pollinators on yield of *A. lebbek* was studied in open and cage pollination experiments. Floral visitor fauna of *A. lebbek* included eight-bees, two wasps, two flies, and two butterflies species. Among them, *Apis dorsata*, *Apis florea*, *Amegilla cingulata*, and *Nomia oxybeloides* had maximum abundance ranging from 349–492, 339–428, 291–342 and 235–255 numbers of individuals, respectively during two flowering seasons. *A. dorsata* had the highest visitation frequency (6.44 ± 0.49 – 8.78 ± 0.48 visits/flower/5min) followed by *Amegilla cingulata* (6.03 ± 0.43 – 7.99 ± 0.33 visits/flower/5min) and *A. florea* (3.61 ± 0.31 – 4.44 ± 0.18 visits/flower/5min). *A. dorsata*, *N. oxybeloides*, and *Amegilla cingulata* had the highest visitation rates (18.904 ± 1.53 – 11.43 ± 1.17 flower visited/min) and pollen load (15333 ± 336.22 – 19243 ± 648.45 pollen grains). The open pollinated flowers had significantly higher capsule weight (4.97 ± 0.21 g), seed weight (1.04 ± 0.05 g), seed numbers per pod (9.80 ± 0.34) and seed germination percentage ($84.0 \pm 1.78\%$) as compared to caged flowers. The results suggested bees especially *A. dorsata*, *N. oxybeloides* and *Amegilla cingulata* could be effective pollinators of *A. lebbek*.

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1. Introduction

Insects play a pivotal role in pollination of flowering plants (Kluser et al., 2010; Mallinger and Prasifka, 2017; Vanbergen and Initiative, 2013). According to Klein et al. (2007), >75% of the wild plant species and agriculture crops depends upon insect pollination. Some crops even do not fruit and produce seeds in the absence of insects' visitation to flowers. Insects are also responsible

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for yield increase in self-pollinated and cross pollinated crops, and ensure global food supply and other services to mankind, for review see [Crenna et al. \(2017\)](#).

Albizia is very important genus belonging to family Fabaceae and sub-family Mimosoideae. The members of this family are fast growing trees and shrubs that are mostly found in tropical and subtropical areas of Asia and Africa ([Allen and Allen, 1981](#); [Council, 1979](#)). Indian siris, *A. lebbeck* (L.) Benth. (Fabaceae: Mimosoideae) is native to Asia and is abundantly located India, Burma, Sri Lanka, Bangladesh including Pakistan ([Babu et al., 2009](#)). This species is known for its multiple uses for example its wood is used for making different furniture items and agriculture implements. The gum obtained from its cut bark is a substitute for gum got from *Acacia senegal* Willd. ([Council, 1979](#); [Farooqi and Kapoor, 1968](#)). The other parts of *A. lebbeck* (leaves, seeds, bark and roots) are used for making different traditional medicines to cure cancer, blood diseases, piles, paralysis and many other diseases of skin teeth and gums ([Ganguly and Bhatt, 1993](#); [Kumar et al., 2007](#); [Tripathi et al., 1979](#); [Verma and Srivastav, 2011](#)). Apart from all its uses, it is also planted as a shade tree and host for lac insects ([Venkataramany, 1968](#)).

The flowers of *A. lebbeck* are cream-coloured mimosa-like with long stamens and appeared on lateral stalks in round clusters during April to May ([Parrotta, 1988](#)). Its flowers are self-compatible plant, but for flower tripping, it requires pollinator's visitation ([Lowry et al., 1994](#)). Although, importance of insect visitors has been proposed in the pollination of *A. lebbeck* but none have conducted detailed studies on its different floral visitors. The visitation and activity rate of pollinators varies among pollinators hence influence the fruit and seed setting in plants ([Cane, 2002](#); [Cane and Schiffhauer, 2003](#)). In the current study, we hypothesized that insects play a significant role in the pollination of *A. lebbeck*. We documented different floral visitors of *A. lebbeck* along with their visitation frequencies and rates for the first time. We also experimentally proved the effectiveness of floral visitors on the yield parameters of *A. lebbeck*.

2. Materials and methods

2.1. Study site

The studies were conducted at Dera Ghazi Khan (longitude 70° 29' 7" E and latitude 29° 57' 38" N) Punjab, Pakistan. The climate of Dera Ghazi Khan is tropical to sub-tropical with erratic rainfall, low humidity, long summer and very short winter. The site has mix plantation of many tree species including *A. lebbeck*, *Acacia arabica*, *Dalbergia sissoo*, *Prosopis cineraria* and *Eucalyptus* spp.

2.2. Foraging behavior of floral visitors

The foraging behavior of floral visitors was assessed in term of their abundance (total numbers of individuals of species in an area), visitation frequency (numbers of visits/flower/5 min) and visitation rate (numbers of flowers visited/min) by following the methodology of [Tidke and Tidke and Thorat \(2011\)](#) and [Saeed et al. \(2012\)](#). The study was conducted during the flowering season (April–May) 2012 and 2013. For visitation frequency, 200 branches from 40 plants were selected randomly each year. Each branch was observed for five minutes at three times of the day (08:00, 12:00 and 16:00 h) to count the numbers of visits of different visitors per flower. The data of visitation frequency were taken on weekly basis for a total of seven weeks during each season. For visitation rate, each floral visitor species was observed at three times a day (08:00, 12:00, and 16:00 h). A total of 40 readings of each visitor were taken with the help of stop watch during each flowering sea-

son. For calculation of visitation frequency and visitation rate of each floral visitor, the data of three readings of each day (08:00, 12:00 and 16:00 h) was pooled together to obtain one reading for one day and then used for data analysis.

2.3. Identification of floral visitors

The different floral visitors of *A. lebbeck* were captured with the help of sweep net, killed by ethyl acetate fumes in killing bottle and preserved in 80% ethanol solution for future identification. Each floral visitor was identified using keys of [Michener \(2000\)](#) and [Vockeroth \(1969\)](#) up to at least genus level. However, identification to species level was done with the help of an expert (see acknowledgment).

2.4. Pollen load analysis

The analysis of pollen load on each floral visitor was done by following the methodology of [Latif et al. \(2016\)](#). Briefly, we carefully captured 40 samples of each floral visitor with the help of sweep net, killed and preserved glass vials separately in ethanol as described earlier. We did all this process with great care to avoid loss of pollen grains from the bodies of floral visitors. Ethanol was added into each vial to make 10 mL volume. A small quantity of detergent was also added into each vial to remove maximum numbers of pollen grains from the body of each floral visitor. After this, 7 μ L volume was taken with the help of micropipette and transferred to haemocytometer. The numbers of pollen in 7 μ L suspension were counted by observing haemocytometer under microscope and then total numbers of pollen grains in 10 mL suspension were calculated using the methodology of ([Sutyemez, 2011](#)).

2.5. Reproductive success of *A. lebbeck* in open and cage pollination experiment

We explored the reproductive success of *A. lebbeck* in open and caged pollination experiments. For open pollination experiment, 40 flower buds from 20 plants were selected and tagged at the start of flowering seasons. The flowers were kept open so that any floral visitor (especially bees in this case) can visit the flowers. However, for cage pollination experiment, 40 flower buds from 20 plants were selected and covered with nylon mesh cages (Seedburo Equipment Co., Des Plaines, IL, USA). The nylon mesh cages ensured the air passage but prevented the visitation of flowers by different floral visitors throughout the experiment that resulted in self-pollination. At the end of both experiments, the capsule weight, seed weight and seed numbers per flower in open and cage pollination experiments were recorded. The germination percentage of obtained seeds was also evaluated. For this purpose, we placed five seeds in glass Petri dish having moist filter paper at room temperature and germination percentage of seeds was calculated by following the methodology of [Islam et al. \(2002\)](#).

2.6. Data analysis

The data of floral visitors' characters (visitation frequency, visitation rate and pollen load) and reproductive success parameters in open and cage pollination experiments were subjected to analysis of variance (ANOVA) and their means were separated by Tukey's HSD by using Statistix version 8.1 ([McGraw-Hill, 2008](#)).

3. Results

3.1. Floral visitors of *A. lebbeck*

The floral visitors of *A. lebbeck* are presented in Table 1 and belonged to insect orders Hymenoptera, Diptera, and Lepidoptera.

3.2. Abundance of floral visitors

Apis dorsata (349–492 numbers) was the most abundant species on the flowers of *A. lebbeck* followed by *Amegilla cingulata* (339–428 numbers), *A. florea* (291–342 numbers) and *Nomia oxybeloides* (235–255 numbers). However, *Paragus serratus* (38–59 numbers), *Ceratina sexmaculata* (57–63 numbers) and *Megachile bicolor* (50–73 numbers) were the least abundant species during 2012 and 2013 (Table 2).

3.3. Visitation frequency of floral visitors

Floral visitors differed significantly in term of visitation frequency ($F_{13,91} = 62.96$; $P < 0.001$, 2012 and $F_{13,91} = 62.17$; $P < 0.001$, 2013) with highest values recorded for *A. dorsata* (6.44 ± 0.49 – 8.78 ± 0.48 visits/flower/5min), *Amegilla cingulata* (6.03 ± 0.43 – 7.99 ± 0.33 visits/flower/5min) and *A. florea* (3.61 ± 0.31 – 4.44 ± 0.18 visits/flower/5min) during 2012 and 2013. *M. bicolor* had significantly low visitation frequency (0.91 ± 0.05 – 1.31 ± 0.12 visits/flower/5 min), followed by *P. serratus* (0.94 ± 0.15 – 1.56 ± 0.22 visits/flower/5 min), *C. sexmaculata* (1.01 ± 0.11 – 1.12 ± 0.11 visits/flower/5 min) (Table 3).

Table 1
Abundance of different pollinator species of *Albizia lebbeck* during 2012 and 2013.

Floral visitor	Order	Taxonomic group	Family
<i>Amegilla cingulata</i>	Hymenoptera	Bees	Apidae
<i>Amegilla sp.1</i>	Hymenoptera	Bees	Apidae
<i>Apis dorsata</i>	Hymenoptera	Bees	Apidae
<i>Apis florea</i>	Hymenoptera	Bees	Apidae
<i>Ceratina sexmaculata</i>	Hymenoptera	Bees	Apidae
<i>Xylocopa aestuans</i>	Hymenoptera	Bees	Apidae
<i>Megachile bicolor</i>	Hymenoptera	Bees	Megachilidae
<i>Nomia oxybeloides</i>	Hymenoptera	Bees	Halictidae
<i>Vespa dorylloides</i>	Hymenoptera	Wasp	Vespidae
<i>Vespa orientalis</i>	Hymenoptera	Wasp	Vespidae
<i>Ischiodon scutellaris</i>	Diptera	Fly	Syrphidae
<i>Paragus serratus</i>	Diptera	Fly	Syrphidae
<i>Eurema hecabe</i>	Lepidoptera	Butterfly	Pieridae
<i>Zizeeria krasandra</i>	Lepidoptera	Butterfly	Lycaenidae

Table 2
Abundance of different pollinator species of *Albizia lebbeck* during 2012 and 2013.

Floral visitor	2012	2013
<i>Amegilla cingulata</i>	428	339
<i>Amegilla sp.1</i>	69	90
<i>Apis dorsata</i>	492	349
<i>Apis florea</i>	291	342
<i>Ceratina sexmaculata</i>	63	57
<i>Xylocopa aestuans</i>	209	228
<i>Megachile bicolor</i>	73	50
<i>Nomia oxybeloides</i>	235	255
<i>Vespa dorylloides</i>	156	184
<i>Vespa orientalis</i>	149	99
<i>Ischiodon scutellaris</i>	87	75
<i>Paragus serratus</i>	59	38
<i>Eurema hecabe</i>	80	114
<i>Zizeeria krasandra</i>	215	270

Table 3

Visitation frequency (numbers of visits/flower/5 min, Mean \pm SEM) of different pollinator species of *Albizia lebbeck* during 2012 and 2013. Means sharing the same letters are not significantly different ($P > 0.05$; Tukey's HSD, Statistix 8.1).

Floral visitor	2012	2013
<i>Amegilla cingulata</i>	7.99 ± 0.33 A	6.03 ± 0.43 A
<i>Amegilla sp.1</i>	1.42 ± 0.20 D-F	1.25 ± 1.00 C
<i>Apis dorsata</i>	8.78 ± 0.48 A	6.44 ± 0.49 A
<i>Apis florea</i>	3.61 ± 0.31 BC	4.44 ± 0.18 B
<i>Ceratina sexmaculata</i>	1.13 ± 0.11 F	1.02 ± 0.11 C
<i>Xylocopa aestuans</i>	3.73 ± 0.26 BC	4.02 ± 0.29 B
<i>Megachile bicolor</i>	1.31 ± 0.12 EF	0.91 ± 0.05 C
<i>Nomia oxybeloides</i>	4.19 ± 0.32 B	6.17 ± 0.36 A
<i>Vespa dorylloides</i>	2.83 ± 0.15 B-D	3.44 ± 0.17 B
<i>Vespa orientalis</i>	2.66 ± 0.38 C-E	1.83 ± 0.14 C
<i>Ischiodon scutellaris</i>	1.57 ± 0.39 D-F	4.67 ± 0.36 B
<i>Paragus serratus</i>	1.56 ± 0.22 D-F	0.94 ± 0.15 C
<i>Eurema hecabe</i>	1.43 ± 0.34 D-F	1.37 ± 0.05 C
<i>Zizeeria krasandra</i>	3.83 ± 0.35 BC	2.07 ± 0.23 C

3.4. Visitation rate of floral visitors

Visitation rates of different floral visitors differed significantly during two years ($F_{13,91} = 25.59$; $P < 0.001$, 2012 and $F_{13,91} = 14.50$; $P < 0.001$, 2013). The highest values were recorded for *A. dorsata* (14.53 ± 1.27 – 18.91 ± 1.53 flower visited/min) followed by *Amegilla cingulata* (15.37 ± 2.41 – 16.16 ± 0.54 flower visited/min) and *N. oxybeloides* (11.43 ± 1.17 – 12.54 ± 0.85 flower visited/min) during 2012 and 2013. Statistically low visitation rates were observed for *P. serratus* (3.57 ± 0.55 flower visited/min) and *Zizeeria krasandra* (5.62 ± 0.79 flower visited/min) during 2012 followed by *C. sexmaculata* (5.61 ± 0.20 – 5.65 ± 0.23 flower visited/min) and *Vespa dorylloides* (5.72 ± 0.64 – 5.84 ± 0.66 flower visited/min) during 2012 and 2013 (Table 4).

3.5. Pollen load of floral visitors

Floral visitors were also significantly different in term of pollen load on their bodies ($F_{13,247} = 114.67$; $P < 0.001$, 2012 and $F_{13,247} = 122.51$; $P < 0.001$, 2013). Pollen load was maximum on the bodies of *A. dorsata* (18298 ± 815.92 – 19243 ± 648.45) followed by *N. oxybeloides* (16028 ± 418.93 – 17449 ± 426.73) and *A. cingulata* (15333 ± 336.22 – 16229 ± 410.06). *Ischiodon scutellaris* had statistically the lowest numbers of pollen grains (4705 ± 186.02 – 4860 ± 174.63) on its body followed by *V. orientalis* (6124 ± 338.12 – 6390 ± 262.34), *Z. krasandra* (6295 ± 285.56 – 6564 ± 291.99) and *P. serratus* (6250 ± 371.22 – 7195 ± 172.82) in two years of study (Table 5).

Table 4

Visitation rate (numbers of flower visited/min, Mean \pm SEM) of different pollinator species of *Albizia lebbeck* during 2012 and 2013. Means sharing the same letters are not significantly different ($P > 0.05$; Tukey's HSD, Statistix 8.1).

Floral visitor	2012	2013
<i>Amegilla cingulata</i>	16.16 ± 0.54 A	15.37 ± 2.41 AB
<i>Amegilla sp.1</i>	7.58 ± 0.65 C	6.33 ± 0.24 D
<i>Apis dorsata</i>	14.53 ± 1.27 AB	18.91 ± 1.53 A
<i>Apis florea</i>	6.42 ± 0.30 CD	10.49 ± 0.67 B-D
<i>Ceratina sexmaculata</i>	5.65 ± 0.23 CD	5.61 ± 0.20 D
<i>Xylocopa aestuans</i>	6.11 ± 0.63 CD	8.98 ± 1.64 CD
<i>Megachile bicolor</i>	5.85 ± 0.49 CD	7.35 ± 0.38 D
<i>Nomia oxybeloides</i>	11.43 ± 1.17 B	12.54 ± 0.85 BC
<i>Vespa dorylloides</i>	5.72 ± 0.64 CD	5.84 ± 0.66 D
<i>Vespa orientalis</i>	6.79 ± 0.83 CD	7.25 ± 0.87 D
<i>Ischiodon scutellaris</i>	6.34 ± 0.69 CD	5.99 ± 0.14 D
<i>Paragus serratus</i>	3.57 ± 0.55 D	9.89 ± 0.86 CD
<i>Eurema hecabe</i>	5.74 ± 0.40 CD	6.40 ± 0.37 D
<i>Zizeeria krasandra</i>	5.62 ± 0.79 CD	8.74 ± 1.00 CD

Table 5

Pollen load (Numbers of pollen grains/visitor, Mean \pm SEM) of different pollinator species of *Albizia lebbek* during 2012 and 2013. Means sharing the same letters are not significantly different ($P > 0.05$; Tukey's HSD, Statistix 8.1).

Floral visitor	2012	2013
<i>Amegilla cingulata</i>	16229 \pm 410.06 AB	15333 \pm 336.22 C
<i>Amegilla</i> sp.1	15572 \pm 244.94 BC	15772 \pm 372.70 BC
<i>Apis dorsata</i>	18298 \pm 815.92 A	19243 \pm 648.45 A
<i>Apis florea</i>	14116 \pm 431.22 C	14095 \pm 495.53 C
<i>Ceratina sexmaculata</i>	8754 \pm 524.25 DE	11689 \pm 689.68 D
<i>Xylocopa aestuans</i>	14529 \pm 665.98 BC	15164 \pm 524.94 C
<i>Megachile bicolor</i>	15019 \pm 383.41 BC	14332 \pm 364.73 C
<i>Nomia oxybeloides</i>	16028 \pm 418.93 BC	17449 \pm 426.73 AB
<i>Vespa dorylloides</i>	7385 \pm 385.03 EF	7818 \pm 322.86 EF
<i>Vespa orientalis</i>	6124 \pm 338.12 FG	6390 \pm 262.34 FG
<i>Ischiodon scutellaris</i>	4705 \pm 186.02 G	4860 \pm 174.63 G
<i>Paragus serratus</i>	6250 \pm 371.22 FG	7195 \pm 172.82 F
<i>Eurema hecabe</i>	10609 \pm 247.45 D	9353 \pm 389.30 E
<i>Zizeeria krasandra</i>	6295 \pm 285.56 FG	6564 \pm 291.99 FG

3.6. Reproductive success of *A. lebbek* in open and cage pollination experiment

3.6.1. Pod weight

The weight of *A. lebbek* pods was significantly different in open and caged pollinated flowers ($F_{1,39} = 112.43$; $P < 0.001$). Statistically higher pod weight was observed in open pollinated flowers (4.97 ± 0.21 g) as compared to the flowers deprived of floral visitors in cages (2.61 ± 0.13 g).

3.6.2. Seed weight

Significant differences were observed in open-pollinated flowers and caged pollinated flowers in term of seed weight ($F_{1,39} = 203.98$; $P < 0.001$). Seeds obtained from open-pollinated flowers had significantly higher seed weight (1.04 ± 0.05 g) compared to those that were recovered from caged flowers (0.41 ± 0.02 g).

3.6.3. Seed number

The numbers of seeds per pod in open and caged flowers differed significantly ($F_{1,39} = 44.82$; $P < 0.001$) with statistically more numbers of seeds produced in open-pollinated flowers (9.80 ± 0.34 numbers) as compared to seeds produced in flowers in cages deprived of pollinators (6.47 ± 0.38 numbers).

3.6.4. Seed germination

Germination (%) was significantly higher ($F_{1,39} = 57.90$; $P < 0.001$) in seeds obtained from open pollinated flowers ($84.0 \pm 1.78\%$) compared to seeds recovered from flowers in cages deprived of pollinators ($57.0 \pm 2.73\%$).

4. Discussion

A. lebbek is an important tree in tropical and sub-tropical areas of the world. It is grown for shade, timber, soil conservation, forage for ruminants and for making medicines (Everist, 1986; Gabhane et al., 1995; Ganguly and Bhatt, 1993; Keating and Bolza, 1982). Gupta (1993) stated that the flowers of *A. lebbek* are rich source of light-coloured honey but none have studied floral visitors. In the current study, we investigated different floral visitors of *A. lebbek* for the first time. We recorded 14 types of insects from its flowers including eight bee species, two wasps, two flies and two butterflies. Bees had highest abundance, visitation frequency, visitation rate and pollen load as compared to other floral visitor groups. Higher yield was obtained in open pollinated flowers as compared to flowers that were deprived of floral visitors in cages.

Based on the findings, bees were regarded as the efficient pollinators of *A. lebbek*.

Our results showed that five bee species, *A. dorsata*, *Amegilla cingulata*, *A. florea*, *N. oxybeloides* and *Xylocopa aestuans* were the most abundant species among all other floral visitors. These five species have been reported as the most abundant species in various crops. According to Anderson and Symon (1988), the species belonging to genus *Nomia* and *Amegilla* were most abundant on the flowers of *Solanum* in Australia. However, *A. dorsata* and *A. florea* have been reported as the most abundant species on the flowers of bitter melon (Saeed et al., 2012), canola (Akhtar et al., 2018; Ali et al., 2011; Kumar and Singh, 2005; Shakeel et al., 2019).

The visitation frequency and rate are often used to determine the pollination effectiveness of floral visitors (Proctor et al., 1996; Singh et al., 2006). The floral visitors having higher values of visitation frequency and rate are considered as effective pollinators of flowering plants (Zameer et al., 2017). In the current study, the visitation frequency and rate values were higher for *A. dorsata*, *Amegilla cingulata*, *N. oxybeloides* and *A. florea* (Tables 3 and 4). Our results are similar to several previous studies. For example, *A. dorsata* and *A. florea* had higher values of visitation frequency and rate on various crops (Saeed et al., 2012; Siregar et al., 2016). In another study, Ali et al. (2011) observed higher visitation rate of *Nomia* sp. on the flowers of pumpkin as compared to all other pollinators. *Amegilla* sp. was the most frequent visitor (along with highest visitation rate) and was regarded as effective pollinators of *Capparis aphylla* flowers in Dera Ghazi Khan, Pakistan (Latif et al., 2017).

Among the other factors that determine the pollination of efficiency of floral visitors, pollen load is also very important (Canto-Aguilar and Parra-Tabla, 2000). In the current study, maximum numbers of pollen grains were recovered from the bodies of *A. dorsata*, *N. oxybeloides* and *Amegilla cingulata*. In previous study, Ali et al. (2011) found that *Nomia* sp. and *A. dorsata* deposited maximum numbers of pollen grains (376.60 ± 23.01 , 204.15 ± 20.63) on the stigma of pumpkin flowers and were considered as the most efficient pollinators as compared to all other pollinators.

The pollination experiment suggested that pollinators had significant effect on the pollination of *A. lebbek*. The flowers that were mostly visited by bees in open pollination experiment had significantly higher capsule weight, seed weight, seed numbers and better seed germination percentage as compared to flowers that were deprived of pollinators in cages. Our results are similar to the findings of Free (1966) who obtained higher numbers of bean seeds in open pollinated flowers as compared to flowers in cages without insect visitation. In other study, higher pods and seeds numbers per pod, seed weights and germination was observed in open pollinated flowers in comparison to caged *Brassica* flowers (Atmowidi et al., 2007). This could be due to the fact that more insect visitation on the flowers accompanied by higher pollen load increase the chances of cross pollination in open flowers leading to higher yield (Heering, 1993).

5. Conclusion

Bees were the most abundant floral visitors of *A. lebbek* as compared to all other groups. Three bee species, *A. dorsata*, *N. oxybeloides* and *Amegilla cingulata* are considered as effective pollinators of *A. lebbek*. The probable higher visitation frequency, rate and pollen load of above three bees caused significant increase in yield of *A. lebbek*.

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