

## PALYNOLOGICAL ANALYSIS OF POLLEN LOADS FROM POLLEN SOURCES OF HONEYBEES IN ISLAMABAD, PAKISTAN

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### Abstract

Palynological studies of pollen loads were carried out to determine the pollen sources for honeybees in Islamabad during January to December 2006. Three different sources for honeybees were identified as Major, Medium and Minor. A total of 40 plant species served as pollen sources for honeybees from amongst the fruit trees, ornamental and agricultural crop and weeds. Major pollen yielding plants viz., *Brassica campestris* L., *Callistemon citrinus* (Curt.) Stapf., *Citrus sinensis* (L.) Osbek, *Eriobotrya japonica* (Thunb.) Lindlay, *Parthenium hysterophorus* L., *Chenopodium album* L., *Abelmoschus esculentus* (L.) Moench, *Ageratum conyzoides* L., *Bauhinia variegata* L., *Bischofia javonica* Blume., *Bombax ceiba* Burm., *Cassia fistula* L., *Convolvulus arvensis* L., *Coriandrum sativum* L., *Cucumis melo-agrostis* L., *Delbergia sisso* Roxb., *Delphinium ajacis* L., *Euphorbia helioscopia* L., *Eucalyptus cammoldulensis* Dehnh., *Grewia asiatica* L., *Hamelia patens* Jacq., *Helianthus annuus* L., *Iberis amara* L., *Jacrandra mimosifolia* Juss., *Justicia adhatoda* Linn, *Lagerstromia indica* L., *Litchi chinensis* Mill., *Melilotus indica* (L.) All., *Nastertium officinale* R. Br., *Prunus armeniaca* L., *P. persica* Benth., and Hook., *P. bokhariensis* Royle., *Pongammia pinnata* (L.) Pierre., *Raphanus sativa* L., *Rosa* sp., *Rumex dentatis* L., *Silibum merianum* L., *Sonchus asper* Hill., *Trifolium alexandrinum* L., *Verbena tunisecta* L., were identified. Although the foraged plants are available throughout the year in the locality but January to April and July to August are major, whereas April and May are minor flow period of pollen while June is the dearth period. This study gives the general idea of range of plant species that occur in the area and their utility to the honeybees which is important for them so that they can make efficient use of these resources.

### Introduction

The present investigation were undertaken at and around National Agriculture Research Centre and Quaid-i-Azam University Islamabad, which lies between 330° and 340° west latitude and 720°-740° east longitude. It makes the Northeast part of Pothohar Plateau, surrounded by Northeast of Margalla Hills. The annual maximum temperature varies between 20°-30°C and minimum from 10°-14°C. (Azhar, 1979). The maximum and the minimum mean temperature in summer is 36.1°C and 23.5°C, whereas in winter it is 23.5°C and 6.9°C respectively.

The area is rich in plants. In upper part areas the dominant trees are *Pinus willichiana* A.B. Jackson, *Taxus willichiana* Zucc., *Populus ciliate* Wall. ex Royle, *Ulmus villosus* Brandis ex Gamble, *U. wallichiana* Zucc., *Populus ciliate* Wall. ex Brandis, *A. cappadocicum* Gled., *Prunus cornata*, *Cornus microphylla* Wall., *Quercus dilatata* Lindle ex Royle, *Q. incana* Roxb., *Juglans regia* L., and *Viburnum feotens* Decne. The climax vegetation of Margalla hills is subtropical with moist Chir pine forest, *Pinus roxburghii* Sargent, *Cedrela serrata* Royle, *Juglens regia* L., *Diospyros lotus* L., *Olea ferrugenia* Royle, *Quercus incana* Roxb., *Pyrus pashia* Ham. ex Don., *Euonymus pendulus* Wall., *Myrsine*  
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*Africana* L., *Jasminum humile* L., *Berberis lyceum* Royle and *Rosa moschata* J. Herrm. In the lower area trees are *Olea europea* L., *Acacia modesta* Wall., *Cedrella toona* M. Roemer, *Melia azadrechta* L., *Dedonia viscosa* L. Jacq. Further down in the lowest area are *Dalbergia sisso* Roxb., *Morus alba* L., *Acacia modesta* Wall. (Beg, 1985).

Four species of Honeybees viz., *Apis cerana*, *A. dorsata*, *A. florea*, *A. mellifera* are found in Pakistan. *A. cerana* are found in foothills or hills in Northern areas. *A. dorsata* is widely distributed almost throughout the plains, foothills and hills (1200 m height). *A. florea* occur almost throughout Pakistan except deserts and high hills (Rafiq *et al.*, 1978). *A. mellifera* has been imported in Pakistan from Australia and Russia in 1979. They are reared here successfully. Palynological analysis of pollen loads was done in the present study to determine bee flora of an area under study. Palynology is the study of the pollen grain produced by the seed plant (Angiosperm and gymnosperm) and spores produced by Pteridophytes, Bryophytes, algae and fungi (Moore *et al.*, 1991). In present study Pollen analysis of Pollen loads were done to determine the bee flora of area under study.

Pollen load is the accumulation of bee pollen in the granular form. Bee pollen is the flower pollen collected by *Apis mellifera*, for the purpose of feeding its larvae in the early stage of development. Collected flower pollen is accumulated as a pellet or pollen load (corbicular pollen) in pouches on the rear legs of the bee and it is the mixture of these pellets that comprises pollen load (Campos *et al.*, 2005). Bee pollen contains the richest known source of vitamins, minerals, proteins amino acids, hormones, enzymes and fats, as well as significant quantities of natural antibiotics scientific curiosity about the origin of the product elaborated by the bees, as well as commercial advantage in determining their quality, stimulated research activities that use the knowledge of the pollen grain morphology as a tool for investigation. Bees collect pollen from the flower and carry them in their basket of their Hindlegs into the hive for storage in other than honey cells. The pollen grain and their morphological character led to the investigation of the species or the taxa of their origin, as well as the quantity that is indicative of properties (Barth, 2004). Singh (2002) studied the pollen sources to the bees in Uttranchal and found that total sixty plants serve as a sources for the bees. Five minor and three major sources were viz., *Ageratum conyzoides*, *Brassica campestris*, *Eucalyptus cammoldulensi*, *Litchi chinensis*, *Parthenium hysterophorus*, *Trifolium alexandrium*. Several palynological studies of pollen loads in different parts of the world have been carried out but in Pakistan not much work has been done which is reported herein.

## Materials and Methods

**Method of trapping pollen loads:** A total 30 samples of honey bee pollen loads were collected fortnightly from the incoming foraging bees into the hive by using pollen trap in front of the hive at two hours interval throughout the period. These pollen samples were removed from the hind legs of honey bees on a rack fitted in trap, as bees passes through the trap the loads on their legs fall down. After two hours interval traps were removed and pollen loads were collected from the rack fitted in the pollen trap. They were spread on the clean white paper, sorted on basis of colour. Similar samples were placed in a packet after counting and weighing. Each packet was labelled with date, colony number, colour of the load and time of collection. Pollen pellets were stored in the freezers and were analyzed afterwards.

**Table 1. Analysis and frequency of occurrence % pollen loads of colony A.**

Sr no.	Taxon	No. of pollen loads	Percentage	Pollen forage value to honey bees
1.	Brassicaceae	1059	19.45	+++
2.	<i>Bombax ceiba</i>	199	3.65	+++
3.	<i>Citrus</i> sp.	153	2.81	+++
4.	<i>Silibum merianum</i>	268	4.92	+++
5.	<i>Eucalyptus cammoldulensis</i>	378	6.94	+++
6.	<i>Callistemon citrinus</i>	525	9.64	+++
7.	<i>Taraxicum officinalis</i>	13	0.23	+
8.	<i>Trifolium alexandrianum</i>	703	12.91	+++
9.	Asteraceae	35	0.64	++
10.	<i>Calendula arvensis</i>	6	0.11	+
11.	<i>Juticia adhatoda</i>	90	1.65	+++
12.	<i>Parthenium hysterophorus</i>	156	2.86	+++
13.	<i>Chenopodium album</i>	378	6.94	+++
14.	<i>Acacia nilotica</i>	55	1.01	+++
15.	<i>Coriandrum sativum</i>	7	0.12	+
16.	<i>Prunus bokhariensis</i>	38	0.69	++
17.	Spores	17	0.31	+
18.	Type# 1	272	4.99	+++
19.	Type# 2	40	0.73	++
20.	Type# 3	0	0	0
21.	Type# 4	180	3.3	+++
22.	Type# 5	63	1.15	+++
23.	Type# 6	143	2.62	+++
24.	Type# 7	53	0.97	+++
25.	Type# 8	0	0	0
26.	Type# 9	885	16.25	+++
Total		5444	100	

Pollen loads + = 1-20 Minor pollen sources, ++ = 21-50 Medium pollen sources, +++ = Pollen loads >50 Major pollen source

**Collection of pollen loads from corbicula of bee while visiting flowers:** A survey was carried out in National Agriculture Research Council fields around the apiaries for trapping of bees foraging the flowers. Honey bees were caught while they were visiting flowers and collecting pollen from them and were anesthetized with Ethyle acetate. The pollen loads were then pushed off the hind legs into an individual specimen tubes of polythene bags. A data labelled with plant species, date and place was also placed in test tube. The bees were released unharmed or some time killed by using killer bottle. The specimen tubes were kept in dark until the pollen loads were colour matched later the same day. The specimens were stored at about the -30°C until checked microscopically to see the whole pollen loads came from the plant species on the bee had been caught. Slides are prepared by mounting with Basis Fuchsin. Pollen size, shape, Aperture #, Aperture type, Surface sculpturing and exine thickness were studies.

Results

**Palynological analysis of pollen loads:** Total pellets collected in study period were 27425 in which a total of 5444 pollen loads of colony A and 21981 pollen loads of colony B were trapped, stored and analyzed. Pollen loads numbers, percentage and pollen forage value of honey bees are given in Tables 1 & 2. Percent share of honey bee pollen load of major, medium and minor sources are presented in Fig. 1. Comparison of the pollen loads collected during the period of research is presented in Figs. 2 & 3. The analysis show that total 24 plant

species serve as a pollen source to colony A and B. Major quantity of pollen was provided by 17 plants to colony A and B. *Brassica campestris*, *Callistemon citrinus*, *Bombax ceiba*, *Chenopodium album*, *Citrus sativa*, *Trifolium alexandrianum*, *Eucalyptus cammoldulensis*, *Parthenium hysterophorus*, *Chenopodium album* and some unknown plant are major pollen sources to colony A. In colony B *Brassica campestris*, *Trifolium alexandrianum*, *Bombax ceiba* and two unknown plants provide the greater quantity of pollen. Minor quantity of pollen was provided by *Citrus* sp., *Silibum merianum*, *Calendula arvensis*, *Justicia adhatoda*, *Parthenium hysterophorus*, *Acacia nilotica*, *Coriandrum sativum*, *Plumb*, Fungal spores and five unknown plants to colony A and *Bombax ceiba*, *Citrus* sp., *Silibum merianum*, *Eucalyptus cammoldulensis*, *Callistemon citrinus*, *Taraxicum officinalis*, *Calendula arvensis*, *Justicia adhatoda*, *Parthenium hysterophorus*, *Chenopodium album*, *Acacia nilotica*, *Coriandrum sativum*, *Prunus bokhariensis* and other unknown plants to colony B. *Callistemon citrinus* to colony A and *Brassica campestris* in colony B are important pollen sources for whole period studied as they provided large amount of pollen loads to bees. Results were compiled by the help of reference slide made with time following the method used by Noor *et al.*, (2004). During survey of field, the flowers in bloom were collected along the notes and their pollen slides were made in the laboratory. Palynological analysis of bee pollen trapped while bees are foraging on the flower are presented in Table 1. Microphotographs of pollens were taken at 1000x. Photos (1-8).

**Table 2. Analysis and frequency of occurrence % pollen loads of colony.**

Sr no.	Taxon	No. of pollen loads	Percentage	Pollen forage value to honey bees
1.	Brassicaceae	2810	12.78	+++
2.	<i>Bombax ceiba</i>	309	1.40	+++
3.	<i>Citrus</i> sp.	319	1.45	+++
4.	<i>Silibum merianum</i>	1330	6.05	+++
5.	<i>Eucalyptus cammoldulensis</i>	1451	6.60	+++
6.	<i>Callistemon citrinus</i>	2376	10.8	+++
7.	<i>Taraxicum officinalis</i>	591	2.68	+++
8.	<i>Trifolium alexandrianum</i>	1187	5.40	+++
9.	Asteraceae	0	0	0
10.	<i>Calendula arvensis</i>	38	0.17	++
11.	<i>Justicia adhatoda</i>	87	0.39	+++
12.	<i>Parthenium hysterophorus</i>	484	2.20	+++
13.	<i>Chenopodium album</i>	752	3.42	+++
14.	<i>Acacia nilotica</i>	322	1.46	++
15.	<i>Coriandrum sativum</i>	57	0.25	++
16.	Plum	710	3.23	++
17.	Spores	574	2.61	++
18.	Type # 1	236	1.07	+++
19.	Type # 2	124	0.56	+++
20.	Type # 3	1520	6.91	+++
21.	Type # 4			
22.	Type # 5	13	0.05	+
23.	Type # 6			
24.	Type # 7	49	0.22	++
25.	Type # 8	189	0.85	+++
26.	Type # 9	6453	29.35	+++
<b>Total</b>		<b>21981</b>	<b>100</b>	

Pollen loads ++= 1-20 Minor pollen sources, += 21-50 Medium pollen sources, +++= Pollen loads >50 Major pollen source

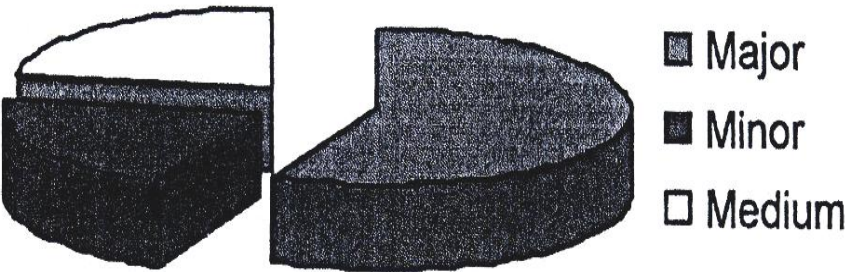


Fig. 1. Percentage share of honey bee pollen loads of major, medium and minor sources of colony A and B.

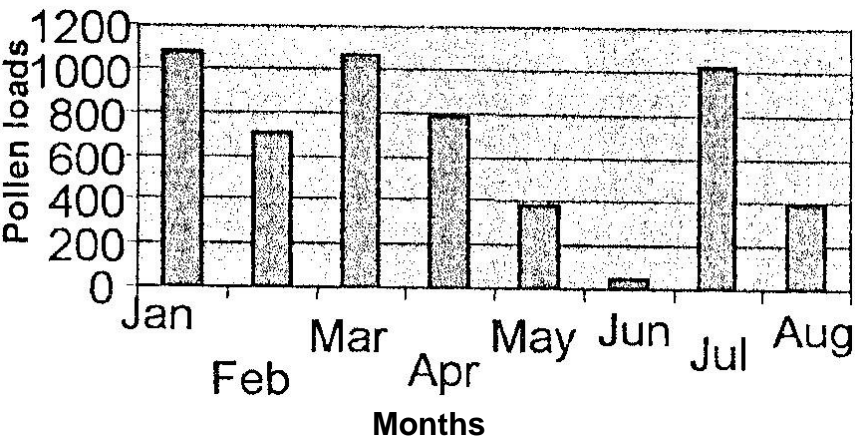


Fig. 2. Month wise collection of pollen loads samples of colony A.

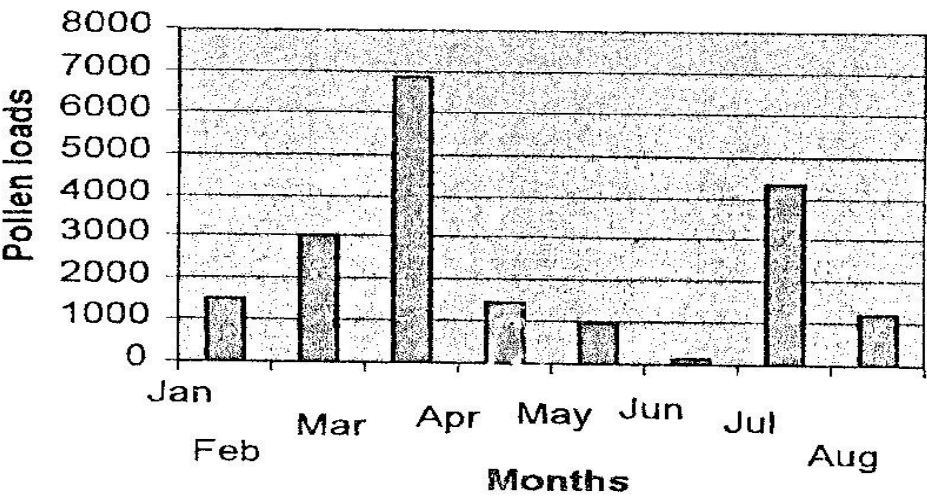


Fig. 3. Month wise collection of pollen loads samples of colony B.

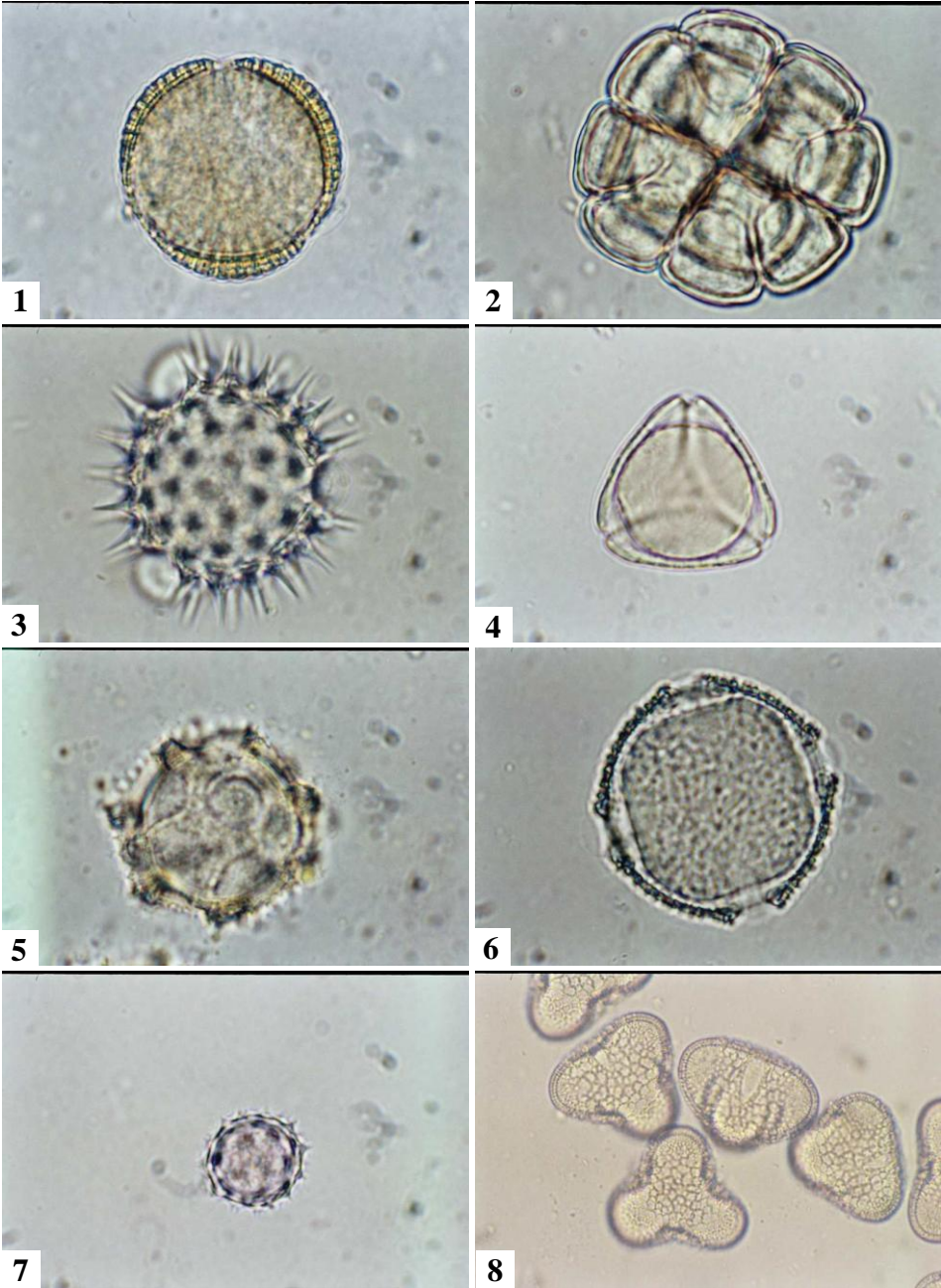


Fig. 4. Microphotographs of pollens in pollen pellets  
1.*Brassica campestris*. 2.*Acacia modesta*. 3.*Silibum merianum*. 4.*Callistemon citrinus*. 5.  
*Taraxicum officinalis*. 6.*Citrus arvensis*. 7.*Parthenium hysterophorus*. 8.*Bombax ceiba*.

## Discussions

An attempt has been made to bring together the available information since knowledge of bee plant species is necessary for the beekeeper so that they can make an efficient use of these resources for honey production. The enumeration does, however, give a general idea of the range of plant species that occur in the region and their utility to bees. Analysis of pollen loads reveal that this region has a good potentiality for the development of beekeeping activities and bee keepers can collect surplus honey during the blooming period. It has been observed that in Islamabad, from January to March, which is a winter season the temperature falls to 0 °C. The activities of bees initially are slow but as most of the plants are in full bloom mostly at the end of February till April it was observed that the colonies construct new combs, build up very rapidly with intensified brood rearing activity. They start rearing queen cells (Naim & Rhodke, 1976). During this period, *Brassica campestris* and *Callistemon citrinus* are exploited the most but as the season changes there is a decline found in their attractiveness to the bees and that attractiveness was overtaken by the *Silibum merianum*, *Taraxicum officinalis*, *Eucalyptus cammoldulensis* and *Citrus arvensis*. It has been concluded after observing change in strategy of foraging by the bees that there is a continuous pattern of flowering sequence coupled with maximum exploitation of nectar and pollen and there is a rapid adaptability which is reflected in above results. As the spring season ends and the summer start, *Trifolium alexandrianum* and *Justicia adhatoda* were found to be exploited most and with the change in season as the summer ends their preference turns to *Parthenium hysterophorus* and *Chenopodium album*. Keeping in view the importance of plants yielding more nectar and pollen than the herbaceous plants as revealed in the results, it is recommended that plants like *Acacia modesta*, *Callestemon citrinus*, *Dalbergia sissoo*, *Eucalyptus cammoldulensis* etc., should be planted on Avenue along side roads, canals, and railway tracks, around the agricultural fields and gardens. Such plants will certainly provide plenty of bees pasturage in addition to the much needed timber and fruit. The shelter and aesthetic value of these plants will be an added use. It has been found that the climate and other factor of ecology in Pakistan are suitable for growth of the rich flora.

## References

- Azhar, J. 1979. A detailed gravity survey to delineate and demarcate the subsurface structure in the vicinity of Quaid-e-Azam University. *M.Sc (geophysics dissertation)*, pp.18-35.
- Barth, O.M. 2004. Pollen analysis of honey, pollen loads and propolis in Brazil. *Science Agricola.*, 61(3) : 342-350.
- Beg, A.R. 1985. Agro-ecological zonation of Potohar. National Agriculture Research Council, Islamabad, 3:17-19.
- Campos, M.G.R., B.A.M. Stephan, S. Teresa and M. Yannia. 2005. *International Beekeeping Congress*, Nov 13-18. Bangalore, India.
- Moore, P. D. 1991. *Pollen analysis*, 2<sup>nd</sup>ed. Blackwell Scientific Publications, Oxford, London. pp. 280-352.
- Naim, M. and K.C. Rhodka. 1976. Bee flora and seasonal activity of *Apis indica* at Pusa. *Indian Bee Journal.*, 38: 13-19.
- Noor, M.J., M. Ahmed, R. Asghar, A. Kanwal and S. Pervaiz. 2004. Palynological studies of Cultivated plant species of University of Arid Agriculture, Rawalpindi, Pakistan. *Asian J. of Plant Sci.*, 3: 467-479.
- Rafiq, A., R. Anwar, S. Khan, S.M.A. Makhdoomi and A Qayyum. 1978. Honey Bee. Flora of Pakistan, *Agriculture Pakistan*, 29(4):
- Singh, R.K. 2003. Studies on pollen and nectar sources to honey bees at Dehradun, Uttaranchal, India *Asian Bee Journal*, 5: 129-138