Research Article

Pollen Spectrum and Biochemical Analysis of Dominant Pollen Types Represented by Local Honey Samples

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ABSTRACT

A pollen spectrum of honey provides a basis for identifying the origin of a honey in terms of locality and floral resources. This information may be used to develop analytical standards for pollen, contributing to quality control of a honey. Qualitative and quantitative biochemical analysis of pollen grains of fifteen plant species was undertaken during the year 2006-2014. The dominant pollen types were Brassica campestris, Moringa oleifera, Syzygium cumini, Coriandrum sativum, and Helianthus annuus. The pollen samples were biochemically investigated for carbohydrates and sugars, free amino acids, protein, free lipids, moisture and ash contents. The maximum amount of reducing sugar and total carbohydrates was found to be 3.08% and 5.76% respectively in Vernonia cineria pollen grains. The crude protein and soluble protein i.e. 48.4% and 32.1% was found in pollen of Parthenium hysterophorus. Major free amino acids 3.09% and lipid 4.10% contents were encountered in Helianthus annuus pollen. The moisture and ash percentage was 12.83% and 7.05% in Tridax procumbens and Brassica campestris respectively.

Some biochemicals are found to be responsible for the visits of bees in general and honey bees (A. dorsata) in particular. The data obtained through pollen biochemical analysis is being interpreted with pollen frequency class and the honey bee visits. Moringa oleifera, Helianthus annuus, Ricinus communis and Parthenium hysterophorus pollen types were dominantly represented in honey samples which have more amount of protein, carbohydrates and amino acids.

Keywords: Biochemical analysis, honey bee, pollen analysis, pollen spectrum

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INTRODUCTION

In the course of evolution a special relationship has been developed between the plants and the bees. The flower provides pollen and nectar as a food to visiting bees while the bees in the course of wandering from flower to flower provide a vehicle for pollen transfer leading to pollination. Pollen is a major source of proteins, fatty substances, minerals and vitamins for the honey bees. Bees deliberately collect the pollen grains to fulfill their protein requirement and store them in pollen chambers in the hive [1]. Furthermore, pollen provides proteins for bees required for building their body tissues especially during early embryonic growth [2].

Pollen is a convenient food source which requires a minimum of adaptations on the part of the users, almost every insect may use it [3]. Pollen grains contain number of metabolites which are essential for different physiological and metabolic activities during growth and development of the pollen [4].

Honey bees collect nectar and pollen from the flowers that provide the nutrients necessary for colony maintenance and development. Nectar is processed to form honey, the major energy source for the colony. Pollen is a source of protein and amino acids for the colonies. The quantity and quality of pollen collected by honey bees affects the reproduction, brood rearing and longevity, thus ultimately the productivity of the colony [5].

Apart from small quantities in nectar, honeybees obtain all the essential nutrients which they need for brood rearing and adult

growth and development from pollen grains [6, 7]. The pollen grains serve as a food for the brood of honey bees and other insect pollinators. The proportion of these nutrients can vary widely amongst the pollen grains of different plant species [8].

There are only few reports pertaining to the studies on biochemical analysis of pollen grains and its relevance to foraging behavior of honey bees [9-15].

During the present investigation, fifteen different pollen types which were dominantly represented by local honey samples; were undertaken for biochemical assessment. The objective of the study is to know the different biochemicals present in the pollen grains of bee pasture plants and to know its relevance with foraging behaviour.

MATERIALS AND METHODS

For the present investigation pollen grains of those plant species were selected, which dominantly represented the pollen spectrum of local honey samples. To know the pollen types, honey samples were analyzed and slides were prepared [16 & 17]. Honey samples were collected from wild *Apis dorsata* colony located at different study sites of Amravati district (MS), India during the period September 2006 to December 2014.

To determine the botanical origin of honey and the percentage of different pollen types, qualitative and quantitative analysis was carried out [16,18]. Pollen preparation was made and observed under the microscope for morphological characterization [19,20].

Pollen grains of dominant pollen types belonging to fifteen selected plant species i.e. *Brassica campestris* L. (Brassicaceae), *Gossypium hirsutum* L. (Malvaceae), *Azadirachta indica* A. Juss. (Meliaceae), *Moringa oleifera* Lamk. (Moringaceae), *Butea monosperma* (Lamk.) Taub.; *Cajanus cajan* (L.) Millsp. (Fabaceae), *Syzygium cumini* (L.) Skeels (Myrtaceae), *Coriandrum sativum* L. (Apiaceae), *Helianthus annuus* L.; *Parthenium hysterophorus* L.; *Tridax procumbens* L.; *Vernonia cineria* (L.) Less. (Asteraceae), *Ipomoea fistulosa* Mart. ex Choisy (Convolvulaceae), *Vitex negundo* L. (Verbenaceae) and *Ricinus communis* L. (Euphorbiaceae) were collected in bulk during the morning before anthesis and sun-dried.

The sun-dried pollen samples were then subjected to biochemical analysis.

Biochemical analysis of pollen grains

The analysis of pollen was carried out for crude proteins, carbohydrates, lipids, amino acids, moisture and ash content.

Total reducing sugars have been quantified using Dinitrosalicylic acid reagent method [21]. The intensity of dark red colour was recorded using UV-Vis. Spectrophotometer (Shimadzu UV-1650PC). The carbohydrates have been quantified according to Anthrone method [22]. For the determination of total nitrogen, 50 mg of pollen samples were digested by Micro-Kjeldahl method [23]. The amount of crude protein was estimated by using 1:6.25 factor. Soluble protein was estimated following the Lowry method using Bovine serum albumin through determination of standard curve [24].

The lipid content was determined as crude fat from a solvent extract [14]. A known amount of sample was weighted out and extracted in 1:2 Chloroform-methanol mixtures and the lipid fraction was estimated from the difference in weight [25]. Moisture content in pollen was determined by difference in weight after oven drying at 105^0C. The pollen ash was prepared by using blast furnace at 500 to 600^0C and weighted after cooling. The Histochemical analysis was carried out to study pollen starch and lipid contents. The starch contents were analyzed by using IKI solution and lipids were tested by using Sudan IV dye [3].

The total content of free amino acids in each sample was determined by using a spectrophotometric procedure based on the reaction of amino acids with a ninhydrin solution [26]. For the extraction and analysis of the free amino acids, 500 mg of pollen sample was homogenized with 5–10 ml of 80% ethanol and centrifuged [27]. The supernatant was preserved and the extraction was repeated twice with the residue. This extract was used for quantitative estimation of total free amino acids with ninhydrin reagent and the intensity of the violet colour developed was read using spectrophotometer (Shimadzu UV-1650PC) and was compared with pure

reagent. A calibrated solution of glycine was used as standard.

Qualitative analysis of the free amino acids was carried out by thin layer chromatography (TLC) on DC-Alufolien Kieselgel 60 aluminium sheets (Merck) using n-butanol: acetic acid: water (80:20:20 v/v) as eluant [28]. Then, 0.1% ninhydrin in acetone was used for the detection of amino acids by heating the sheets at 110°C for 5 minutes and the Rf values were calculated [29].

Thin layer chromatography (TLC) of major sugars has been carried using using n-butanol: acetic acid: water (40:10:50 v/v) as eluant [28]. Then 5:5:1 of 1% Aniline, 1% Diphenylamine and Orthophosphoric acid was used for detection of sugars.

Honey Bee visit

The target plants were selected from the field to know the honey bee visits.

OBSERVATIONS AND RESULTS

1. During the present investigations, honey samples were subjected to pollen analysis, in which eight pollen types were identified as secondary dominant types and seven types were considered as important minor types (**Table 1**). The secondary dominant pollen types were *C. cajan, M. oleifera, P. hysterophorus, V. cineria, Gossypium* sp., *H. annuus, S. cumini* and *A. indica.* The important minor types were *V. negundo, R. communis, T. procumbens, Ipomoea* sp., *B. campestris, C. sativum* and *B. monosperma.* Some pollen types were found to be as common in both secondary dominant and important minor types i.e. *P. hysterophorus, C. cajan, Gossypium* sp., *V. cineria* and *S. cumini* (**Table 1**).

Table 1: Dominant pollen types represented

S. N.	Honey Sample No.	Date of Collection	Site of Collection	Pollen types in different frequency classes	
				Secondary (16 – 45%)	Important Minor (3-15%)
1.	MGT-SUS-01	10/09/06	Susarda (Dharni)	Acanthaceae type, *Moringa oleifera, Terminalia* sp.	*Ageratum conyzoides, Justicia procumbens, Parthenium hysterophorus, Prosopis juliflora, Tridax procumbens*
2.	MGT-SUS-02	13/10/06	Susarda (Dharni)	Acanthaceae type, Asteraceae type, Poaceae type	*Moringa oleifera, Syzygium cumini*
3.	POH-SAV-03	12/11/06	Vithoba Savanga (Chandur Rly.)	Acanthaceae type,	*Cassia siamea, Moringa oleifera, Prosopis juliflora, Ricinus communis, Terminalia* sp.
4.	POH-AMT-04	27/12/06	Amravati	*Eucalyptus globulus, Prosopis juliflora*	*Allium cepa, Brassica campestris, Moringa oleifera*
5.	MGT-SUS-05	16/02/07	Susarda (Dharni)	*Prosopis juliflora*	Acanthaceae type, *Bombax ceiba, Moringa oleifera, Ricinus communis, Terminalia* sp., *Tridax procumbens*
6.	POH-CRL-06	21/02/07	Chandur Rly.	*Coriandrum sativum*	*Brassica campestris, Tridax procumbens, Ziziphus jujuba*
7.	SAL-MOR-07	16/09/07	Morshi	*Ceiba pentandra, Parthenium hysterophorus,*	*Aster* sp., *Brassica campestris, Cassia siamea, Tridax procumbens*

8.	SAL-MOR-08	28/09/07	Morshi	Acanthaceae type, *Parthenium hysterophorus, Tridax procumbens*	*Ageratum conyzoides, Cassia siamea, Coriandrum sativum,* Poaceae type, *Ricinus communis*
9.	MGT-SUS-09	20/10/07	Susarda (Dharni)	*Cajanus cajan, Gossypium* sp.	*Ipomoea* sp., *Parthenium hysterophorus, Tecoma stans, Tridax procumbens*
10.	MGT-SUS-10	11/11/07	Susarda (Dharni)	*Prosopis juliflora*	Asteraceae type, *Cajanus cajan, Gossypium* sp., *Ipomoea* sp., *Parthenium hysterophorus, Tridax procumbens*
11.	POH-DUP-11	24/11/07	Daryapur	*Brassica campestris Coriandrum sativum*	*Moringa oleifera, Prosopis juliflora*
12.	POH-CRL-12	16/12/07	Chandur Rly.	*Eucalyptus globulus,*	Acanthaceae type, *Moringa oleifera, Ricinus communis, Tridax procumbens*
13.	POH-AMT-13	20/12/07	Amravati	*Eucalyptus globulus, Moringa oleifera, Prosopis juliflora*	*Crotalaria* sp.
14.	POH-SAV-14	23/12/07	Vithoba Savanga (Chandur Rly.)	*Moringa oleifera, Prosopis juliflora, Tridax procumbens*	*Cajanus cajan, Cassia siamea, Ricinus communis, Syzygium cumini*
15.	SAL-MOR-15	13/01/08	Morshi	*Cassia siamea, Vitex negundo*	Asteraceae type, *Ricinus communis, Tridax procumbens*
16.	POH-CRL-16	17/03/08	Chandur Rly.	*Bombax ceiba, Moringa oleifera*	Poaceae type, *Tecoma stans, Vitex negundo*
17.	MGT-SUS-17	20/03/08	Susarda (Dharni)	*Moringa oleifera, Terminalia* sp.	Asteraceae type, *Mangifera indica*
18.	MGT-SUS-18	27/03/08	Susarda (Dharni)	*Terminalia* sp. *Moringa oleifera,*	*Butea monosperma, Delonix regia, Mangifera indica, Peltophorum pterocarpum,* Poaceae type
19.	MGT-SUS-19	19/04/08	Susarda (Dharni)	*Prosopis juliflora, Moringa oleifera, Syzygium cumini*	*Azadirachta indica,* Poaceae type, *Terminalia* sp., *Tridax procumbens*
20.	POH-SAV-20	05/05/08	Vithoba Savanga (Chandur Rly.)	*Moringa oleifera Butea monosperma,*	*Azadirachta indica, Ipomoea* sp., Poaceae type, *Tecoma stans*
21.	SAL-MOR-21	10/10/08	Morshi	*Moringa oleifera Cajanus cajan,*	*Vitex negundo*
22.	POH-AMT-22	17/10/08	Amravati	*Vernonia cineria*	*Ricinus communis, Tridax procumbens, Vitex negundo*

23.	POH-DUP-23	28/10/08	Daryapur	*Gossypium* sp.	*Ipomoea* sp., *Tridax procumbens*
24.	SAL-MOR-24	05/11/08	Morshi	*Moringa oleifera*	*Cajanus cajan*, *Ricinus communis*
25.	POH-DUP-25	15/11/08	Daryapur	*Helianthus annuus*	*Tridax procumbens*, *Vernonia cineria*, *Brassica campestris*, *Cajanus cajan*, *Gossypium* sp.
26.	POH-CRL-26	17/01/09	Chandur Rly.	----	*Brassica campestris*
27.	MGT-SUS-27	18/02/09	Susarda (Dharni)	*Moringa oleifera* *Helianthus annuus*,	*Syzygium cumini*
28.	MGT-SUS-28	23/03/09	Susarda (Dharni)	*Moringa oleifera*, *Syzygium cumini*	*Brassica campestris*, *Coriandrum sativum*
29.	SAL-MOR-29	27/03/09	Morshi	*Azadirachta indica*	*Coriandrum sativum*
30.	SAL-MOR-30	08/04/09	Morshi	----	*Butea monosperma*
31.	POH-DUP-31	24/11/09	Daryapur	*Coriandrum sativum* *Brassica campestris*	*Moringa oleifera*, *Prosopis juliflora*
32.	POH-CRL-32	16/12/09	Chandur Rly.	*Eucalyptus globulus*, *Parthenium hysterophorus*	Acanthaceae type, *Moringa oleifera*, *Ricinus communis*, *Tridax procumbens*
33.	SAL-MOR-33	13/01/10	Morshi	*Cassia siamea*, *Parthenium hysterophorus*, *Vitex negundo*	Asteraceae type, *Ricinus communis*, *Tridax procumbens*
34.	POH-CRL-34	17/03/10	Chandur Rly.	*Bombax ceiba*, *Moringa oleifera*	Poaceae type, *Tecoma stans*, *Vitex negundo*
35.	MGT-SUS-35	19/04/10	Susarda (Dharni)	*Moringa oleifera*, *Prosopis juliflora*, *Syzygium cumini*	*Azadirachta indica*, Poaceae type, *Terminalia* sp., *Tridax procumbens*
36.	POH-SAV-36	23/12/10	Vithoba Savanga (Chandur Rly.)	*Moringa oleifera*, *Prosopis juliflora*, *Tridax procumbens*	*Cajanus cajan*, *Cassia siamea*, *Ricinus communis*, *Syzygium cumini*
37.	MGT-SUS-37	20/03/11	Susarda (Dharni)	*Moringa oleifera*, *Terminalia* sp.	Asteraceae type, *Azadirachta indica*, *Delonix regia*, *Mangifera indica*
38.	MGT-SUS-38	27/03/11	Susarda (Dharni)	*Moringa oleifera*, *Terminalia* sp.	*Butea monosperma*, *Delonix regia*, *Mangifera indica*, *Peltophorum pterocarpum*, Poaceae type
39.	POH-SAV-39	05/05/11	Vithoba Savanga (Chandur Rly.)	*Butea monosperma*, *Moringa oleifera*	*Azadirachta indica*, *Ipomoea* sp., Poaceae type, *Tecoma stans*
40.	POH-AMT-40	20/12/11	Amravati	*Eucalyptus globulus*, *Moringa oleifera*, *Prosopis juliflora*	*Crotalaria* sp.

41.	POH-CRL-41	17/01/12	Chandur Rly.	----	*Brassica campestris*
42.	MGT-SUS-42	18/02/12	Susarda (Dharni)	*Helianthus annuus, Moringa oleifera*	*Syzygium cumini*
43.	MGT-SUS-43	23/03/12	Susarda (Dharni)	*Moringa oleifera, Syzygium cumini*	*Brassica campestris, Coriandrum sativum*
44.	SAL-MOR-44	10/10/12	Morshi	*Cajanus cajan, Moringa oleifera*	*Vitex negundo*
45.	POH-AMT-45	17/10/12	Amravati	*P. hysterophorus, Vernonia cineria*	*Ricinus communis, Tridax procumbens, Vitex negundo*
46.	POH-DUP-46	28/10/12	Daryapur	*Gossypium* sp.	*Ipomoea* sp., *P. hysterophorus, Tridax procumbens*
47.	SAL-MOR-47	27/03/13	Morshi	*Azadirachta indica*	*Coriandrum sativum*
48.	SAL-MOR-48	08/04/13	Morshi	*Azadirachta indica*	*Butea monosperma*
49.	SAL-MOR-49	05/11/13	Morshi	*Moringa oleifera*	*Cajanus cajan, Ricinus communis*
50.	POH-DUP-50	15/11/13	Daryapur	*Helianthus annuus*	*Brassica campestris, Cajanus cajan, Gossypium* sp., *P. hysterophorus, Tridax procumbens, Vernonia cineria*
51.	POH-CRL-51	16/02/14	Chandur Rly.	*Coriandrum sativum*	*Brassica campestris, Ziziphus jujuba*
52.	SAL-MOR-52	10/09/14	Morshi	*Ceiba pentandra*	*Aster* sp., *Brassica campestris, Tridax procumbens*
53.	SAL-MOR-53	16/09/14	Morshi	Acanthaceae type, *Tridax procumbens*	*Ageratum conyzoides, Cassia siamea, Coriandrum sativum*
54.	MGT-SUS-54	28/09/14	Susarda (Dharni)	Acanthaceae type, *Moringa oleifera,*	*Ageratum conyzoides, Justicia procumbens, Tridax procumbens*
55.	MGT-SUS-55	13/10/14	Susarda (Dharni)	Acanthaceae type, Poaceae type	*Moringa oleifera, Syzygium cumini*
56.	MGT-SUS-56	20/10/14	Susarda (Dharni)	*Cajanus cajan,*	*Ipomoea* sp., *Tecoma stans, Tridax procumbens*
57.	MGT-SUS-57	11/11/14	Susarda (Dharni)	*Prosopis juliflora*	Asteraceae type, *Parthenium hysterophorus, Tridax procumbens*
58.	POH-SAV-58	12/11/14	Vithoba Savanga (Chandur Rly.)	Acanthaceae type,	*Cassia siamea, Ricinus communis, Terminalia* sp.
59.	POH-AMT-59	21/12/14	Amravati	*Eucalyptus globulus, Prosopis juliflora*	*Brassica campestris, Moringa oleifera*
60.	MGT-SUS-60	27/12/14	Susarda (Dharni)	*Prosopis juliflora*	Acanthaceae type, *Bombax ceiba, Tridax procumbens*

Table 2: Biochemical composition of represented pollen grains

Sr. No.	Name of Plant Species		Reducing Sugar (NS Method)	Total Carbohy. (Anthrone Method)	Micro-Kjeldahl Medhod		Soluble Protein Content (Lowry's Method)	Total Free Amino Acid Content	Total Lipids Content (Chloro.-Metha. Mixture)	Moisture Content	Total Ash Content
					Nitrogen Content	Total Protein Content					
1.	*Brassica campestris*	**mg/gm**	11.70	22.60	36.50	228.20	26.10	20.80	18.70	105.80	70.50
		%	1.17	2.26	3.65	22.82	2.61	2.08	1.87	10.58	7.05
2.	*Gossypium hirsutum*	**mg/gm**	10.50	27.30	28.10	176.00	47.00	13.40	12.40	110.30	22.80
		%	1.05	2.73	2.81	17.60	4.70	1.34	1.24	11.03	2.28
3.	*Azadirachta indica*	**mg/gm**	2.70	12.20	38.70	242.00	22.20	10.40	35.40	88.20	60.40
		%	0.27	1.22	3.87	24.20	2.22	1.04	3.54	8.82	6.04
4.	*Moringa oleifera*	**mg/gm**	18.70	38.40	67.30	420.70	200.40	12.30	30.40	100.30	62.80
		%	1.87	3.84	6.73	42.07	20.04	1.23	3.04	10.03	6.28
5.	*Butea monosperma*	**mg/gm**	25.20	42.80	46.80	293.00	213.00	14.30	24.30	110.40	38.40
		%	2.52	4.28	4.68	29.30	21.30	1.43	2.43	11.04	3.84
6.	*Cajanus cajan*	**mg/gm**	7.30	28.70	17.90	112.00	101.00	6.80	34.80	88.20	40.30
		%	0.73	2.87	1.79	11.20	10.10	0.68	3.48	8.82	4.03
7.	*Syzygium cumini*	**mg/gm**	15.30	37.20	43.80	274.00	200.80	6.80	23.70	100.80	28.20
		%	1.53	3.72	4.38	27.40	20.08	0.68	2.37	10.08	2.82
8.	*Coriandrum sativum*	**mg/gm**	8.70	35.40	26.40	165.00	113.00	7.20	28.70	90.40	25.20
		%	0.87	3.54	2.64	16.50	11.30	0.72	2.87	9.04	2.52
9.	*Helianthus annuus*	**mg/gm**	10.90	32.20	20.60	129.70	24.10	30.90	41.00	98.70	33.60
		%	1.09	3.22	2.06	12.90	2.41	3.09	4.10	9.87	3.36
10.	*Parthenium hysterophorus*	**mg/gm**	22.80	43.60	77.40	484.00	321.00	14.00	22.20	110.80	34.20
		%	2.28	4.36	7.74	48.40	32.10	1.40	2.22	11.08	3.42
11.	*Tridax procumbens*	**mg/gm**	12.40	34.80	21.40	134.00	34.00	10.20	32.70	128.30	37.80
		%	1.24	3.48	2.14	13.40	3.40	1.02	3.27	12.83	3.78
12.	*Vernonia cineria*	**mg/gm**	30.80	57.60	36.80	230.40	170.40	8.50	20.30	110.20	33.30
		%	3.08	5.76	3.68	23.04	17.04	0.85	2.03	11.02	3.33
13.	*Ipomoea fistulosa*	**mg/gm**	15.80	37.20	23.50	147.00	8.40	7.20	11.00	85.70	27.40
		%	1.58	3.72	2.35	14.70	0.84	0.72	1.10	8.57	2.74
14.	*Vitex negundo*	**mg/gm**	27.20	42.50	37.40	234.00	26.30	6.30	38.70	120.30	48.70
		%	2.72	4.25	3.74	23.40	2.63	0.63	3.87	12.03	4.87
15.	*Ricinus communis*	**mg/gm**	8.70	27.20	58.80	368.00	32.70	15.20	22.70	118.20	50.40
		%	0.87	2.72	5.88	36.80	3.27	1.52	2.27	11.82	5.04

Biochemical analysis of pollen grains

i) Sugars and carbohydrates

Total reducing sugars were found to be maximum i.e. 3.08% in *Vernonia cineria* and minimum i.e. 0.27% in *Azadirachta indica*. The total carbohydrates have been also found to be maximum i.e. 5.76% in *Vernonia cineria* and minimum i.e. 1.22% in *Azadirachta indica* (**Table 2**). From thin layer chromatography (TLC) of sugars, sucrose was found to be observed in all pollen samples of studied plant species except *Ricinus communis.* Presence of glucose was recorded in pollen samples of *B. campestris, S. cumini, H. annuus, I. fistulosa* and *V. negundo.* However, fructose was observed in pollen samples of *C. cajan, S. cumini, H. annuus, V. negundo* and *R. communis* (**Table 3**).

The pollen grains of species *S. cumini, H. annuus* and *V. negundo* represented all the three sugars i.e. glucose, fructose and sucrose (**Table 3**). The unknown sugars were observed in pollen grains of *H. annuus* and *V. negundo.*

Table 3: Qualitative analysis of pollen sugar content by TLC

Sr. No.	Name of Plant Species	Type of Sugar Present			
		Glucose	Fructose	Sucrose	Unknown
1.	*Brassica campestris*	+	–	+	-
2.	*Gossypium hirsutum*	–	–	+	-
3.	*Azadirachta indica*	–	–	+	-
4.	*Moringa oleifera*	–	–	+	-
5.	*Butea monosperma*	–	–	+	-
6.	*Cajanus cajan*	–	+	+	-
7.	*Syzygium cumini*	+	+	+	-
8.	*Coriandrum sativum*	–	–	+	-
9.	*Helianthus annuus*	+	+	+	01
10.	*Parthenium hysterophorus*	–	–	+	-
11.	*Tridax procumbens*	–	–	+	-
12.	*Vernonia cineria*	–	–	+	-
13.	*Ipomoea fistulosa*	+	–	+	-
14.	*Vitex negundo*	+	+	+	02
15.	*Ricinus communis*	–	+	–	-

\+ Present – Absent

ii) Free amino acids

The total free amino acids content was found to be maximum i.e. 3.09% in *H. annuus* and minimum i.e. 0.63% in *V. negundo* (**Table 2**). From thin layer chromatography (TLC) for free amino acids, 12 different types of free amino acids along with few unknown amino acids were recorded in all plant species studied. The unknown amino acids may belong to different complex amino acids or amines.

The qualitative analysis showed the presence of different free amino acids in which Alanine, Histidine monohydrochloride, Methionine, Ornithine monohydrochloride and Arginine monohydrochloride were found to be dominant. The other free amino acids were Cysteine, Glutamic acid, Isoleucine, Lysine monohydrochloride, Proline, Threonine and Valine. Maximum eight free amino acids were observed in *M. oleifera* and minimum three in *V. negundo* pollen (**Table 4**).

iii) Total nitrogen and protein

The maximum amount 7.74% of nitrogen and 48.4% of crude protein was observed in *P. hysterophorus* pollen. The minimum amount 1.79% of nitrogen and 11.2% of crude protein was recorded in *C. cajan* pollen (**Table 2**). Maximum soluble protein 32.1% was observed in *P. hysterophorus* and minimum 0.84% in *I. fistulosa* pollen.

iv) Lipids

The amount of free lipids was found to be minimum i.e. 1.1% in *I. fistulosa* and maximum i.e. 4.1% in *H. annuus* (**Table 2**).

v) Moisture and ash

The amount of moisture content was found to be minimum i.e. 8.57% in *I. fistulosa* and maximum i.e. 12.83% in *T. procumbens.* The amount of ash content was found to be minimum i.e. 2.28% in *G. hirsutum* and maximum i.e. 7.05% in *B. campestris* (**Table 2**).

Table 4: Types of free amino acids observed in pollen grains by TLC

S. N.	Name of Plant Species	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	Unk.	Total
1.	*Brassica campestris*	+	+	–	–	+	–	–	+	–	+	–	+	01	07
2.	*Gossypium hirsutum*	+	+	–	–	+	–	–	+	–	–	–	–	02	06
3.	*Azadirachta indica*	–	+	–	–	+	–	+	–	+	–	–	–	01	05
4.	*Moringa oleifera*	+	–	+	+	–	+	–	+	+	–	+	+	-	08
5.	*Butea monosperma*	+	–	–	–	+	–	–	–	+	–	–	–	02	05
6.	*Cajanus cajan*	+	+	–	–	–	–	–	+	–	–	–	–	01	04
7.	*Syzygium cumini*	+	–	–	–	+	–	–	+	–	–	–	–	02	05
8.	*Coriandrum sativum*	+	+	–	–	+	–	–	–	–	–	–	–	01	04
9.	*Helianthus annuus*	+	+	–	–	+	–	–	+	+	+	–	–	-	06
10.	*Parthenium hysterophorus*	–	–	–	–	–	–	+	–	+	–	–	–	03	05
11.	*Tridax procumbens*	–	–	–	–	–	–	+	–	+	–	–	–	02	04
12.	*Vernonia cineria*	+	+	–	–	+	–	–	+	–	–	–	–	01	05
13.	*Ipomoea fistulosa*	–	–	–	–	–	–	+	–	+	+	–	–	02	05
14.	*Vitex negundo*	–	–	–	–	–	–	–	–	–	–	+	+	01	03
15.	*Ricinus communis*	–	–	–	–	–	+	+	+	+	+	–	–	02	07

Types of free amino acids observed:

A1	Alanine	A2	Arginine monohydrochloride
A3	Cysteine	A4	Glutamic acid
A5	Histidine monohydrochloride	A6	Isoleucine
A7	Lysine monohydrochloride	A8	Methionine
A9	Ornithine monohydrochloride	A10	Proline
A11	Threonine	A12	Valine
Unk.	Unknown	+	Present
–	Absent		

vi) Histochemicals

From histochemical studies, starch was found to be present in all pollen samples of studied plant species excluding *A. indica* and *V. cineria.* Lipids were observed for its presence in all studied plant species excluding *V. cineria, I. fistulosa* and *V. negundo* (**Table 5**).

Table 5: Histochemical test of pollen grains

Sr. No.	Name of Plant Species	IKI Test for Starch	Sudan IV Test for Lipids
1.	*Brassica campestris*	+	+
2.	*Gossypium hirsutum*	+	+
3.	*Azadirachta indica*	–	+
4.	*Moringa oleifera*	+	+
5.	*Butea monosperma*	+	+
6.	*Cajanus cajan*	+	+
7.	*Syzygium cumini*	+	+
8.	*Coriandrum sativum*	+	+
9.	*Helianthus annuus*	+	+
10.	*Parthenium hysterophorus*	+	+
11.	*Tridax procumbens*	+	+
12.	*Vernonia cineria*	–	–
13.	*Ipomoea fistulosa*	+	–
14.	*Vitex negundo*	+	–
15.	*Ricinus communis*	+	+

+ Present – Absent

Honey bee visits

In all studied 15 plant species honeybee *Apis dorsata* was found to be regular visitor to the flowers. It visited 2 to 6 flowers per bout. The frequency of honeybee visitor was found to be more. Honeybee visits the flower during morning hours 08:00 hrs. to 12:30 hrs. and in evening 15:30 hrs. to 18:00 hrs. The activity of honeybee varied according to floral types and its seasonal availability. The movements of honeybees between flowers are strongly influenced by the amount of reward present.

DISCUSSION

Pollen spectra of the regional honey samples varied according to the vegetation type utilized by the bees within the floristically diverse regions. Some of the pollen types were considerably observed to serve as important nectar and pollen sources to honey bees. From the pollen spectra it was observed that Amravati district includes both naturalized flora as well as cultivated crops. The investigation revealed that in addition to already known bee forage (e.g. *Brassica, Coriandrum* and *Moringa*) some other species including *S. cumini, B. monosperma, R. communis* and *P. pinnata* were also heavily utilized as pollen and nectar sources by honey bees from this region. The agricultural crops like *H. annuus*, *Gossypium* sp. and *C. cajan* were also found to be very useful food resources for honey bees as observed during the investigation.

Honey samples were also represented with the pollen types belonging to anemophilous plant species such as *P. hysterophorus, R. communis, T. procumbens* and *V. cineria*. As the pollen grains are having nutritional value, the honey bees collect them as a source of proteins. Pollen grains are collected by honey bees from a wide range of floral species. The chemical composition of pollen and its subsequent nutritional value to honey bees varies considerably between different floral types. It was observed that pollen from anemophilous plants was not greatly different from pollen of entomophilous plants in their nutritional value [30]. Pollen load analysis provides valuable floral resource and foraging behavior of visitor [31].

A melissopalynological analysis including qualitative and quantitative analysis. It is carried out in order to identify the principal pollen types in Indian honeys and therefore, the important plants exploited by *Apis dorsta* in the country [32]. *A. dorsata* is a voracious forager, collect nectar and pollen from diversified flora and produce multifloral honey useful to mankind. Understanding its floral source would help reveal floral status of the region and knowledge on pollen types would provide a greater insight into Melissopalyonology of the region [33,34]. Fabaceae, Asteraceae and Myrtaceae were most represented families. There was a dominance of tree species which are the most preferred and highest contribution for nectar and pollen source for honeybees [35].

Pollen is a source of proteins, lipids and vitamins which are essential to growth and development of honey bees rather than energy production [8,10,15,36]. In particular, nitrogen is crucial for development of larvae and longevity of adults [37]. In the pollen types such as *P. hysterophorus, M. oleifera* and *R. communis* the amount of nitrogen is found to be higher.

It is important to detect which are the main pollen sources of a region and to determine their protein value as pollen is a major component of honey bees' diet. In present study the pollen types such as *P. hysterophorus, M. oleifera* and *R. communis* were found to be with higher amount of protein contents i.e. 48.40%, 42.07% and 36.80% respectively.

The amino acids are also required to the honey bee colony as a protein source [14]. The amino acid composition defines the nutritional value of pollen more accurately than protein contents [15]. The nutritional value is reduced when inadequate amounts of the essential amino acids are present [38]. The most pollen contain all common amino acids however, pollen sometime lacks phenylalanine, tryptophane, hydroxyproline, tyrosine and aminobutyric acid [39]. Tryptophane and phenylalanine are the only essential amino acids which frequently found to be absent [40]. In the present investigation, the amino acids i.e. alanine, histidine monohydrochloride,

ornithine monohydrochloride and methionine were found to be common in pollen grains of all studied plant species.

Proline is one of the most abundant free amino acids observed in pollen grains and can account for 1-2% of the total weight of pollen grains [4]. The presence of proline was observed in pollen grains of *H. annuus, B. campestris, I. fistulosa* and *R. communis* (**Table 4**).

It was revealed that the honey bees are attracted towards the pollen grains having high lipid levels even though the general nutritional value of some of the pollen grains is low when using protein and amino acid level as a measure of honey bee nutritional value [14]. During the present investigation, pollen samples of *H. annuus, V. negundo, C. cajan* and *A. indica* were found to be with higher amount of lipid contains (**Table 2**).

Fructose, glucose and sucrose are the free sugars present in the pollen grains and particularly sucrose is present in higher amount [4]. These observations were corroborating with present findings. Sucrose was found to be present in all pollen samples of studied plants excluding *R. communis.* The amount of sucrose is generally higher in mature angiospermic pollen [41]. Glucose and fructose were also observed in most of the pollen samples (**Table 5**).

Pollen histochemistry is possibly related to pollination mode, pollinator foraging behavior and phenology [3]. All Angiosperm pollen grains contain stored food reserves in the forms of starch and /or lipids and can be classified in two classes, 'starchy' and 'starch less' [6]. Studies on histochemistry have shown that all Angiosperm pollen contains some lipids; while starch is not always present [42]. But in the present histochemical investigations, in almost all pollen samples starch and lipids were found to be present (**Table 5**).

Histochemicals like starch and oil are the main calorific reserves of pollen. Plant groups which offer pollen as the main or the only source of energy, tend to have oil-rich pollen. It should be noted that pollen has a higher energy content investment per gram of organic tissues than other plant parts [3].

The anemophily as a concept of pollination ecology is not relevant in case of social insects like honey bees, whose foraging behavior is controlled by a different set of factors [43]. It was very surprising that *P. hysterophorus* was represented in most of honey samples although it is anemophilous plant species. It is a troublesome weed and is now widely spread. The pollen grains of *P. hysterophorus* are allergenic and cause skin eruptions; however, it is found to be a chief nectar source for the honey bees [44]. The observation indicates that pollen of *P. hysterophorus* is not toxic to the bees while it provides the nectar and pollen almost throughout the year.

Since the time of Aristotle, it has been written that honeybees show remarkable fidelity to a plant species when visiting a patch of flowers to forage. This pollinator-flower constancy, in fact, is not limited to a few flowers in a set of sequentially visited flowers. The legendary flower fidelity of honeybees actually arises for different reasons. Like many other species, honeybee flower fidelity can arise from energetic considerations involving nectar reward quality, quantity or work considerations [45].

CONCLUSION

The biochemicals from the pollen grains especially amino acids, carbohydrates and proteins, were found to be responsible for the visits of honey bees to the flowers of particular taxa. From the chemical point of view, pollen grains with high protein content and other essential nutrients proved to be an excellent food for flower visitors like honey bees.

From the biochemical investigations, it can be concluded that some pollen types represented by regional honey samples viz; *P. hysterophorus, M. oleifera, H. annuus, R. communis* and *V. cineria* were found to be with more amount of proteins, carbohydrates and amino acids, which also have an impact on frequency, fidelity and constancy of honeybee visitors.

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