Plant Archives Vol. 19 No. 2, 2019 pp. 3421-3427 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

STUDY ON POLLEN GRAINS IN THE HONEY SAMPLES OF HOWRAH DISTRICT, WEST BENGAL, WITH REFERENCE TO THEIR OCCURRENCE IN AIR IN THREE DIFFERENT SEASONS.

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Abstract

Honey is an important food supplement of medicinal and nutritive value produced by honeybees. Pollen grains are important components which are included in honey, during foraging activities. Pollen diversity and quantity of honey of a particular locality determines its quality and characteristic feature. In the present study, pollen components are studied in honey samples (n = 12) collected from four sites (Tikiapara-Howrah, Domjur, Santragachhi and Uluberia) of Howrah district of West Bengal state, India, during winter (December-January), spring (February-March) and summer (April-May) of 2017-18. In microscopic analysis of acetolysed honey samples, most are found as multifloral type with presence of the pollen of *Allium*, *Brassica, Coriandrum, Phoenix sylvestris,* etc in winter, Asteraceae, *Azadirachta indica, Mangifera indica, Psidium,* etc. in spring and *Borassus flabellifer, Cassia, Delonix,* etc. in summer. Unifloral honey is also found in winter with predominance of *Coriandrum* pollen (>45% of absolute pollen count, APC).

Areopalynological survey was carried in Howrah using Burkard 7-day volumetric sampler during study period to observe the occurrence of pollen grains in air. Altogether 38 pollen types are found to be present in honey samples and atmosphere of Howrah. There are more than 25 pollen types, including *Parthenium hysterophorus*, which are already reported to be airborne and allergenic, contributed 10-25% of APC of honey. Consumption of honey containing allergenic pollen, may cause health hazard by inducing hypersensitive reaction in susceptible individual. In this context, proper analytical quality control of honey and general awareness development is extremely necessary for food safety.

Key words: Honey, pollen grains, atmosphere, allergenicity, Howrah district.

Introduction

Honey is a supersaturated sugary liquid based on floral nectars with some pollen grains collected (during foraging activities) and stored in the hives by honey bees. It is one of the widely used and popular natural liquid sweeteners in human civilization, with strengthening effect and several health benefits. India is one of the important producers (FAOSTAT, 2017) of honey in global market (61,335 tonnes of total 1,786,996 tonnes in the world in 2016, *i.e.,* 5th in position).

Melissopalynology is the branch of Palynology (study of pollen grains) in Plant Science, which deals with the study of pollen grains present in honey, indicating the source of nectars in honey and provides useful guide in beekeeping in a particular geographical region. During nectar collection, pollen gets attached with bees and hence included in honey. Sometimes bees collect pollen from the flowers for nutritional advantage as a source of protein. Honey bee (*Apis mellifera* L.) usually collect pollen from entomophilous nectariferous plants during flowering period. However, they also collect pollen from anemophilous plants during unfavourable weather conditions by habitual foraging activities (Giovanetti and Aronne, 2011).

Pollen gains are well known source of soluble allergenic protein to initiate IgE-mediated hypersensitive reaction in susceptive individuals. Majority of the allergenic pollen grains are airborne, which cause allergic reaction when inhaled (Bastl *et al.,* 2018). The concentration level of airborne allergenic pollen grains can reach up to the level of several thousand per cubic meter air (Sauliene *et al.,* 2014). However, study on the incidence and subsequent adverse health effect of allergenic pollen present in honey is not common (Sauliene *et al.,* 2015). From the available literature, it seems that,

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Table 1: Pollen members present in air (A) and honey samples (H) of Howrah district in three seasons during 2017-18.

A. Herb and shrub pollen

Pollen Types	Winter December-January	Spring February-March	Summer April-May
1. *Allium sp.*	H	AH	
2. *Argemone mexicana*		AH	
3. Asteraceae	AH	AH	AH
4. *Brassica* sp.	AH	H	
5. *Cassia* sp.			
6. Chenopodiaceae- Amaranthaceae	AH	A	A
7. *Coriandrum* sp.	AH	AH	
8. Cucurbitaceae	AH	AH	
9. Cyperaceae	A	AH	AH
10. Euophorbiaceae		AH	
11. Fabaceae members	AH	AH	AH
12. *Helianthus annuus*		AH	
13. Liliaceae		AH	
14. *Lunurus sibiricus*		H	H
15. *Parthenium hysterophorus*		H	AH
16. Poaceae	A	A	A
17. *Rumex* sp.		AH	
18. Solanaceae		H	
19. *Tridax* sp.		AH	
Shrub Members			
20. *Cleroodendrum* sp.		AH	
21. *Lantana camara*			AH

B. Tree pollen

Pollen Types	Winter December-January	Spring February-March	Summer April-May
1. *Aegle marmelos*		A H	
2. *Alstonia scholaris*	AH		
3. *Areca catechu*		AH	A
4. *Azadirachta indica*		AH	
5. *Bombax ceiba*		AH	AH
6. *Borassus flabellifer*		AH	AH
7. *Carica papaya*		A	AH
8. *Cocos nucifera*	AH	A	A
9. *Delonix regia*		A	AH
10. *Eucalyptus* sp.		AH	
11. Fabaceae	A	AH	AH
12. *Mangifera indica*		AH	
13. *Peltophorum pterocarpum*			AH
14. *Phoenix sylvestris*	AH		
15. *Psidium guajava*		AH	H
16. *Syzygium* sp.		AH	H
17. *Trema orientalis*	A	A	A
18. *Vitex negundo*			H

A: Airborne pollen H: Pollen present in honey

there is no such report of comparative occurrence of pollen grain in honey and the air of a locality from Indian subcontinent.

The objective of the present study is to highlight the pollen spectrum in honey (collected by *Apis mellifera*) with reference to the distribution of airborne pollen in Howrah district of West Bengal, India. Knowledge on the airborne pollen spectrum is also significant and demands pre-cautionary measures, as most of such pollen grains cause allergic symptoms on susceptible human individuals.

Materials and Methods

Study area

The study was conducted in Howrah district of West Bengal, India during 2017-18. The area is characterized by tropical mesophytic vegetation of lower Gangetic river plain. Ecofloristic surveys were carried out to record the vegetation and collection of pollen sample from study area for preparation of reference pollen slide following acetolysis technique as suggested by Erdtman (1960), for identification.

Honey sample collection

Squeezed honey samples (*Apis mellifera*) were collected during the period of December, 2017 to May, 2018 from four localities (Fig. 1) of Howrah district: Howrah (Tikiapara), Santragachhi, Domjur and Uluberia (sites 1- 4). Site 1-3 are basically congested urban areas with natural tropical vegetation. In the site 2 and 4, there were additional agricultural fields with *Brassica nigra*, *Solanum tuberosum, Coriandrum sativum*, cereals like *Oryza sativa,* among others. A total of twelve honey samples were collected, directly from bee hives, three from each site–in winter (December-January), spring (February-March) and summer (April-May) respectively.

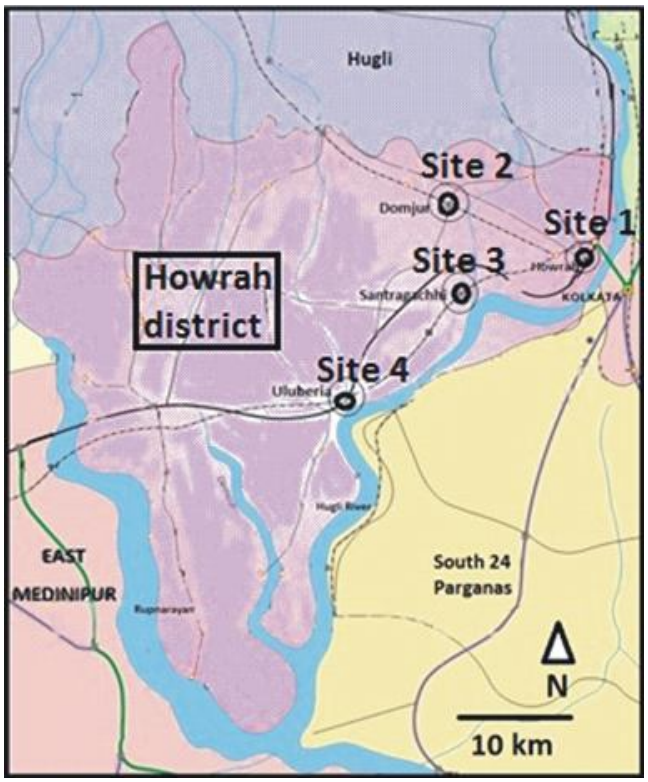

Fig. 1: Location map of the study area in Howrah District showing aeropalynlogical survey and honey collection sites [Site 1: Howrah (Tikiapara), Site 2: Domjur, Site 3: Santragachhi and Site 4: Uluberia].

Survey of airborne pollen grains

Airborne pollen survey was continuously carried out with 7-day recording volumetric trap (Burkard Manufacturing Co., UK) at Narasinha Dutt College, Belilious Road, Howrah, at site 1 (Howrah-Tikiapara).

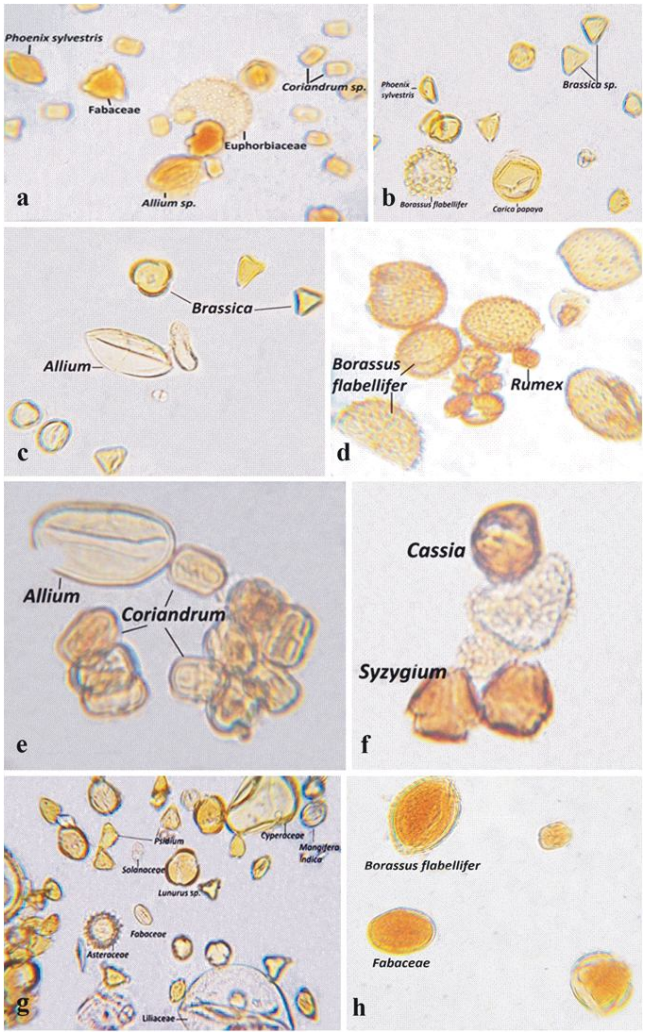

Fig. 2: Photomicrographs (500X) of palynoflora in honey samples of different sites of Howrah district in different season (2017-18). **a.** Site 1-Winter, **b.** Site 1-Spring, **c.** Site 2-Winter, **d.** Site 2-Summer, **e.** Site 3 – Winter, **f.** Site 3 – Summer, **g.** Site 4- Spring, **h.** Site 4- Summer.

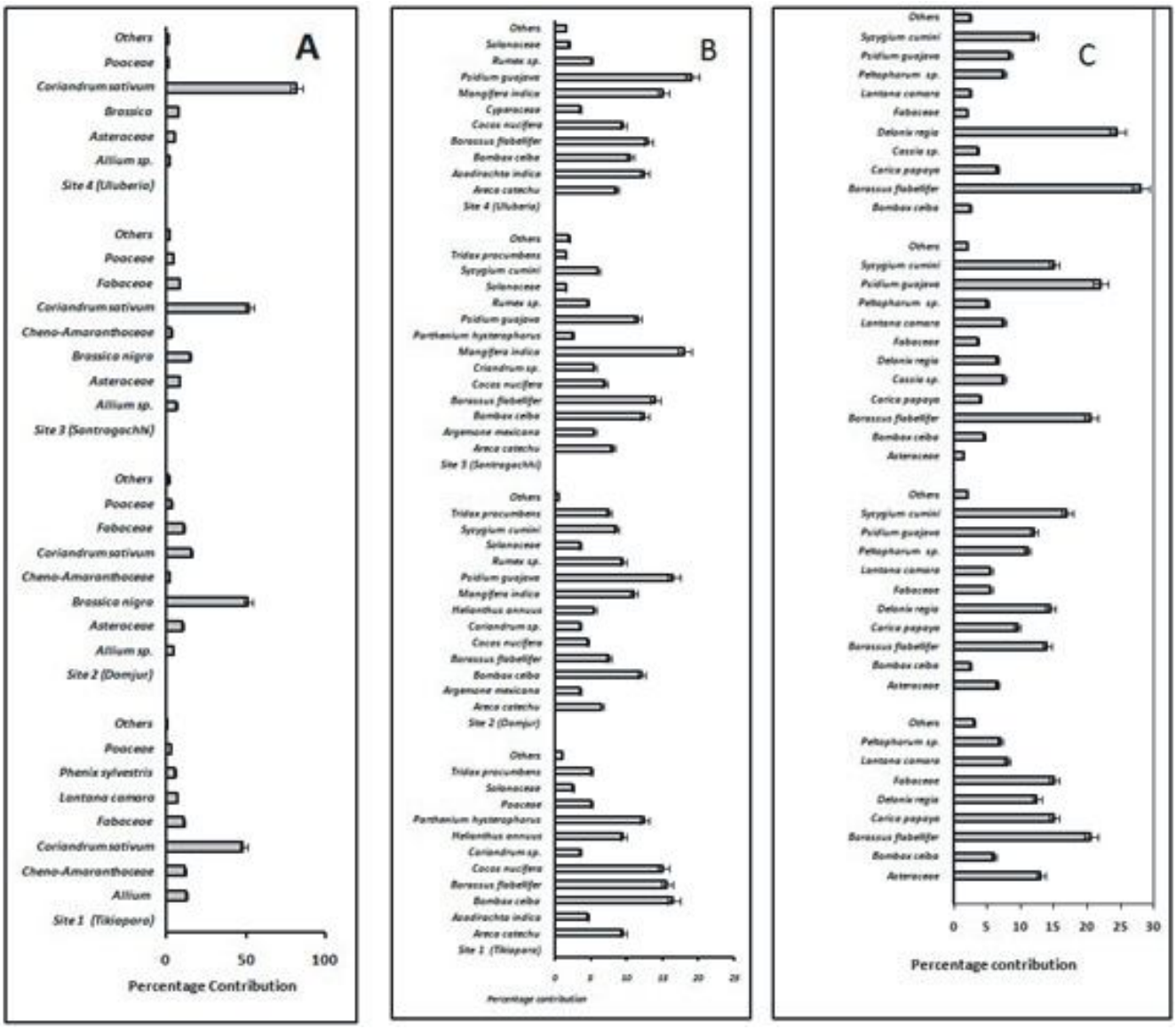

Fig. 3: Percentage contribution of different pollen types to overall palynoflora of honey (2017-18) in Howrah district. **a.** Winter, **b.** Spring and **c.** Summer. Bars indicate standard deviation.

Site 2 (Domjur) is situated at 5.6 km North-West to the aeropalynological sampling site (site 1). The distance of site 3 (Santragachhi) and 4 (Uluberia) was respectively 9 km and 36 km South-East to site 1 (Fig. 1).

The exposed melinex tapes segments of Burkard 7-day volumetric sampler representing a day (48 mm) were mounted on glass slide and observed under microscope following the guideline of The British Aerobiological Federation (1995) to record the airborne pollen grains.

Identification of pollen grains

The pollen analysis of honey samples were carried out by acetolysis as following:

1. From each sample 5 ml was taken out and diluted in 20 ml distilled water, centrifuged at 4500 rpm for 15 minutes and the pellet was collected. The pellet was then repeatedly (four times) washed in double distilled water by centrifugation and decantation of supernatant.
2. The pellets were then treated with glacial acetic acid, centrifuged twice and the supernatant was decanted.
3. The collected pellet was treated with Acetolysis mixture (the mixture of acetic anhydride and concentrated H_2SO_4 at 9:1 ratio) according to Erdtman (1960) in a boiling water bath for five minutes.
4. The samples were centrifuged and decanted to remove acidic solution.
5. To each sample residue, 1 ml 50% glycerine was added along with 20 µl phenol to stop microbial/fungal

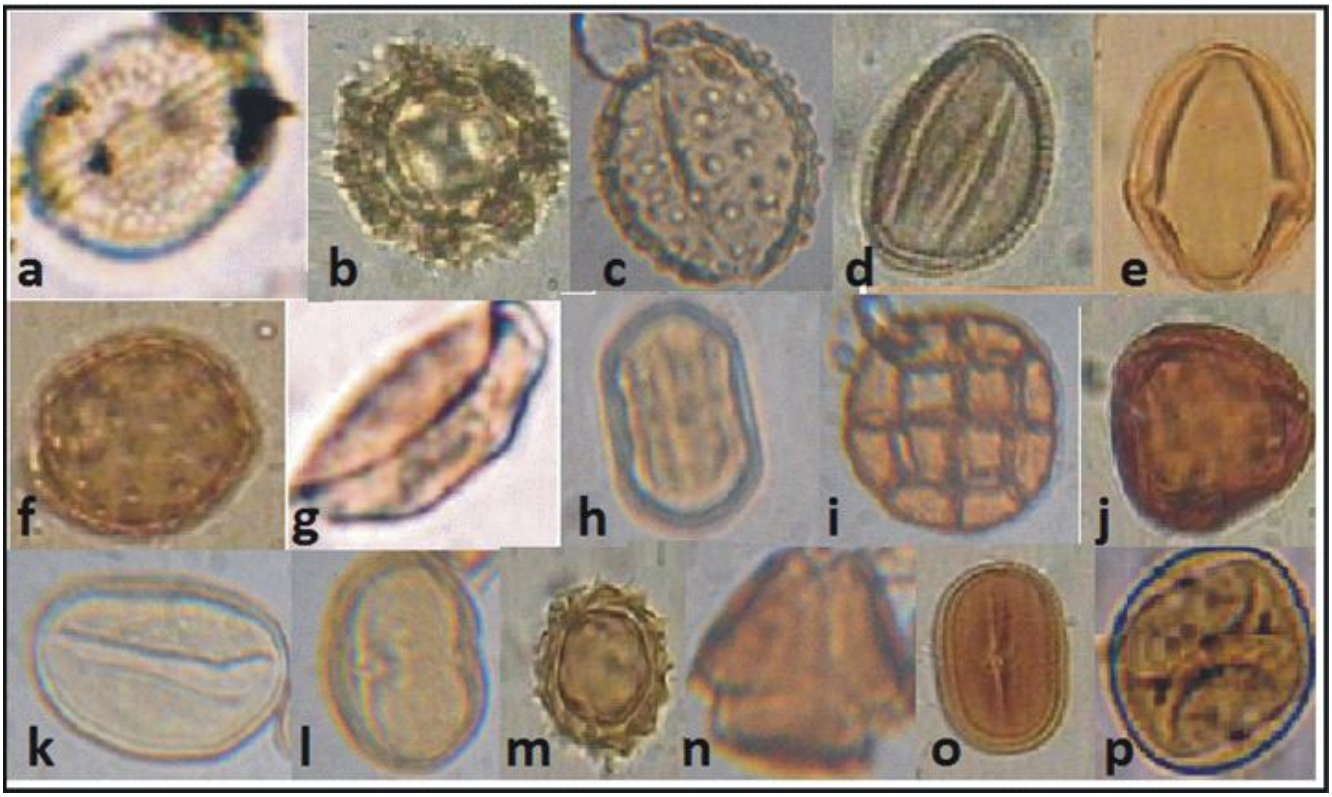

Fig. 4: Pollen types present in the air of Howrah area (EV = equatorial view, PV = Polar view) exposed in Burkard tape, as observed under microscope. **a.** *Areca catechu* (EV,450X), **b.** Asteraceae (500X), **c.** *Borassus flabellifer* (EV, 450X), **d.** *Brassica* sp. (EV, 500X), **e.** *Cassia* sp. (EV, 450X), **f.** Chenpodiaceae-Amaranthaceae (400X), **g.** *Cocos nucifera* (EV, 450X), **h.** *Coriandrum* sp. (EV, 450X), **i.** Fabaceae-polyad (550X), **j.** *Lantana camara* (PV, 450X), **k.** Liliaceae (EV, 500X), **l.** *Mangifera indica* (EV, 450X), **m.** *Parthenium hysterophorus* (450X), **n.** *Psidium guajava* (PV, 550X), **o.** *Rumex* sp. (EV, 450X), **p.** *Trema orientalis*(EV, 450X);

degradation.

6. From each sample, five slides were prepared and scanned thoroughly under microscope. Pollen grains (about 300) were counted at random for assessment of frequency of different pollen types in honey following International Commission of Bee Botany (Louveaux *et al.*, 1978). The pollen types were categorized as Predominant (>45% of counted pollen grains), secondary type (16-45%), important minor type (3-15%) minor type (<3%) and pollen present (<1%).

   Identification of pollen grains in honey was carried out with the help of acetolysed reference pollen slides prepared in laboratory after pollen sample collection by eco-floristic survey in the study area.

7. With the help of a haemocytometer, absolute pollen count (APC) in each honey sample was determined as suggested by Suryanarayan *et al.,* (1981).

Results and Discussion

Analyses reveal that there are 38 pollen types belonging to 25 different families present in honey and air samples of three seasons in the study area (Fig. 1). These pollen types are depicted in table 1 and fig. 2-3.

Study on pollen grains in honey

In the winter, the recorded pollen grains are predominantly from *Coriandrum sativum* (Apiaceae), *Phoenix sylvestris* (Arecaceae), *Allium sativum* (Liliaceae), *Brassica* sp (Brassicaceae) and members of Amaranthaceae, Asteraceae, Chenopodiaceae, Fabaceae, Poaceae, etc. (Table 1). In site 1, 3 and 4 the honey samples are found to be unifloral type with predominant *Coriandrum* pollen (>45% of APC), whereas in site 2, *Brassica* pollen is predominant. The other recorded pollen types are secondary or important minor types.

In spring, pollen grains of *Borassus flabellifer*, *Coriandrum sativum*, Cucurbitaceae, Solanaceae, *Cocos nucifera*, *Areca catechu*, Euphorbiaceae, Fabaceae, *Rumex* sp., *Argemone mexicana*, *Helianthus annuus*, *Tridax* sp., etc. are found to be present in the multifloral honey samples (Fig. 2 & 3). In spring, no one particular type is found as predominant. In site 1 (Table 1), Asteraceae pollen members including *Tridax* sp.,

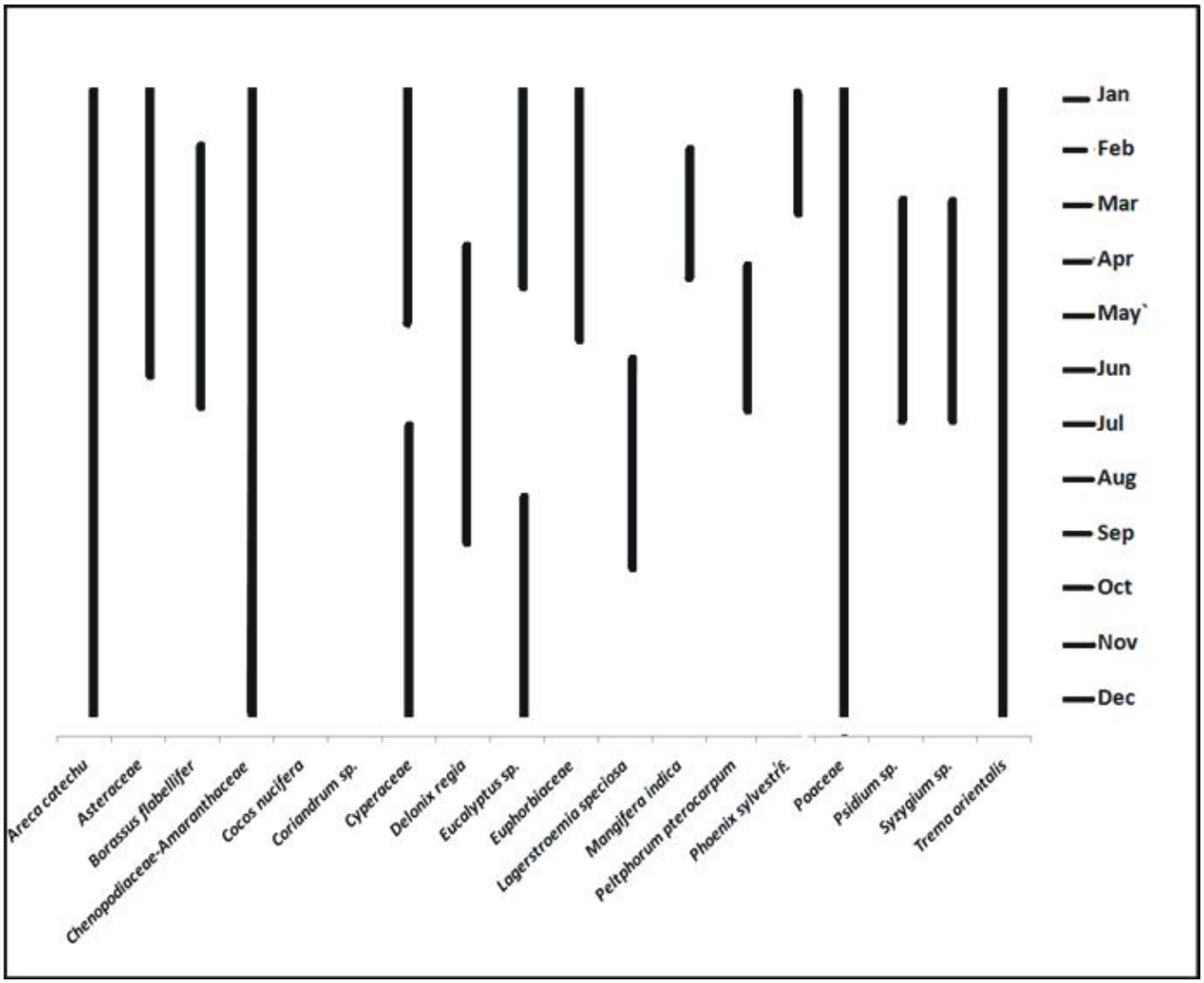

Fig. 5: Distribution pattern of airborne pollen in the air of Howrah during study period.

Parthenium hysterophorus are secondary type/ important minor (3-20% contribution to APC). In site 2 and 3, there are pollen grains of Cyperaceae, *Borassus flabellifer, Mangifera indica*, *Psidium guajava, Sygygium cumini,* Fabaceae, Liliaceae and other members. In site 4, pollen grains of *Cocos nucifera, Rumex* sp., Cucurbitaceae and Asteraceae are common contributors as secondary type.

Honey samples of summer are represented by the pollen grains of *Borassus flabelliofer, Cassia* sp., *Bombax ceiba*, *Carica papaya*, *Psidium* sp., *Syzygium* sp, *Lantama camara*, *Delonix regia*, *Peltophorum pterocarpum*, *Carica papaya*, etc. Honey samples from all four sites shows the presence of pollen from *Borassus flabellifer*, *Cassia* sp., *Fabaceae*, *Delonix regia*, and other members as important minor types with 3-15% contribution to APC.

In case of airborne pollen grains, the dominant type is noted from Poaceae, Chenopodiaceae, Amaranthaceae, Cyperaceae, Asteraceae, *Cocos nucifera, Trema orientalis* for all the three seasons (Fig. 4 and 5). In winter, the frequent types are recorded from *Alstonia scholaris*, *Phoenix sylvestris, Brassica*, Apiaceae, Solanaceae, etc. (Fig. 3). Rumex, *Areca catechu, Azadirachta indica,* Fabaceae, are frequently airborne in spring. In summer, *Delonix regia*, *Borassus flabellifer, Cassia, Peltophorum pterocarpum*, Fabaceae, *Bombax ceiba*, etc. are predominantly present in the air of Howrah. The comparative view of the pollen grains in honey is depicted in table 1, where pollen of *Trema orientalis* and Poaceae are totally found to be airborne. However, pollen grains of Solanaceae and *Vitex negundo* are present in honey samples only. Remaining all the pollen types are found both within honey and atmosphere of the study area. In the present investigation, among the recorded pollen types in honey, except *Aegle marmelos, Allium, Coriandrum,* Liliaceae, *Psidium,*

Rumex, Solanaceae, *Syzygium* and *Vitex* pollen, all are reported to be airborne as well as allergenic by previous clinical and immunochemical studies (Mandal *et al.,* 2008, Hossain *et al.,* 2013, Chakraborty *et al.,* 2016, Ghosh *et al.*, 2016). These pollen grains with allergenic potential can initiate allergenic reaction in sensitive people. Soluble protein present in these pollen can triggers IgE mediated allergic disorders. Consumption of honey containing allergenic pollen (*e.g., Parthenium* and others) can cause health hazards (Hebling *et al.,* 1992). There is also report of anaphylaxis induced by honey consumption (Tuncel *et al.,* 2011) in infants.

Conclusion

Thus the present study is significant as it provides an airborne pollen map of the district of Howrah in three seasons towards bee keepers' aspect as well as for clinical diagnosis of allergy. Assessment of honey is required prior to its marketing.

Acknowledgements

The study was funded by University Grants Commission, New Delhi, India [(UGC MRP reference no. F.PSW/098/15-16 (ERO)]. The infrastructure for aeropalynological survey was provided by the administration of Narasinha Dutt College, Howrah. Thanks are also due to Prof. (Dr.) Swati Bhattacharya and Mr. Chanchal Chakraborty, Division of Plant Biology, Bose Institute, Kolkata, for the valuable help in laboratory studies.

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