



Revista de Biología Tropical

ISSN: 2215-2075

ISSN: 0034-7744

Universidad de Costa Rica

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Revista de Biología Tropical, vol. 66, no. 4, 2018, pp. 1597-1605
Universidad de Costa Rica

DOI: 10.15517/rbt.v66i4.32697

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Apis mellifera pollen loads to understand the pollen foraging pattern used for apicultural practice in a potentially agricultural belt in Bengal, India

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Received 12-III-2018. Corrected 21-VIII-2018. Accepted 24-IX-2018.

Abstract: For accurate determination of polleniferous taxa vis-a-vis floral fidelity of *Apis mellifera* L. in North 24 Pargana, West Bengal, we carried out pollen analyses of individual corbicular pollen loads collected from four apiaries during 2015-2016. Among the 2 434 analyzed loads, 72.97 % were unifloral type, 19.0 % bifloral and 8.10 % were multifloral in pollen composition. We identified 43 different types of palynomorphs belonged to 28 botanical families. Major polleniferous plant species include: *Alangium salviifolium*, *Borassus flabellifer*, *Brassica nigra*, *Coriandrum sativum*, *Croton bonplandianum*, *Cyanotis axillaris*, *Luffa cylindrica*, *Neolamarckia cadamba*, *Phoenix sylvestris*, *Poa gangetica*, *Sesamum indicum* and *Trema orientalis*. Among them, *T. orientalis* is newly reported from West Bengal especially during June to August (monsoon season). The plant family provided maximum number of loads was Arecaceae (20.91 %), followed by Brassicaceae (16.2 %), Poaceae (6.70 %), Pedaliaceae (6.38 %), Apiaceae (6.16 %) and Fabaceae (5.38 %). Month wise highest number of pollen diversity were obtained during March (13 pollen types) and a minimum of 6 types in November. The present investigation will help the beekeepers to maintain their hives in the region for sustainable apicultural practices.

Kew words: beekeeping; floral fidelity; pollen load; polleniferous flora.

Nandi, T., & Karmakar, P. (2018). *Apis mellifera* pollen loads to understand the pollen foraging pattern used for apicultural practice in a potentially agricultural belt in Bengal, India. *Revista de Biología Tropical*, 66(4), 1597-1605.

Pollen is the bee's major sources of proteins, amino acids, lipids, minerals and vitamins (Marchini, Reis, & Moreti, 2006). It is essential for the growth of larvae and young adult bees (Dietz, 1975; Modro, Silva, Cynthia, Luz, & Message, 2009). Rearing one worker bee from larval to adult stage requires approximately 120-145 mg of pollen (Alfonsus, 1933; Haydak, 1935). Honeybee transports pollen to the hive in specialised structures of its legs (corbicula), in which pollen moistened with nectar and hypopharyngeal and mandibular secretions, is packaged forming pasty pellets called 'pollen loads' (Thorpe, 2000). The pollen

composition in the pollen loads is a reflection of the local flora preferred by the workers of the bee species surrounding hives (Diaz-Losada, Ricciardelli-d'Albore, & Saa-Otero, 1998). Therefore determination of polleniferous flora in a given area is very important for development of any apiculture industry (Sajwani, Farooq, & Bryant, 2014). In West Bengal some work on polleniferous flora was done by Pal and Karmakar (2013), More, Ghorai, & Bera, 2010. Though few works on pollen analyses of *A. mellifera* form outside the state as well as from other countries were reported by Suryanarayana, Rao, & Singh, 1992; Noor,

Khan, & Camphor, 2009; Lopez, Vives, & Boi, 2013; Saavedra-Carhuatacto, Aguinago-Castro, Rojas-Indrogo, & Delgado-Paredes, 2014 and Freitas, Sattler, Souza, Almeida-Muradian, Sattler, & Barth, 2015.

The present study aims to investigate the botanical origin of pollen loads collected in North 24 Parganas district, West Bengal to provide a guide to the optimal utilization of floral resources by honeybees for sustainable apiculture industries.

MATERIALS AND METHODS

Study site: North 24 Pargana is a district in southern West Bengal of Eastern India. The district situated between (22°11' - 23°15' N & 88°20' - 89°5' E) with a total area of 4094 km². The climatic condition is tropical and experiences hot, humid and exhausting summer (mid April-mid June) with daily temperature ranging from 20 °C to 40 °C. The next season is monsoon which continued up to August, with an average annual rainfall of 1579 mm and with high relative humidity level (RH 90 %). The cold and dry winter approach in early

December and stay up to mid February with day temperature ranges from 8 to 28 °C. Major rivers flows through the district are Ganges, Ichhamati, Jamuna and Bidyadhari. The soil of the northern part of the district is sandy, in the central middle part it is sandy and clay loam and in the southern side it is clay loam. The area harbours a diversified flora with a productive agricultural output as the river Ganges flows its long course through the district.

Collection of samples: A total of 2434 pollen loads were collected from four apiaries viz. Amdanga (634 pollen loads), Bagdaha (586 pollen loads), Barrackpur (610 pollen loads) and Haripur (604 pollen loads) located at four different areas of North 24 Parganas district, West Bengal (Fig. 1). Pollen samples were collected for 12 months (January to December) and three to four sampling days per month. 30 to 64 loads were collected for each sampling day from one apiary. Incoming pollen foragers were captured at the entrance of the hive, and the two corbicular loads were collected. Pollen loads were preserved separately in (5 mL) white glass vials containing FAA (Formalin-Aceto-Alcohol, 5:5:90) solution.

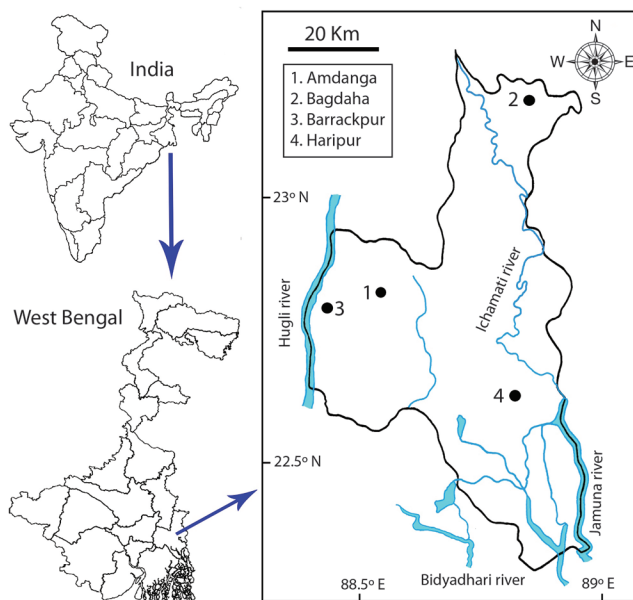


Fig. 1. Location areas of collection of bee pollen load for analysis.

Palynological analyses: Each pollen load was analyzed separately. The pollen loads were suspended in 50 mL of 95 % ethanol and thoroughly mixed (Jones & Bryant, 2004). The final suspension was then centrifuged at 4 000 rpm for 5 min. After decanting supernatant, the sediment was subjected to acetolysis (Erdtman, 1960) with a 9:1 ratio of acetic anhydride to conc. sulphuric acid. After thoroughly mixing, the mixture containing tube was placed into a water bath (at 80 °C) for 3 min. Then 5 mL of glacial acetic acid was added to each sample. The samples were rinsed twice with distilled water, centrifuging and decanting each time, and then once with 95% ethyl alcohol. After centrifuging, the pellet was taken on a small piece of glycerine jelly and transferred to the centre of a glass slide. Glycerine jelly was used as a mounting medium to prepare the samples for light microscopy (Ohe, Oddo, Piana, Morlot, & Martin, 2004). Then warmed gently to melt the jelly containing pollen sediment and covered by cover glass and sealed with paraffin wax. Identification of pollen types was done (on the basis of 500 grains counted per slide) with the help of reference slides prepared from the local flora as well as from published accounts (Nayar, 1990; Layek & Karmakar, 2016). Analysis was conducted using Leica DM1000 and photomicrographs of suitable magnifications were made with Leica DFC295

Digital camera. The contents of each pollen load were designated as being unifloral (with single pollen type ≥ 90 %), bifloral (with pollen of two types, one 80-90 % and other 20-10 %) and multifloral (with pollen of more than two types ≥ 10 %). Month wise percentages of the pollen types were also determined. Then we classified the obtained pollen types into three groups: very frequent (> 20 %), frequent (10-20 %) and less frequent (< 10 %) (Layek & Karmakar, 2018).

Data Analysis: Statistical analyses of the pollen materials were done to get the arithmetic mean and standard deviation. To assess the association between different variables we followed Kernal Pearson's correlation coefficient method.

RESULTS

The analyzed pollen loads were designated as unifloral, bifloral and multifloral according to their pollen composition. The overall number of unifloral loads was 1 776 (72.97 %), bifloral was 462 (18.98 %) and multifloral was 196 (8.05 %). Among the multifloral loads, the majority had three pollen types and only eight loads were with four pollen types in composition. The highest proportion of unifloral loads was recorded during April (89.8 %), followed

TABLE 1
Pollen loads of *A. mellifera* collected in North 24-Parganas, West Bengal (India)

Months	No. of loads analyzed	Percentages of different types of pollen load (mean \pm std. deviation)		
		Unifloral	Bifloral	Multifloral
January	226	82.3 \pm 4.2	13.2 \pm 2.7	4.4 \pm 1.5
February	242	79.3 \pm 8.9	14.9 \pm 3.4	5.8 \pm 5.7
March	244	86.0 \pm 4.3	11.5 \pm 2.1	2.4 \pm 3.1
April	216	89.4 \pm 4.7	5.5 \pm 2.1	5.0 \pm 4.1
May	184	61.3 \pm 7.6	28.9 \pm 4.9	9.8 \pm 4.1
June	120	53.3 \pm 9.4	35.0 \pm 6.4	11.6 \pm 6.4
July	166	50.5 \pm 4.2	39.9 \pm 7.1	9.5 \pm 3.3
August	202	50.1 \pm 5.6	31.7 \pm 2.9	18.1 \pm 4.3
September	188	65.0 \pm 4.3	20.2 \pm 0.4	14.8 \pm 4.4
October	210	71.0 \pm 5.4	17.1 \pm 3.4	11.9 \pm 5.9
November	214	84.1 \pm 7.8	12.0 \pm 6.5	3.9 \pm 2.9
December	222	80.4 \pm 7.0	15.2 \pm 3.7	4.3 \pm 4.1

by March (86.1 %), November (85.05 %), January (82.30 %), December (80.18 %), February (79.34 %), October (71.4 %), September (64.89 %), May (61.96 %), June (53.33 %), July (50.60 %) and August (49.50 %). The sequences were almost reverse in cases of bifloral and multifloral loads (Table 1, Fig. 2). A total of 43 pollen types belonging to 28 plant families were identified (Table 2). Month wise analysis of loads revealed highest number of pollen types during March (13 types), followed by July (11 types), February, June and August (each of them with ten types), January, April, May and September (each with 8 types), October and December (both of each with seven types), and November with 6 pollen types (Table 2). According to frequency classes very frequent (> 20 %) pollen types were *Brassica nigra*, *Phoenix sylvestris* (during January); *Coriandrum sativum* (in February); *Borassus flabellifer* (during March and April); *Sesamum indicum* (in May); *Acacia auriculiformis*, *Cocos nucifera* (in September); *Cocos nucifera*, *Poa gangetica* (in October) and *Brassica nigra* during November and December. Besides the above mentioned pollen types, other frequent pollen types were *Alangium salviifolium*, *Citrus × aurantiifolia*, *Citrus maxima*, *Croton bonplandianum*, *Cyanotis axillaris*, *Luffa cylindrica*, *Neolamarckia cadamba* and *Trema orientalis*. Plants that accounted for the higher number of pollen loads were *Brassica nigra* (7.59 %), followed by *Cocos nucifera* (4.57 %), *Poa gangetica* (3.34 %), *Borassus flabellifer* (3.20 %), *Sesamum indicum* (3.19 %), *Coriandrum sativum* (3.08 %), *Phoenix sylvestris* (2.68 %), *Trema orientalis* (2.37 %) and *Acacia auriculiformis* (2.16 %). Plant families that accounted for a large number of loads were Arecaceae (21 %), Brassicaceae (16.23 %), Poaceae (6.68 %), Pedaliaceae (6.38 %), Apiaceae (6.16 %) and Fabaceae (5.38 %). The families that represented the greater number of taxa were Asteraceae and Euphorbiaceae (each of them with 4 plant taxa); followed by Arecaceae and Cucurbitaceae (each with 3 plant taxa); each of the family viz. Brassicaceae, Fabaceae, Myrtaceae and Rutaceae represented

by 2 plant taxa. The remaining 20 families represented by single plant taxon.

DISCUSSION

Major proportion of unifloral loads implies the floral fidelity behaviour of the bee species. Bifloral and multifloral loads could be due to the variety of flowering plants that were blooming simultaneously. This multifloral availability appears to divert the attention of some foraging bees (Alves & Santos 2014). Greater proportion of unifloral loads were obtained during March-April (spring-summer) and November-January (winter) derived from *Borassus flabellifer* and *Brassica nigra*, *Phoenix sylvestris* respectively. The importance of these plant taxa as polleniferous plant were also reported by Layek, Nandi, & Karmakar (2016) from West Bengal and Suryanarayana et al. (1992) from Bihar in India. In addition to the above mentioned taxa, other important polleniferous plants were: *Acacia auriculiformis*, *Cocos nucifera*, *Coriandrum sativum*, *Poa gangetica*, *Sesamum indicum* and *Trema orientalis*. The significance of some the above taxa as pollen contributor were also recorded in India as well as from other countries (by Noor et al., 2009; Karmakar, Layek, & Pal, 2011; Layek, Bhakat, & Karmakar, 2015). But the significance of *Trema orientalis* as polleniferous plant was not previously recorded by any author in West Bengal, India. The small percentage of pollen from: *Bombax ceiba*, *Euphorbia tithymaloides*, *Flacourtia jangomas*, *Litchi chinensis*, *Nelumbo nucifera*, *Tridax procumbens* indicate that these plants are either sporadic in distribution, fairly rare in abundance, do not produce sufficient amount of pollen for bee foraging, or produce pollen that is not preferred by honeybees due to low nutritional value (Dórea, Novais & Santos, 2010). Among the major pollen supplied families, Apiaceae, Pedaliaceae and Poaceae comprises of single plant taxon. On the other hand, largest represented families Asteraceae, Cucurbitaceae and Euphorbiaceae contributed moderate pollen to the bee species. Hence, the correlation between represented plant taxa of a

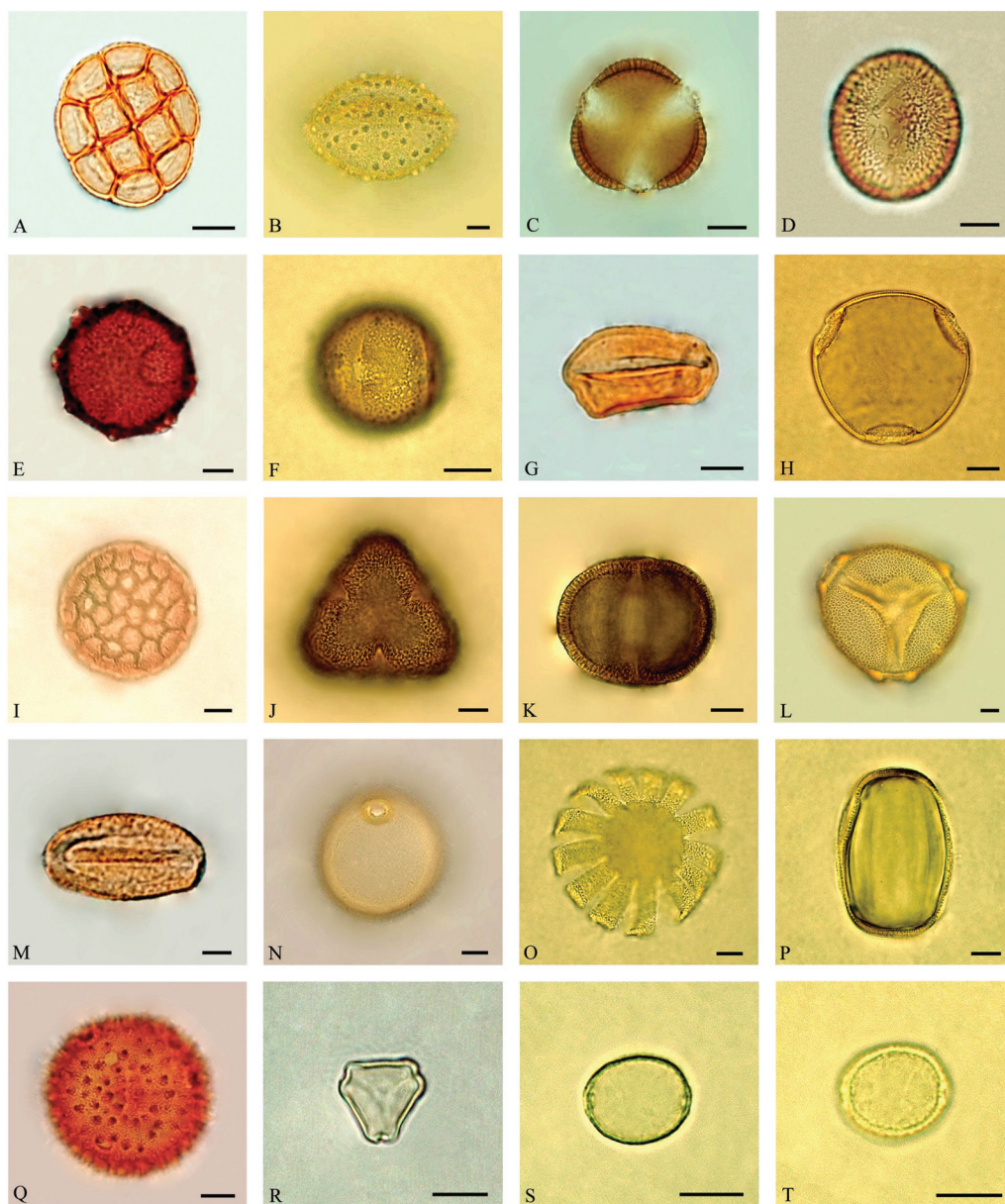


Fig. 2. Some pollen types obtained from pollen loads of *Apis mellifera*: A) *Acacia auriculiformis*; B) *Borassus flabellifer*; C, D) *Brassica nigra*; E) *Chrozophora rotleri*; F) *Citrus x aurantiifolia*; G) *Cocos nucifera*; H) *Cucumis sativus*; I) *Delonix regia*; J, K) *Euphorbia tithymaloides*; L) *Luffa cylindrical*; M) *Monochoria hastate*; N) *Poa gangetica*; O, P) *Sesamum indicum*; Q) *Sida acuta*; R) *Syzygium cumini*; S, T) *Trema orientalis*. Scale bars = 10 μ m.

TABLE 2
Pollen types obtained from loads of *Apis mellifera* in North 24 Parganas, West Bengal (India)

Pollen types	Months												No. of loads
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Amaranthaceae													7
<i>Amaranthus spinosus</i>							LF	LF					7
Apiaceae													75
<i>Coriandrum sativum</i>	F	VF										F	75
Arecaceae													254.49
<i>Borassus flabellifer</i>			VF	VF									78
<i>Cocos nucifera</i>	LF	LF	LF			LF	F	F	VF	VF		LF	111.32
<i>Phoenix sylvestris</i>	VF	F										F	65.17
Asparagaceae													11.50
<i>Polianthes tuberosa</i>							LF	LF					11.50
Asteraceae													36.83
<i>Helianthus annuus</i>		LF	LF										8
<i>Mikania scandens</i>	LF										LF	LF	16.33
<i>Tagetes erecta</i>	LF											LF	8
<i>Tridax procumbens</i>						LF							4.50
Brassicaceae													197.50
<i>Brassica nigra</i>	VF	F	LF								VF	VF	184.67
<i>Raphanus sativus</i>	LF											LF	12.83
Cannabaceae													57.67
<i>Trema orientalis</i>				LF	LF	F	F	F	LF				57.67
Capparaceae													11
<i>Capparis zeylanica</i>			LF	LF									11
Cleomaceae													7.33
<i>Cleome viscosa</i>						LF	LF						7.33
Combretaceae													10
<i>Terminalia arjuna</i>				LF									10
Commelinaceae													17.67
<i>Cyanotis axillaris</i>						LF	LF		LF	F	LF		17.67
Cornaceae													27.67
<i>Alangium salviifolium</i>			F	LF									27.67
Cucurbitaceae													61.67
<i>Cucumis sativus</i>							LF	LF	LF				21
<i>Cucurbita maxima</i>			LF										5.50
<i>Luffa cylindrica</i>			LF		LF	F	LF	LF	LF				35.17
Euphorbiaceae													45.17
<i>Chrozophora rotleri</i>					LF	LF							9.17
<i>Croton bonplandianum</i>				LF	LF	F							22
<i>Euphorbia tithymaloides</i>					LF								2
<i>Ricinus communis</i>									LF	LF			12
Fabaceae													65.50
<i>Acacia auriculiformis</i>							LF	LF	VF	LF			52.50
<i>Delonix regia</i>				LF	LF								13
Malvaceae													10.17
<i>Bombax ceiba</i>			LF										3.17
<i>Sida acuta</i>										LF	LF		7

CUADRO 2 (Continuación) / TABLE 2 (Continued)

Pollen types	Months												No. of loads
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Moringaceae													6.33
<i>Moringa oleifera</i>	LF	LF											6.33
Myrtaceae													28.83
<i>Eucalyptus globulus</i>										LF	LF	LF	22.83
<i>Syzygium cumini</i>			LF										6
Nelumbonaceae													2.50
<i>Nelumbo nucifera</i>					LF								2.50
Pedaliaceae													77.67
<i>Sesamum indicum</i>				F	VF								77.67
Poaceae													81.33
<i>Poa gangetica</i>								F	LF	VF	F		81.33
Pontederiaceae													7.67
<i>Monochoria hastata</i>								LF					7.67
Ranunculaceae													8.33
<i>Nigella sativa</i>		LF											8.33
Rubiaceae													21.84
<i>Neolamarckia cadamba</i>						LF	F	LF					21.84
Rutaceae													32.17
<i>Citrus × aurantiifolia</i>						LF	F						11
<i>Citrus maxima</i>		LF	F										21.17
Salicaceae													5
<i>Flacourtia jangomas</i>			LF										5
Sapindaceae													1.50
<i>Litchi chinensis</i>		LF											1.50
Ulmaceae													18.50
<i>Holoptelea integrifolia</i>		LF	LF										18.50
No. of pollen types	8	10	13	8	8	10	11	10	8	7	6	8	

VF: very frequent, F: frequent, LF: less frequent.

family and their contribution as pollen supplier is low to medium ($r = 0.40$, $n = 28$).

The present study was undertaken to identify the polleniferous flora of the study area as well as floral fidelity behaviour of the bee species. Regarding pollen composition of each load, it was found that majority were unifloral type which signifies the floral fidelity behaviour of the bee species. Floral constancy level increases with the presence of excellent and huge polleniferous plants during March-April and November-January, whereas decreases during dearth period from June-August due to absence of very frequent pollen types in these regions. However, *Trema orientalis* play a

major role to sustain the pollen flow for the bee colony during the dearth period in the studied area. The present work provides us with a considerable knowledge about plant diversity and the annual flowering pattern. By knowing the flowering pattern and the preferred plant species for pollen collection by *A. mellifera*, the apiculturists will get a proper knowledge regarding successful establishment of apiaries in that region.

Ethical statement: authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we

followed all pertinent ethical and legal procedures and requirements. A signed document has been filed in the journal archives.

ACKNOWLEDGMENTS

We are thankful to authorities of Vidyasagar University for providing necessary laboratory and internet facilities. We are also grateful to Apiarists for help us during collection of pollen loads of the bee species. Thanks are also due to Ujjwal Layek for helping us by preparing the tables and photo plates of the manuscript.

RESUMEN

Cargas polínicas de *Apis mellifera* para entender los patrones de forrajeo de polen usados en apicultura en una franja potencialmente agrícola en Bengala, India. Para una determinación precisa de taxa polinífero y fidelidad floral de *Apis mellifera* en Pargana Norte 24, Bengala Occidental, realizamos análisis de cargas individuales de polen corbicular, recolectados en cuatro apiarios durante 2015-2016. De las 2 434 cargas analizadas, 72.97 % fueron de tipo unifloral, 19.0 % bifloral y 8.10 % multifloral, en la composición del polen. Identificamos 43 tipos diferentes de palinomorfos pertenecientes a 28 familias botánicas. Las especies principales de plantas poliníferas incluyen: *Alangium salviifolium*, *Borassus flabellifer*, *Brassica nigra*, *Coriandrum sativum*, *Croton bonplandianum*, *Cyanotis axillaris*, *Luffa cylindrica*, *Neolamarckia cadamba*, *Phoenix sylvestris*, *Poa gangetica*, *Sesamum indicum* y *Trema orientalis*. Entre estas, *T. orientalis* ha sido reportada recientemente en Bengala Occidental, especialmente de junio a agosto (temporada de monsoones). La familia de plantas que presentó máxima cantidad de cargas fue: Arecaceae (20.91 %), seguida por Brassicaceae (16.2 %), Poaceae (6.70 %), Pedaliaceae (6.38 %), Apiaceae (6.16 %) y Fabaceae (5.38 %). De acuerdo al mes, el número mayor de diversidad de polen se obtuvo durante marzo (13 tipos de polen) y un mínimo de seis tipos en noviembre. La presente investigación ayudará a los apicultores a mantener sus colmenas en la región para prácticas apícolas sostenibles.

Palabras clave: apicultura; fidelidad floral; carga polínica; flora polinífera.

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