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Determination of Nectar Resources through Body Surface Pollen Analysis: A Study with the Stingless Bee *Tetragonula iridipennis* Smith (Apidae: Meliponini) in West Bengal, India

Sourabh Bisui[1], Ujjwal Layek[2], Prakash Karmakar[1]

1 - Department of Botany & Forestry, Vidyasagar University, Midnapore- 721102, India

2 - Department of Botany, Rampurhat College, Birbhum - 731224, India

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Corresponding author
Prakash Karmakar
Department of Botany & Forestry, Vidyasagar University
Midnapore - 721102, India.
E-Mail: prakashbot1973@gmail.com

Abstract

Knowledge about floral resources is essential for bee management and conservation. Pollen analysis of honey is the most traditional method for determining the nectar resources of a bee species. However, the collection of honey samples is difficult in cavity-nesting natural stingless bee colonies. Furthermore, it is detrimental to the wild bee's colony and may threaten their survivability. We analyzed adhered body surface pollen of incoming nectar foragers (which were smeared incidentally during nectar foraging) as an alternative method to determine nectariferous flora of *Tetragonula iridipennis* in West Bengal, India. By this method, we have identified 75 pollen types. The number of obtained pollen types was lower in the human-altered habitats of Midnapore city (44 pollen types) than the semi-natural habitats of Garhbeta (71 pollen types). Excluding a few pollen types of non-nectariferous plants, most of the pollen types came from nectariferous plants of both crop and non-crop species. Non-crop flowering plants (viz. *Ailanthus excelsa*, *Borassus flabellifer*, *Eucalyptus tereticornis*, *Lannea coromandelica*, *Peltophorum pterocarpum*, and *Tectona grandis*) provided a significant amount of nectar to the bee species and, therefore, play an important role in the conservation of the bee species.

Introduction

Stingless bees form a monophyletic tribe of corbiculate bees with a few hundred recognized species of about 60 genera (Rasmussen & Cameron, 2010). They are highly eusocial bees that inhabit the tropical and sub-tropical regions of the world. Stingless bees form perennial colonies with a single queen, a few hundred to several thousand workers, and a few hundred males (Michener, 2007). To sustain their colony, foragers collect nectar, pollen, resin, mud, sap, honeydew, animal protein, fungal spores for nutrition, or nest-building materials (Roubik, 1989; Eltz et al., 2002). Nectar is the principal source of carbohydrates from which bees obtain their energy (Ramalho et al., 1991; Nicolson, 2011). Stingless bees are generalist foragers, i.e., explore diverse angiosperm taxa to collect nectar (Ramalho et al., 1990; Roubik & Moreno Patiño, 2009; Vossler et al., 2014) and maintain high floral constancy (Layek & Karmakar, 2018a). While collecting nectar, some pollen grains incidentally adhered to the hairy body parts of the bees are transmitted inadvertently to the hives. After a meticulous investigation of pollen morphology, the origin of the plant resources collected by bees can be established (Jones & Jones, 2001). Thus, pollen grains act as dependable 'fingerprints' of plants, evidenced in the colonies' food materials and smeared on the foragers' body surface (Eltz et al., 2001). Stingless bees performed as one of the most important pollinators of native plants and economic crops (Slaa et al., 2006; Rallanawannee & Duangphakdee, 2019). Therefore, stingless bees management is considered an effective way to enhance crop yield via increasing pollination

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services. Knowledge of bee flora vis-à-vis bee diet is essential for sustainable meliponiculture, management, and proper conservation strategies for a sustainable livelihood in a region.

The dammar bee, *Tetragonula iridipennis* Smith is the most abundant stingless bee in India, including West Bengal. The management and utilization of the bee species for pollination purposes are also minimal. Only a few people have utilized this bee species to pollinate crops like cucumber (Kishan et al., 2017) and bitter gourd (Bisui et al., 2020). Inadequate development of meliponiculture using the bee species has several reasons, including scarcity of data about its foraging behavior for nectar resources. Quantitatively, nectariferous plants were depicted through palynological analysis of pot honey (Vijayakumar & Jeyaraaj, 2016; Layek & Karmakar, 2018a). Pollen analyses of honey (i.e., melissopalynology) have been used to accurately determine the botanical and geographic origin of honey (Louveaux et al., 1978, Von der Ohe et al., 2004; Ponnuchamy et al., 2014). But, the collection of honey samples from natural nests of stingless bees is difficult due to their cavity-nesting habit.

Although field observation was considered a way to determine bee-visited plants (Layek & Karmakar, 2016; Roopa et al., 2017), this procedure has some impediments like spatio-temporal inaccessibility by foraging bees. Moreover, the collection method (honey-sampling) may destroy natural nests and intimidates their survivability. Therefore, it is essential to develop an alternative method for the determination of the nectar resources of the bee species.

To contribute to the knowledge of the floral resources of *T. iridipennis* in semi-natural and human-altered areas of West Bengal, the goals of the paper were to: (1) Identify the nectar resources through body surface pollen analysis (2) Estimate the relative nectar contribution of the bee-visited plants (3) Provide additional information about the acceptability of the used method as an alternative to palynological analysis of honey.

Material and methods

Study areas

The present work was conducted in Garhbeta (22.86° N and 87.35° E) and Midnapore town (22.43° N and 87.32° E) in Paschim Medinipur district of West Bengal, India (Fig 1). We considered the study sites in Garhbeta as semi-natural areas (having dense vegetation and greater agricultural activities) and the study sites in Midnapore town as human-altered areas (less vegetation and little or no agricultural activities). Within the semi-natural areas of Garhbeta, major cultivated crops were *Brassica juncea* (L.) Czern., *Coriandrum sativum* L.,

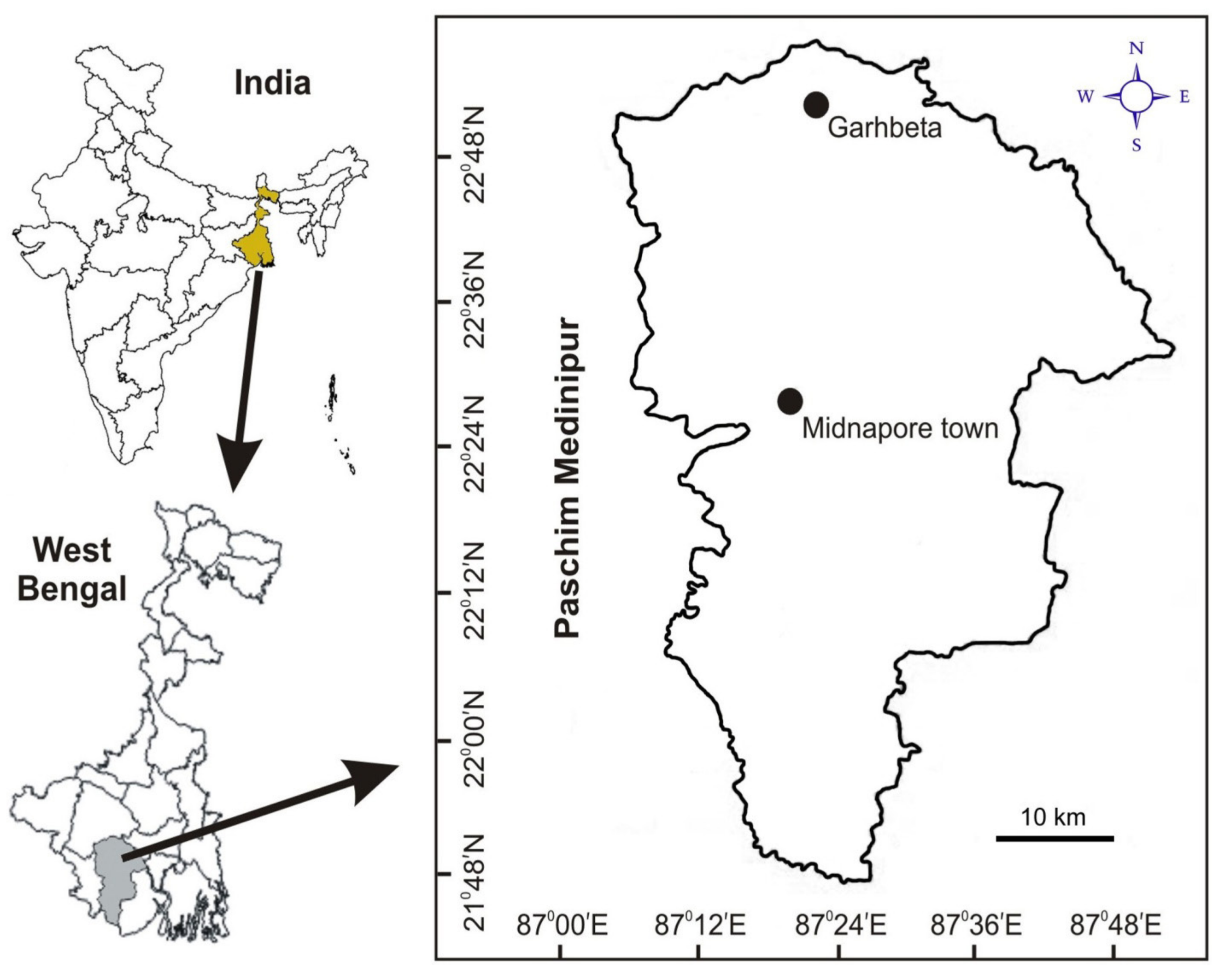

Fig 1. Map showing sampling sites (circles).

Oryza sativa L., *Sesamum indicum* L., *Solanum melongena* L., *Solanum tuberosum* L., *Triticum aestivum* L., and some other cucurbits. Several weeds like *Alternanthera* spp., *Celosia argentea* L., *Parthenium hysterophorus* L., *Mimosa pudica* L., *Sida* spp., *Taraxacum officinale* (L.) Weber ex F. H. Wigg, *Trianthema portulacastrum* L., and *Tridax procumbens* L. were grown in croplands as well as other uncultivated areas. A few tree species (*Acacia auriculiformis* A. cunm. ex Benth., *Ailanthus excelsa* Roxb., *Anacardium occidentale* L., *Delonix regia* (Boj. ex Hook.) Raf., *Eucalyptus tereticornis* Sm., *Lannea coromandelica* (Houtt.) Merr., *Peltophorum pterocarpum* (DC.) K. Heyne, *Shorea robusta* Roth, and *Tectona grandis* L. f.) were also found. In addition to the above-mentioned tree species, the human-altered areas of Midnapore town contain only a few non-crop flowering plants along the roadside and within the Vidyasagar University campus.

Sampling colonies

Samples were collected from 7 colonies of *T. iridipennis*. Four colonies (namely, A, B, C and D) were selected from Garhbeta, and three colonies (namely, E, F and G) were selected from Midnapore town. In Garhbeta, the distance between two nearby selected colonies ranged from 0.63 km (between colony B and colony C) to 1.46 km (between colony A and colony D). In Midnapore town, colony distance was 0.87 km (between colony E and colony F), 1.06 km (between colony E and colony G), and 1.62 km (between colony F and colony G). Nesting substrates were building walls (colony B, C, E, and F), tree trunks of *Tectona grandis* (colony D), and *Terminalia arjuna* (Roxb.) Wight & Arn. (colony A). The colony 'G' was within a broken wall and placed outside the window of the Palynology and Plant Reproductive Biology laboratory of Vidyasagar University. The nests were oriented southward (colony B, C, and D), northwest (colony E and F), east (colony A), and westward (colony G) direction with an elevation of >2 m from the ground level.

Collection of nectar foragers

Samples were collected at three time-slots (8.00–10.00 h, 12.00–14.00 h, and 16.00–18.00 h) on a sampling day. To capture the incoming nectar foragers, we closed the entrance of the selected colonies for 5–10 minutes. Among the incoming bees, we selected the bees who have swollen abdomen but without corbicular loads. We captured the bees by using clean forceps and kept them within a glass vial (single bee per vial) using 1 mL FAA (formaldehyde, glacial acetic acid, 95% ethanol, and distilled water) a ratio of 5:5:50:40) solution. Then bee container having FAA solution was stirred to dislodge the pollen grains smeared to the bee's body surface and removed the bees from the solution. During 2016–2019, month-wise samples were collected, and in each sampling day, we collected 10–20 samples. In this way, we collected 1413 samples (= bees) from Garhbeta and 1319 samples from Midnapore town (Table 1).

Palynological analyses

After shaking (to homogenize the pollen solution), 10 µL of solution were taken from the samples (pollen containing FAA solution) by micropipette. The solution was slid on a clear glass slide. The non-acetolysed pollens present within the taken sample were examined under a light microscope. Based on the morphology of the pollen types, the stock

Table 1. Month wise sample size in semi-natural areas of Garhbeta and human-altered areas of Midnapore town in West Bengal.

Month	Number of captured bees					
	Garhbeta			Midnapore		
	With body-surface pollen	Without body-surface pollen	Total	With body-surface pollen	Without body-surface pollen	Total
January	117	6	123	92	04	96
February	114	5	119	91	03	94
March	124	11	135	105	07	112
April	126	16	142	117	08	125
May	100	18	118	112	12	124
June	119	21	140	138	17	155
July	99	09	108	93	07	100
August	90	05	95	85	04	89
September	101	06	107	89	05	94
October	81	03	84	106	06	112
November	105	04	109	100	04	104
December	127	06	133	109	05	114
Total	1303	110	1413	1237	82	1319

pollen samples (collected on a sampling day) were sorted into different sub-groups for further analysis. Additionally, we counted the number of pollen grains suspended in the 10 µL solution. Noticeably, we did not consider all the samples (to reduce experimental time and hardness) and counted only those samples (n = 166 for Garhbeta, n = 150 for Midnapore town) that were homogenous in pollen content. We repeated the counting procedure three times for a sample and estimated the average number of pollen grains in 10 µL. Then, we calculated the total number of pollen grains present within the sample according to the initial volume of the solution.

Samples of each sub-group (having similar pollen types) were taken together for further analysis. Samples were centrifuged for 10 min at 2500 rpm (1036 *g*). After discarding the supernatant, pollen sediment was used for palynological preparation through the acetolysis method (Erdtman, 1960). The acetolysed pollen was mounted on a glass slide using glycerine jelly. Then, pollen types were identify using the reference slides prepared from the local flora and with the help of published articles (Pal & Karmakar, 2013; Layek & Karmakar, 2016, 2018b; Bisui et al., 2019; Layek et al., 2020b) and an unpublished Ph.D. thesis (Ghosh, 2018). Microscopy was done by using a Nikon Eclipse LV100 POL polarising microscope and Leica DM 1000 Ergo trinocular microscope. Microphotographs of some pollen types were taken. Pollen was classified according to the pollen type system of Joosten and De Klerk (2002) and De Klerk and Joosten (2007), which was based on morphological features.

Samples without any pollen content were not considered during the percentage calculation of identified pollen types. A few samples were heterogeneous, which means they have more than one pollen type. To calculate the occurrence of pollen types, we attributed a value of 1 for a particular pollen type per homogenous (containing one kind of pollen type) sample. For a heterogeneous sample, the given value was 1/x for each pollen type present within the sample. Here, x is the number of pollen types present within this sample. We estimated the percentage of pollen types by considering the total number of pollen samples, month-wise and year-round.

After the estimation of month-wise occurrence (%) of the pollen types, we classified the pollen types into four categories: very frequent (> 30%), frequent (10–30%), less frequent (3% to <10%), and rare (< 3%).

Floral morphology of some nectar contributing plants

We selected a few nectariferous plants (based on pollen types within the analyzed samples) to collect data about their floral morphology. We considered flower size and shape, the position of anthers, the number of pollen grains per flower, and the pollen size of those plants. According to the length of the flower, we classified them into small-sized (< 1 cm), medium-sized (1–3 cm), and large-sized (> 3 cm). Based on the multiplication values between polar diameter (PD) and equatorial diameter (ED), we classified the pollen grains into three size groups (Layek & Karmakar, 2018a; Layek et al., 2020a): small-sized (PD x ED < 625 µm^2), medium-sized (PD x ED 625–2500 µm^2) and large-sized (PD x ED > 2500 µm^2).

Statistical analysis

Statistical analyses of the samples were conducted to obtain the arithmetic mean and standard deviation. One-way ANOVA and Duncan's multiple range test (DMRT) were used to analyze data. A generalized linear model (GLM) with a Poisson distribution and log link function was used to test if there was a difference in pollen richness among the studied colonies. Results were treated as significant if $P \leq 0.05$. We analyzed the monthly utilized pollen type's similarity (agglomerative hierarchical clustering) using the Jaccard similarity coefficient. These statistical analyses were performed using SPSS (16.0) statistical packages and Microsoft Excel. To check the extent to which the floral characters explained the number of pollen grains on the bee's body surface, principal component analysis (PCA) was performed using PAST software version 3.26 (Hammer et al., 2001).

Results

Among the analyzed samples (1413 in Garhbeta and 1319 in Midnapore town), most of them contained pollen types (Table 1). A total of 75 pollen types belonging to 39 plant families were identified (Table 2). Photomicrographs of some pollen types were depicted in Fig 2. Higher represented families were Fabaceae (9 pollen types), Asteraceae (5 pollen types), Lamiaceae (4 pollen types), and Myrtaceae (4 pollen types). All most all the obtained pollen types were indicating nectariferous plants. However, a few pollen types viz. *Capparis zeylanica*, *Chenopodium album*, *Holoptelea integrifolia*, *Luffa aegyptiaca*, *Ricinus communis*, *Solanum sisymbriifolium*, *Streblus asper,* and *Trema orientalis* came from non-nectariferous plants (particularly for this bee species). The issue of plant resource types is more likely to be species-specific, and some of these non-nectariferous plants (viz. *Luffa aegyptiaca*, *Ricinus communis*) may provide nectar to other insect species. Major nectar contributed families (based on the occurrence of pollen types of nectariferous plants) were Myrtaceae, Fabaceae, Lamiaceae, Brassicaceae, Arecaceae, and Anacardiaceae. If we see the picture round the year, frequent pollen types obtained in semi-natural areas of Garhbeta were *Eucalyptus tereticornis* (14.51%), *Brassica juncea* (11.34%), *Tectona grandis* (9.62%), *Peltophorum pterocarpum* (7.12%), *Borassus flabellifer* (3.71%), *Lannea coromandelica* (3.26%), and *Delonix regia* (3.22%) (Table 2). In human-altered areas of Midnapore town, frequent pollen types were *Eucalyptus tereticornis* (24.66%), *Peltophorum pterocarpum* (13.20%), *Delonix regia* (6.95%), *Tectona grandis* (6.01%), *Borassus flabellifer* (4.21%), and *Lannea coromandelica* (4.61%).

Table 2. Year-round occurrence of different pollen types (obtained from bee's body surface pollen analyses) in Garhbeta and Midnapore town.

Family	Pollen type	Occurrence (%)	
		Garhbeta	Midnapore
Acanthaceae	*Hygrophila auriculata*	0.38	-
	Justicia adhatoda	0.46	0.81
	Justicia simplex	0.23	-
Aizoaceae	*Trianthema portulacastrum*	0.46	-
Amaranthaceae	***Chenopodium album***	0.31	-
Amaryllidaceae	*Allium cepa*	0.46	-
Anacardiaceae	*Lannea coromandelica*	3.26	4.61
	Mangifera indica	1.00	1.61
	Spondias pinnata	0.61	-
Apiaceae	*Coriandrum sativum*	2.55	-
Apocynaceae	*Alstonia scholaris*	0.61	1.29
	Wrightia tinctoria	-	1.05
Arecaceae	*Borassus flabellifer*	3.71	4.21
	Cocos nucifera	1.07	5.37
	Phoenix sylvestris	1.66	-
Asteraceae	*Chrysanthemum indicum*	-	0.81
	Eupatorium odoratum	0.15	0.73
	Mikania scandens	1.53	2.18
	Tridax procumbens	1.07	1.37
	Xanthium strumarium	0.31	-
Bignoniaceae	*Millingtonia hortensis*	0.46	-
Brassicaceae	*Brassica juncea*	11.34	-
	Raphanus sativus	0.15	-
Cannabaceae	***Trema orientalis***	0.90	2.12
Capparaceae	***Capparis zeylanica***	0.04	-
Convolvulaceae	*Evolvulus nummularius*	1.15	-
Cornaceae	*Alangium salviifolium*	0.69	0.64
Cucurbitaceae	*Coccinia grandis*	2.53	2.67
	Cucumis sativus	0.46	-
	Luffa aegyptiaca	0.65	0.40
	Momordica charantia	0.31	-
Dipterocarpaceae	*Shorea robusta*	0.92	0.81
Euphorbiaceae	*Chrozophora rottleri*	0.31	-
	Croton bonplandianus	1.61	1.05
	Ricinus communis	0.27	0.49
Fabaceae	*Acacia auriculiformis*	2.30	3.47
	Albizia lebbeck	0.46	0.40
	Cassia fistula	0.15	0.32
	Dalbergia sissoo	0.31	0.57
	Delonix regia	3.22	6.95
	Lablab purpureus	0.46	-
	Leucaena leucocephala	0.38	0.57
	Millettia pinnata	0.46	-
	Peltophorum pterocarpum	7.12	13.20
Lamiaceae	*Ocimum tenuiflorum*	0.38	0.65
	Salvia splendens	-	0.24
	Tectona grandis	9.62	6.01
	Vitex negundo	1.77	-
Lythraceae	*Lagerstroemia speciosa*	-	0.32
Malvaceae	*Bombax ceiba*	0.23	-
	Ceiba pentandra	1.00	-
	Grewia asiatica	0.38	-
Meliaceae	*Azadirachta indica*	1.07	0.57
	Melia azedarach	0.84	-
	Swietenia mahagoni	0.15	0.40
Menispermaceae	*Tinospora cordifolia*	0.92	0.89
Moraceae	***Streblus asper***	0.23	-
Moringaceae	*Moringa oleifera*	1.92	-
Myrtaceae	*Eucalyptus tereticornis*	14.51	24.66
	Syzygium cumini	1.46	1.62
	Syzygium jambos	0.15	0.24
	Syzygium reticulatum	0.38	0.49
Nyctaginaceae	*Boerhavia diffusa*	1.07	0.40
Oxalidaceae	*Oxalis corniculata*	0.23	-
Pedaliaceae	*Sesamum indicum*	0.69	-
Phyllanthaceae	*Bridelia retusa*	2.53	0.81
Rhamnaceae	*Ziziphus mauritiana*	0.69	0.40
Rubiaceae	*Meyna spinosa*	0.08	-
Rutaceae	*Citrus* type	0.38	0.16
Sapindaceae	*Sapindus mukorossi*	0.08	-
Sapotaceae	*Mimusops elengi*	0.23	1.62
Simaroubaceae	*Ailanthus excelsa*	1.07	2.06
Solanaceae	*Petunia × alkinsiana*	-	0.32
	Solanum sisymbriifolium	0.38	0.75
Ulmaceae	***Holoptelea integrifolia***	0.81	-

Note: in bold - pollen types came from non-nectariferous plants; others pollen types are indicating nectar sources.

Monthly pollen richness significantly differed among the studied colonies (GLM, type III: coefficient = -0.11, Wald χ^2 = 32.70, d.f. = 1, P < 0.001; Intercept: coefficient = 2.56, Wald χ^2 = 1106.76, d.f. = 1, P < 0.001). In general, colonies within semi-natural areas of Garhbeta have greater pollen richness than the colonies within human-altered areas of Midnapore town. Zone-wise, 71 pollen types were identified in semi-natural areas of Garhbeta (Table 3) and 44 pollen types in human-altered areas of Midnapore town (Table 4). Important pollen types (including very frequent and frequent) were *Acacia auriculiformis*, *Ailanthus excelsa*, *Borassus flabellifer*, *Brassica juncea*, *Bridelia retusa*, *Cocos nucifera*, *Coriandrum sativum*, *Croton bonplandianum*, *Delonix regia*, *Eucalyptus tereticornis*, *Lannea coromandelica*, *Moringa oleifera*, *Peltophorum pterocarpum*, *Phoenix sylvestris*, *Syzygium cumini*, *Tectona grandis,* and *Vitex negundo*. The number of pollen types obtained per month was also varied (Fig 3). From September to December, the number of obtained pollen types was lesser than the rest of the months. Monthly pollen type's similarity analysis showed two clusters in both the studied areas (Fig 4). Pollen types from October to February were grouped into cluster 1, and the remaining (during March to September) constituted cluster 2. January and February (similarity index of 0.67 in Garhbeta and 0.57 in Midnapore town) and July and August (similarity index of 0.58 in Garhbeta and 0.62 in Midnapore town) periods showed higher pollen type similarity in both study areas.

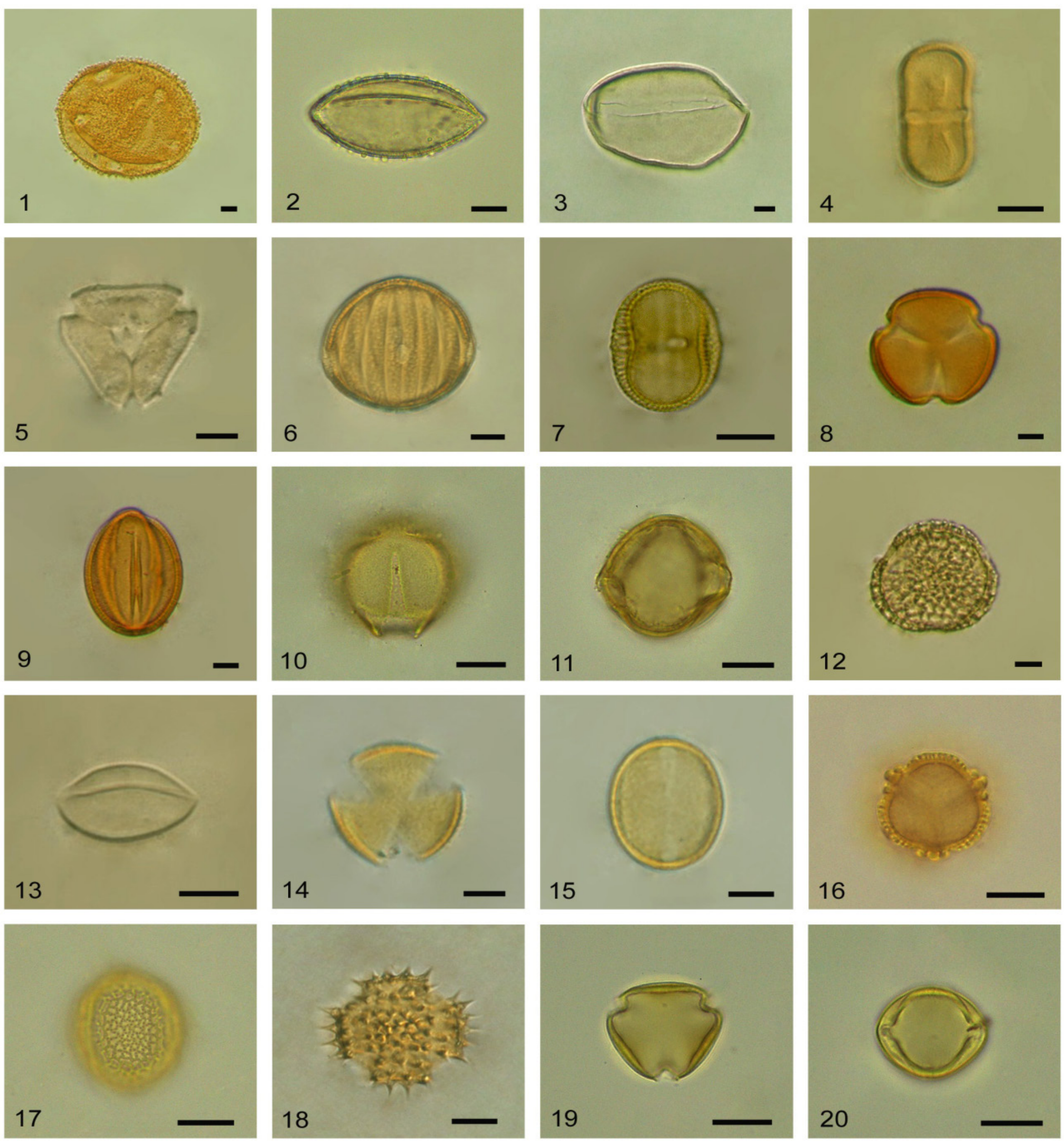

Fig 2. Micrographs of some pollen types obtained from body surface of *T. iridipennis*. 1. *Alangium salviifolium*. 2. *Borassus flabellifer*. 3. *Cocos nucifera*. 4. *Coriandrum sativum*. 5. *Eucalyptus tereticornis*. 6. *Hygrophila auriculata*. 7. *Justicia simplex*. 8–9. *Leucaena leucocephala*. 10–11. *Moringa oleifera*. 12. *Peltophorum pterocarpum*. 13. *Phoenix sylvestris*. 14–15. *Tectona grandis*. 16–17. *Tinospora cordifolia*. 18. *Tridax procumbens*. 19–20. *Ziziphus mauritiana*. Scale bars - 10 µm.

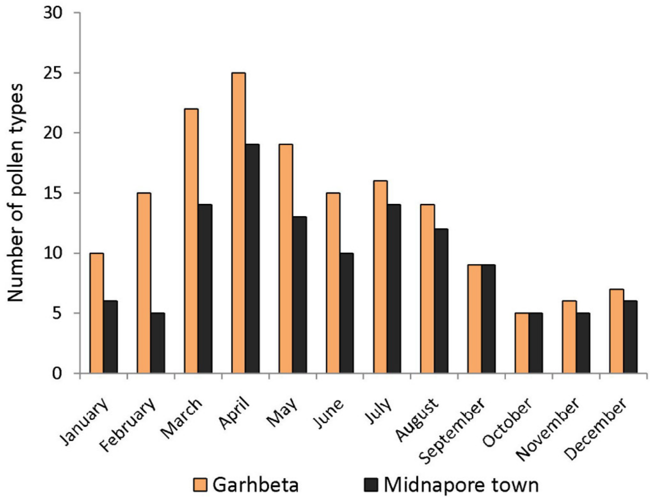

Fig 3. Month-wise richness of pollen types in Garhbeta and Midnapore town of West Bengal.

Pollen content per bee's body surface significantly differed among the studied plant species ($F_{9, 306}$ = 152.52, P = 1.6E-107). Foragers bore a higher number of pollen grains when they foraged on *Ailanthus excelsa*, *Eucalyptus tereticornis*, *Lannea coromandelica,* and *Tectona grandis* (Table 5; Fig 5). On the other hand, bees foraged on *Brassica juncea* have low pollen content on their body surface. Regarding the floral morphology of these plants, it was revealed that small-sized, dish or stellate or brush flowers having exposed anthers with small-sized pollen grains delivered a larger amount of pollen to the forager's body surface.

Discussion

At present, desertification of natural and semi-natural habitats because of deforestation is one of the most significant reasons for the loss of biodiversity and certain key pollinators, such as bees, in terrestrial ecosystems throughout the world (Kevan, 1999; Farig 2003; Brown & Paxton, 2009). In most cases, various human activities that cause deforestation eventually result in habitat fragmentation and are expected to negatively influence the population of a bee species (Winfree et al., 2011; Senapathi et al., 2015). However, bee species can even thrive in human-altered landscapes (Sirohi et al., 2015). Floral resources serve as critical components for maintaining bee fauna (Laha et al., 2020). Field surveillance, melissopalynological study, or gut content analysis are three well-known methods employed by scientists to identify nectar sources used by bee species. However, each of these methods has some limitations. In field observations and gut content analyses, it would be difficult to do quantitative estimations. Melissopalynological analyses also have limitations like

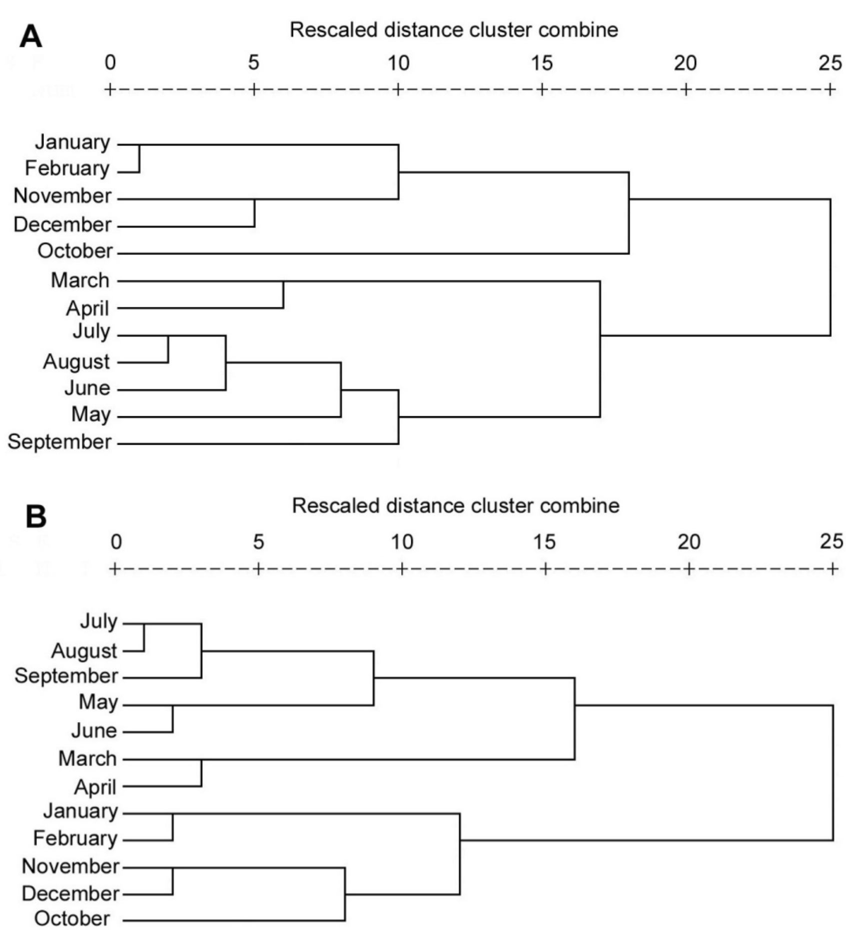

Fig 4. Dendrogram of utilized pollen type's similarity. **A**. Garhbeta. **B**. Midnapore town.

Table 3. Month-wise pollen types obtained from bee's body surface pollen analysis in Garhbeta, West Bengal.

Month	Pollen types			
	Very frequent	Frequent	Less frequent	Rare
January	*Brassica juncea*	*Eucalyptus tereticornis*	*Ailanthus excelsa*, *Coriandrum sativum*, *Mangifera indica*, *Moringa oleifera*, *Phoenix sylvestris*	***Chenopodium album***, ***Holoptelea integrifolia***, *Mikania scandens*
February		*Brassica juncea*, *Lannea coromandelica*	*Ailanthus excelsa*, *Coriandrum sativum*, *Eucalyptus tereticornis*, ***Holoptelea integrifolia***, *Lablab purpureus*, *Mangifera indica*, *Moringa oleifera*, *Phoenix sylvestris*, *Spondias pinnata*	*Allium cepa*, *Chenopodium album*, *Mikania scandens*, ***Streblus asper***
March		*Lannea coromandelica*, *Syzygium cumini*	*Alangium salviifolium*, *Albizia lebbeck*, *Borassus flabellifer*, *Ceiba pentandra*, *Coccinia grandis*, *Millettia pinnata*, *Momordica charantia*, *Shorea robusta*, *Ziziphus mauritiana*	*Allium cepa*, *Bombax ceiba*, ***Capparis zeylanica***, *Cocos nucifera*, *Dalbergia sissoo*, *Grewia asiatica*, *Justicia adhatoda*, *Mangifera indica*, *Melia azedarach*, *Raphanus sativus*, ***Ricinus communis***, *Spondias pinnata*, *Tridax procumbens*
April		*Borassus flabellifer*, *Delonix regia*	*Alangium salviifolium*, *Azadirachta indica*, *Citrus* type, *Coccinia grandis*, *Croton bonplandianus*, *Evolvulus nummularius*, *Lannea coromandelica*, *Melia azedarach*, *Shorea robusta*, *Syzygium cumini*, *Tinospora cordifolia*	*Albizia lebbeck, Bombax ceiba*, *Cassia fistula*, *Grewia asiatica*, *Justicia adhatoda*, *Oxalis corniculata*, ***Ricinus communis***, *Spondias pinnata*, *Syzygium jambos*, ***Trema orientalis***, *Tridax procumbens*, *Ziziphus mauritiana*
May		*Croton bonplandianus*, *Delonix regia*, *Peltophorum pterocarpum*, *Tectona grandis*	*Azadirachta indica*, *Borassus flabellifer*, *Coccinia grandis*, *Evolvulus nummularius*, *Melia azedarach*, *Sesamum indicum*, *Syzygium reticulatum*, *Tridax procumbens*	*Cocos nucifera*, *Justicia adhatoda*, *Meyna spinosa*, *Oxalis corniculata*, *Tinospora cordifolia*, ***Solanum sisymbriifolium***, *Trianthema portulacastrum*, ***Trema orientalis***
June		*Delonix regia*, *Peltophorum pterocarpum*, *Tectona grandis*	*Boerhavia diffusa*, *Coccinia grandis*, *Cocos nucifera*, *Evolvulus nummularius*, *Sesamum indicum*, *Trianthema portulacastrum*	*Chrozophora rottleri*, *Croton bonplandianus*, *Hygrophila auriculata*, *Justicia simplex*, ***Solanum sisymbriifolium***, *Tinospora cordifolia*, *Trema orientalis*
July	*Tectona grandis*		*Acacia auriculiformis*, *Boerhavia diffusa*, *Bridelia retusa*, *Coccinia grandis*, *Croton bonplandianus*, *Mimusops elengi*, *Peltophorum pterocarpum*, *Tridax procumbens*	*Chrozophora rottleri*, *Evolvulus nummularius*, *Hygrophila auriculata*, *Ocimum tenuiflorum*, ***Solanum sisymbriifolium***, *Swietenia mahagoni*, *Tinospora cordifolia*, *Trema orientalis*
August	*Tectona grandis*	*Peltophorum pterocarpum*, *Vitex negundo*	*Acacia auriculiformis*, *Bridelia retusa*, *Coccinia grandis*, *Cucumis sativus*, *Tinospora cordifolia*	*Cocos nucifera*, *Evolvulus nummularius*, *Hygrophila auriculata*, *Ocimum tenuiflorum*, ***Trema orientalis***, *Tridax procumbens*
September		*Acacia auriculiformis*, *Bridelia retusa*, *Peltophorum pterocarpum*, *Vitex negundo*	*Boerhavia diffusa*, *Eucalyptus tereticornis*	*Ocimum tenuiflorum*, ***Trema orientalis***, *Tridax procumbens*
October	*Eucalyptus tereticornis*		*Acacia auriculiformis*, *Cucumis sativus*, *Leucaena leucocephala*, ***Luffa aegyptiaca***	*Chromolaena odoratum*
November	*Eucalyptus tereticornis*	*Brassica juncea*	*Alstonia scholaris*, *Mikania scandens*, *Millingtonia hortensis*, *Xanthium strumarium*	***Luffa aegyptiaca***
December	*Brassica juncea*	*Coriandrum sativum*, *Eucalyptus tereticornis*, *Moringa oleifera*, *Phoenix sylvestris*	*Mikania scandens*	*Alstonia scholaris*

Note: in bold - pollen types came from non-nectariferous plants; others pollen types are indicating nectar sources.

difficult sample collection (from wild hives) and mode of collection, which is an important criterion for an accurate depiction of nectar sources (Layek et al., 2020b). Besides, the presence of over-represented and under-represented pollen grains in honey samples may again provide a faulty result. In the case of social bees of populated colonies, the method (analysis of pollen content on the bee's body surface) is poorly destructive compared to killing a whole colony.

Furthermore, by utilizing this method, we determined the nectar contributing potential of the bee-visited plants.

Table 4. Month-wise pollen types obtained from bee's body surface pollen analysis in Midnapore town, West Bengal.

Month	Pollen types			
	Very frequent	Frequent	Less frequent	Rare
January	*Eucalyptus tereticornis*	*Ailanthus excelsa*	*Chrysanthemum indicum*, *Mangifera indica*, *Mikania scandens*	*Petunia × alkinsiana*
February	*Eucalyptus tereticornis, Lannea coromandelica*	*Ailanthus excelsa*	*Mangifera indica*, *Mikania scandens*	
March		*Borassus flabellifer*, *Lannea coromandelica*, *Syzygium cumini*	*Alangium salviifolium*, *Albizia lebbeck*, *Coccinia grandis*, *Cocos nucifera*, *Dalbergia sissoo*, *Mangifera indica*, *Shorea robusta*, *Ziziphus mauritiana*	*Justicia adhatoda*, ***Ricinus communis***, *Salvia splendens*, *Tridax procumbens*
April		*Borassus flabellifer*, *Delonix regia*	*Alangium salviifolium*, *Azadirachta indica*, *Cassia fistula*, *Coccinia grandis*, *Cocos nucifera*, *Justicia adhatoda*, *Lannea coromandelica*, *Shorea robusta*, *Syzygium cumini*	*Albizia lebbeck*, *Citrus* type, *Croton bonplandianus*, ***Ricinus communis***, *Syzygium jambos*, *Tinospora cordifolia*, *Tridax procumbens*, *Ziziphus mauritiana*, ***Trema orientalis***
May		*Delonix regia*, *Peltophorum pterocarpum*, *Tectona grandis*	*Borassus flabellifer*, *Cocos nucifera*, *Coccinia grandis*, *Croton bonplandianus*, *Justicia adhatoda*, *Syzygium reticulatum*, ***Trema orientalis***, *Tridax procumbens*	*Azadirachta indica*, ***Solanum sisymbriifolium***, *Tinospora cordifolia*
June		*Delonix regia*, *Peltophorum pterocarpum*, *Tectona grandis*	*Cocos nucifera*, *Mimusops elengi*, ***Trema orientalis***	*Boerhavia diffusa*, *Coccinia grandis*, *Croton bonplandianus*, ***Solanum sisymbriifolium***, *Tinospora cordifolia*
July	*Tectona grandis*	*Peltophorum pterocarpum*	*Acacia auriculiformis*, *Coccinia grandis*, *Croton bonplandianus*, *Lagerstroemia speciosa*, *Mimusops elengi*, *Ocimum tenuiflorum*, *Swietenia mahagoni*, ***Trema orientalis***	*Boerhavia diffusa*, *Bridelia retusa*, ***Solanum sisymbriifolium***, *Tinospora cordifolia*, *Tridax procumbens*
August		*Acacia auriculiformis*, *Peltophorum pterocarpum*	*Bridelia retusa*, *Coccinia grandis*, *Cocos nucifera*, *Tectona grandis*, ***Trema orientalis***, *Tridax procumbens*, *Wrightia tinctoria*	*Boerhavia diffusa*, *Ocimum tenuiflorum*, *Tinospora cordifolia*
September	*Peltophorum pterocarpum*	*Acacia auriculiformis*	*Bridelia retusa*, *Eucalyptus tereticornis*, *Ocimum tenuiflorum*, ***Trema orientalis***, *Tridax procumbens*, *Wrightia tinctoria*	*Boerhavia diffusa*
October	*Eucalyptus tereticornis*	*Cocos nucifera*	*Acacia auriculiformis*, *Chromolaena odoratum*, *Leucaena leucocephala*	***Luffa aegyptiaca***
November	*Eucalyptus tereticornis*		*Alstonia scholaris*, *Chromolaena odoratum*, *Cocos nucifera*, *Mikania scandens*	***Luffa aegyptiaca***
December	*Eucalyptus tereticornis*	*Cocos nucifera*	*Alstonia scholaris*, *Chrysanthemum indicum*, *Mikania scandens*	*Petunia × alkinsiana*

Note: in bold - pollen types came from non-nectariferous plants; others pollen types are indicating nectar sources.

We can also eliminate the effect of over and under-representation of pollen types. Pollen content on a bee's body surface indicates the representation potential of a pollen type within honey samples. Therefore, counting the bee's body surface pollen may give information about the less-represented and over-represented pollen types. In this regard, we considered *Brassica juncea* as a less-represented pollen type and *Ailanthus excelsa*, *Eucalyptus tereticornis*, *Lannea coromandelica,* and *Tectona grandis* as over-represented pollen types. Over-representation of some pollen types mentioned above was also reported by several workers (Layek & Karmakar, 2018b). However, the over-representation of *Tectona grandis* as a nectar source will be the first time report made by the present authors from West Bengal.

While considering the floral morphology of some low, medium, and over-represented pollen taxa, we provided a relation between flower shapes and pollen representation. In general, small dish/stellate flowers with exposed anthers and easily accessible short columns of nectar (*Ailanthus excelsa*, *Lannea coromandelica,* and *Tectona grandis*) and brush flower (*Eucalyptus tereticornis*) containing a copious amount of nectar along with exposed anthers having small-sized pollen grains favored over-representation. Workers of *T. iridipennis* may visit a greater number of flowers in a single bout on these plants, and hairy body parts contact with a higher number of pollen grains than the plants bearing large-sized flowers with large-sized pollen grains in hidden anthers.

Besides the merits of the utilized method, there are some limitations: (i) absence of pollen grains on the body surface of some incoming bees, (ii) sometimes occurrence of more than one pollen type from a single incoming bee, and (iii) body surface of some captured bees contain pollen types of non-nectariferous but polleniferous plants. The absence of pollen on the bee's body surface of a returning forager may have several reasons – (i) bee is a water forager, (ii) extrafloral nectar or another sugary liquid forager (iii) foraged exclusively over female flowers of some unisexual plant species (iv) the forager may be a nectar thief/robber. Considering the occurrence of more than one pollen type or non-nectariferous pollen types on a bee's body surface, we inferred that it is due to foragers (i) shifting from one plant resource to another plant resource during its entire foraging span, (ii) picking up residual pollen from the hive environment, (iii) having air-born pollen from wind-pollinated plants adhering to their body, or (iv) visiting flowers of two or more plant species in a single bout. However, the first and second reasons are more agreeable for the occurrence of more than one pollen type or pollen types of non-nectariferous plants on bee's body surface.

Regarding the obtained pollen types, most of them were common to the pollen types of polleniferous plants obtained in our previous study (Bisui et al., 2019). The utilization of bee-visited plants as a source of both nectar and pollen has been regarded as a profitable foraging strategy and was previously documented for honey bees (Layek et al., 2015; Taha et al., 2019; Layek & Karmakar, 2020) and stingless bees (Layek & Karmakar, 2018a). Few pollen types like *Alstonia scholaris*, *Oxalis corniculata*, and *Wrightia tinctoria* are new record from West Bengal. The presence of *Azadirachta indica* pollen types indicated that the bee species might collect toxic rewards (pollen and nectar) from the

Table 5. Pollen presentation (on bee's body surface) of some nectariferous plants and their floral morphology.

Plant species	Pollen content/bee's body surface	Floral morphology				
		Flower size	Flower shape	Pollen presentation	Pollen yield/flower	Pollen size
Acacia auriculiformis	46.75^{d} ± 10.70	small	brush	exposed	low	medium
Ailanthus excelsa	229.50^{a} ± 69.45	small	dish	exposed	medium	large
Borassus flabellifer	41.08^{de} ± 10.23	small	tubular	exposed	low	large
Brassica juncea	21.63^{e} ± 9.14	medium	cruciform	exposed	medium	medium
Cocos nucifera	44.80^{de} ± 15.00	small	stellate	exposed	high	medium
Delonix regia	38.39^{de} ± 18.82	large	flag	exposed	medium	large
Eucalyptus tereticornis	192.32^{b} ± 47.55	small	brush	exposed	high	small
Lannea coromandelica	180.24^{b} ± 50.13	small	stellate	exposed	medium	small
Peltophorum pterocarpum	28.33^{de} ± 9.20	medium	dish	exposed	medium	large
Tectona grandis	143.06^{c} ± 41.47	medium	dish	exposed	medium	small

Values are given in mean ± standard deviation. Different letters indicate significant differences (DMRT at 5%).

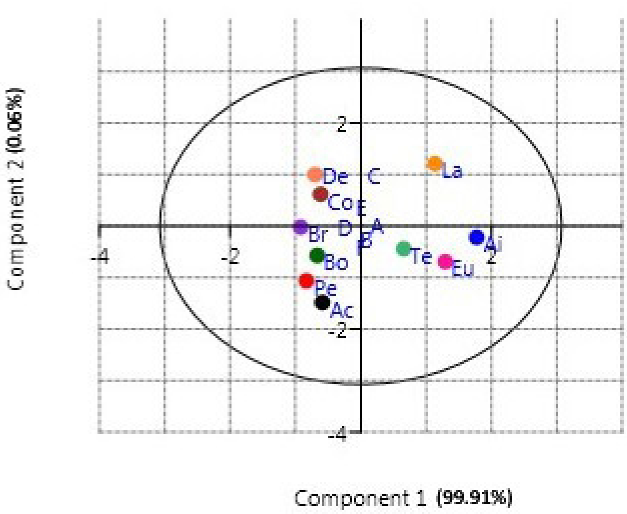

Fig 5. PCA chart showing difference between body surface pollen content and floral characteristics. Ac = *Acacia auriculiformis*, Bo = *Borassus flabellifer*, Br = *Brassica juncea*, Co = *Cocos nucifera*, De = *Delonix regia*, Eu = *Eucalyptus tereticornis*, La = *Lannea coromandelica*, Pe = *Peltophorum pterocarpum*, Te = *Tectona grandis*; A = body surface pollen contents, B = Flower size, C = Flower shape, D = pollen presentation, E = pollen yield per flower, F = pollen size.

plant species during its flowering period (March–April). An enormous collection of toxic rewards may lead to the collapse of the bee colonies. Therefore, beekeepers need to precaution during this time period to avoid collecting these toxic rewards by managed bees. The bee species utilized a greater number of nectariferous plants in semi-natural areas of Garhbeta than the human-altered areas of Midnapore town.

In the semi-natural areas of Garhbeta, *T. iridipennis* vigorously utilized crop plants (e.g., *Brassica juncea*) and non-crop plants (viz. *Borassus flabellifer*, *Delonix regia*, *Eucalyptus tereticornis*, *Lannea coromandelica*, *Peltophorum pterocarpum*, and *Tectona grandis*). However, within the human-altered areas of Midnapore town, the bee species mainly depends on the non-crop flowering plants. Progressive urbanization with increasing population density in human-altered areas of Midnapore town leads to a gradual decline in natural forest and agricultural fields. Nevertheless, the trees mentioned above are present in the University premises and along the roadside and became available to the studied colonies. The maximum amount of nectar contributed family was Myrtaceae, followed by Fabaceae, Lamiaceae, Arecaceae, Anacardiaceae, and Brassicaceae. The nectar contribution of a plant family depends on the plant species' nectar yielding potential and their abundance around the studied colonies (within their foraging range). The significance of these families (except Lamiaceae) as a source of nectar was well established in India (Vijaykumar & Jeyaraaj, 2016; Roopa et al., 2017) as well as outside the country (Obregon & Nates-Parra, 2014; Novais et al., 2015). The high nectar contributing potential of the family Lamiaceae to the bee species is newly registered for West Bengal. In addition, the most important member of this family is *Tectona grandis* which served as a vital nectar source during monsoon (July–August), i.e., the dearth period for most of the bees in West Bengal. Therefore, the plant played an important role in the sustenance of the bee species.

Conclusions

Our results show that most incoming foragers (those are without any corbicular load and of the regarded as probably nectar forager) contain pollen types over the body surface. With the elimination of a few pollen types of non-nectariferous plants, the obtained pollen spectra can be regarded as nectariferous flora for a given bee species. Therefore, bee's body surface pollen analysis seems to be an effective alternative to the traditional methods (i.e., pollen analysis of honey and field observation) in regards to (i) accuracy of the determining nectariferous plants, (ii) avoiding honey-sampling difficulties, (iii) avoiding the destruction of natural nests, and (iv) survivability of the bee species. This method allows characterizing a large number of plants foraged by the stingless bee (*T. iridipennis*). However, only a few plants (viz. *Borassus flabellifer*, *Brassica juncea*, *Delonix regia*, *Eucalyptus tereticornis*, *Lannea coromandelica*, *Peltophorum pterocarpum*, and *Tectona grandis*) have contributed a greater amount of nectar and thereby played an important role in the conservation of the bee species.

Moreover, we found that the bee species utilized more diverse floral resources in semi-natural areas of Garhbeta (utilize both crop and non-crop plants) than the human-altered areas of Midnapore town (depends on mainly non-crop trees). Thus, non-crop flowering plants play an important role in colony health and conservation of the bee species. Finally, stingless bees act as key species in maintaining ecosystem function and ecosystem health; bee pasturage and nesting sites will maintain the proper bee growth and pollination services with the untamed diversity of flowering plants and crops.

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Authors' Contribution

SB: investigation, formal analysis, and writing the original draft.
UL: investigation, formal analysis, revising the draft, and final approval of the version to be published.
PK: conceptualization, revising the draft, and final approval of the version to be published.

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