RESEARCH ARTICLE

Floral Sources for Stingless Bees (*Tetragonula iridipennis*) in Nellithurai Village, Tamilnadu, India

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Study Area: Nellithurai Village, Tamilnadu, India

Coordinates: 11°16'59.74"N & 76°53'6.24"E

Key words: Bee flora, Non-floral sources,
Melissopalynology, Meliponiculture.

Abstract

We documented 45 plant taxa belonging to 29 families and non-floral sources utilized by *Tetragonula iridipennis* for pollen, nectar and resin. The foragers of *T. iridipennis* were also found to collect non-floral resources like fruit juice, fruits kept in the market for sales and from falling and damaged mango and jasmine fruits. The mutualistic association between *T. iridipennis* colonies and Hemipterans was observed and documented. According to pollen analysis, all are appeared to be multifloral honeys. The families Arecaceae and Fabaceae had a significant importance amongst the samples represented by four pollen types. Coconut, Sunflower and Banana pollen types occurred most constantly among the samples. The present palynological analysis of honey samples can provide the accurate depiction of the bee flora in Nellithurai village. The present study to help the beekeepers to know the stingless bee flora and to identify the botanical origins of honey.

Introduction:

Stingless bees comprise a highly diverse and abundant group of eusocial bees that inhabit the tropical and subtropical parts of the world. These are the major biomass component of the nectar and pollen foraging insect community in many tropical areas. Stingless bees are effective pollinators of wild and cultivated crops. According to Kerr *et al.* (2001), 30-80% of plants are pollinated by one or more species of stingless bees in their biomes. In India, *Tetragonula iridipennis* is widely distributed and nests in varying substrates, becoming also abundant in disturbed areas. The colonies of stingless bees make less honey, as compared to other contemporarily honey bees.

In the tropical regions, there are a variety of trees, shrubs and agricultural crops that provide pollen and nectar to the bees. *Apis* uses large amounts of pollen to support its brood production and swarming, while the stingless bees do not often swarm and uses less pollen (Roubik, 2006). *T. iridipennis* is a gentle species and easily adjusts to beekeeping used to collect honey or to pollinate crops (Kumar *et al.* 2012; Vijayakumar *et al.* 2013). They are generalists in their habits and their efficient pollination on the tropical flora is remarkable.

Studies have been made in areas harbor different types

of vegetation such as natural forests, grasslands, agricultural land, pasture land, or urban areas. Most of the methods for obtaining information about bee plants in an area are based on direct field observation of foraging bees on flowers. Absy & Kerr (1977) using pollen analysis of honey began the study of stingless bee floral visitation in the Amazon region, which comprises different forest types, namely savannas and riversides including human-disturbed landscapes and urban areas. In Brazil, Oliveira *et al.* (2009) demonstrated that stingless bees are using variety of pollen resources, but they are not commonly utilized the unique plant species. The analysis of bee plant pollen loads and palynological analysis of honey samples can provide the accurate depiction of the bee flora of a particular area/landscape (Lakshmi, 2012).

The melissopalynological studies of stingless bee honey are rare, limited to *Melipona seminigra merrillae* and *Melipona rufiventris paraensis* (Absy & Kerr 1977), *Melipona compressipes*, *Melipona favosa*, *Melipona trinitatis*, *Frieseomelitta nigra*, *Frieseomelitta* sp. aff. *varia*, *Plebeia* sp., *Scaptotrigona* sp. aff. *depilis*, *Scaura latitarsis* and *T. angustula* (Vit *et al.* 1994), *Melipona scutellaris* (Carvalho *et al.* 2001), *Melipona mandacaia* (Alves *et al.* 2006), *Tetragonisca angustula* (Flores & Sanchez 2010), and *M. favosa* (Vit *et al.* 2012). In India, significant work

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has been carried out by various authors (Ramanujam *et al.*, 1992; Bhargava *et al.*, 2009 & Shilpa & Ratan, 2011). Melissopalynology of *Syzygium* honey has been studied by Suryanarayana (1966) and Bhargava *et al.* (2009). Foraged pollen loads are good indicators of the surrounding flowering plant species that provide pollen for the bees. They also indicate availability of dominant food resources for the different pollinators in an ecosystem. The purpose of this study has been to use two different methods to identify the plant species visited most frequently by *T. iridipennis*.

Materials and methods:

Study Area: The study was carried out in the Nellithurai village of Coimbatore district, Tamil Nadu, India. Field observations were conducted between April 2013 to March 2014. In situ identification of plants visited by stingless bee forages was done by direct observation in the field. The bee floral survey carried out during peak foraging activity (6.00 am - 6.00 pm). Plant species were considered attractive to bees when they were observed to visit by more than five bees during a particular time interval (10 min). The identification of pollen, nectar and resin foragers visually based on either pollen or resin load on corbiculae and honey stomach. The forager bees were caught with sweep nets and preserved for further studies.

Melissopalynological study: Present melissopalynological investigation was carried out in 15 different honey samples collected from different hived colonies of *Tetragonula iridipennis* located at Nellithurai village, Tamil Nadu, India. Pollen slides of honey samples were prepared using the method of Loureaux *et al.* (1978) and acetolysis (Erdtman, 1960). The pollen grains obtained from honey samples were identified and compared with the reference slides made from identified plants and from standard works of Erdtman (1960) and Lakshmi (2012). The absolute pollen count and percentages of pollen types in each sample were calculated (Lakshmi, 2012) on the basis of total number of pollen grains counted in each sample. Samples were observed using light and polarized light microscopy.

The pollen spectra were constructed on the basis of these percentages. Honey samples having 45% or more grains of a single pollen type were termed as “unifloral honey” and those having several pollen types in considerable percentage were termed as “multifloral honey”. Frequencies and frequency classes of the pollen types were determined in accordance with Loureaux *et al.* (1978) and the pollen types were placed under four pollen frequency classes (Predominant pollen D (Above 45%), Secondary pollen S (16% to 45%), Important minor pollen - IM (3% to 15%), Minor pollen m (Less than 3%)) recommended by the International Commission for Bee Botany (ICBB) (Moore & Webb, 1978).

Results

Bee floral survey

Predominant families of flowering plants in the study area: A total of 45 plant species belonging to 28 families were recorded. The important flowering families were Arecaceae and Fabaceae representing the highest frequency of 4 (8.89% N=45), Cucurbitaceae, Euphorbiaceae, Poaceae and Solanaceae with frequency of 3 (6.67% N=45), Lamiaceae and Rutaceae with frequency of 2 (4.44% N=45) and remaining 20 families (Amaranthaceae, Anacardiaceae, Apiaceae, Apocynaceae, Calenduleae, Commelinaceae, Convolvulaceae, Lythraceae, Malvaceae, Meliaceae, Moringaceae, Musaceae, Myrtaceae, Nyctaginaceae, Phyllanthaceae, Portulacaceae, Rubiaceae, Verbenaceae, Vitaceae and Zygophyllaceae) with frequency of 1 (2.22% N=45) (Figure:1).

Figure 1. Important families of floral resources utilized by *T. iridipennis*

Extra-floral nectarines and non- floral resources: honeydew produced by sap-sucking Homoptera is an important resource for a variety of organisms. The foragers of *T. iridipennis* were also found to collect non-floral resources like fruit juice, fruits kept in the market for sales and from falling and damaged mango and jasmine fruits. Foragers of *T. iridipennis* were also found to collect sugar sap from leaf nectaries (Plate 1a), sugar cane sap from crushed sugarcane stems (Plate 1b), watermelon juice and coconut neera. The foragers collected honey dew actively from papaya mealy bug (*Paracoccus marginatus*) (Plate 1c). The forager of *T. iridipennis* collects fresh resin from falling flowers (Plate 1d) and tree wounds. The forager of *T. iridipennis* were observed landing on mealybug assemblages and ingesting excess honeydew and bees were found to collect the honeydew from the mealy bug in the morning and late afternoon during observation times.

Melissopalynological study: melissopalynological investigations carried out on 15 honey samples collected from hived *T. iridipennis* colonies and indicated that the bees were found to forage on 45 pollen types belonging to 29 families for pollen, nectar and resin. The pollen micrograph was showed in plate 2. During this study, the pollen samples are taken from 15 hived *T. iridipennis*

Plate 1: *T. iridipennis* collecting - a) nectar from leaf, b) from crushed sugarcane, c) excretes from animal waste, d) resin from falling flowers

colonies on April 2013 to March 2014 and were subjected to microscopic analysis.

Results from the palynological studies show that stingless bees collect pollen from a wide range of plant species. Pollen grains of *Cocos nucifera*, *Helianthus annuus* and *Musa paradisiaca* were identified as a secondary pollen group. The important minor pollen groups (IM) and minor pollen groups (m) tabulated in table 3. Other plants were also visited by *T. iridipennis* are *Alternanthera sessilis*, *Tamarindus indica*, *Helianthus annuus*, *Cajanus cajan*, *Citrus medica*, *Calotropis gigantea*, *Parthenium hysterophorus*, *Pongamia pinnata*, *Azadirachta indica*, *Aerva tomentosa*, *Digera muricata*, *Evolvulus alsinoides*, *Mimosa pudica*, *Jatropha curcas*, *Phyllanthus emblica*, *Capsicum frutescens*, *Eucalyptus* sp., *Soalnum melongena*, *Mangifera indica*, *Benincasa hispida*, *Prisopis julifera* and *Sorghum halepense*. The lowest pollen frequency of 11 to 20 was noted in *Vitex negundo*, *Coriandrum sativum*, *Tribulus terrestris* and *Pennisetum americanum*.

Discussion:

Floral sources and non flora sources for stingless bees: the present study site located near the Nilgiri Biosphere Reserve located within the Western Ghats and is a region having high ecological and sociological importance (Prabhakar, 1994). Recently, Sasidharan & Kunhikannan (2010) recorded 113 plant species belonging to 93 genera and 42 families which associated with bees in the Nilgiri Biosphere Reserve. During the study, a total of 45 plant species of 12 families was recorded at the source of Pollen/nectar/resin for stingless bees (*T. iridipennis*). The plant species found in agricultural fields are of great economic importance. Results showed that the stingless

bee-flora consists of mostly timber, medicinal, fruits, vegetables, ornamentals and other commercially important plants like pulses, cereals, oil yielding, fiber, fodder etc. Multivarious of the economic importance of the bee plants must be protected.

Honeydew produced by sap-sucking Homoptera is an important resource for a variety of organisms. In the present study, the mutualistic association between *T. iridipennis* colonies and Hemipterans was observed and documented. The forager bees found to collect the honeydew from the mealy bug. Similarly, the association between Hemipterans and stingless bees were discussed by Camargo & Pedro (2002) and found only the genus *Schwarzula* had mutualistic association with Hemipterans. Roubik (1989) observed the mutualistic association between stingless bees and Hemipterans. In addition, some species of stingless bees with obligate nectarophagic habits, three species (*Trigona necrophaga*, *T. hypogea* and *T. crassipes*) were found to collect flesh of dead animals is their only protein source and sugars obtained from ripe or rotting fruit, extra floral nectaries, fallen flowers on the ground (Roubik, 1982).

Melissopalynological study: the results of the pollen analyses reveals that the forages of *T. iridipennis* are mostly preferred the 12 plants species belonging to four families (Asteraceae, Musaceae, Fabaceae and Poaceae). The highest frequency (71 to 100%) was noted in pollen of *Cocos nucifera*, *Zea mays*, *Musa paradisiaca* and *Commelina benghalensis*. The presence of the above pollen types were noted annually, which confirmed the attractiveness of their pollen and nectar to stingless bees.

Similarly, Singh & Kar (2011) recorded a diverse spectrum of 27 pollen types belonging to 19 families from 12 Sunderbans honey samples (Bangladesh) and Andaman (India). Jhansi *et al.* (1994) recorded 51 pollen types referable to 36 families in the samples studies from Andhra Pradesh, India. Atwal *et al.* (1970) studied different bee flora for four species of *Apis mellifera* for Lucknow region based on the pollen analysis. Recently, Padmavathy & Rehel (2014) recorded 12 species belonging

Plate 2: Pollen micrographs

to 11 families were identified as floral sources for *Apis dorsata* in Conoor, Nilgiri hills, Tamil Nadu. They recorded the pollen grains of *Syzygium cumini* and *Acacia* spp. frequently from the honey samples of *A. dorsata*.

A pollen spectrum revealed that the pollen grains of *Cocus nucifera* as a major source of pollen and it was found throughout the year in the present study area. Similarly, Bhargava *et al.* (2009) reported that *Cocus nucifera* as the pollen source available throughout the year in Karnataka, India. In addition, Sekhar (2000) revealed that the dominant pollen sources for bees in Bangaluru region are also *Cocus nucifera* and *Eucalyptus tereticornis*. Many Asteraceae species produce nectar (Terrab *et al.*, 2001) and according to our results the taxa of this family are of great importance both as nectar and pollen source in the study area. In addition, Suryanarayana *et al.* (1991) in their study at Muzaffarpur (Bihar), India, reported that members of Asteraceae provided forage for a major part of the year.

The present study confirmed the availability of high diversity vegetation around Nellithurai village. In addition, melissopalynology of stingless bee honey helps to characterize vegetation surrounding the collection site and provide detailed information on stingless bee flowering plants. The present study helps the beekeepers and farmers to know the flowering plants utilized by stingless bees. Thus they can take appropriate steps to develop meliponiculture.

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Table-1: Stingless bee floral sources in Nellithurai village

No	Scientific name	Common name /Tamil name	Family	Habitat
1	Adhatoda zeylanica	Malabarnut/ Adathoda	Acanthaceae	Herb
2	Alternanthera sessilis	Dwarf copperleaf Ponnanganni Keerai	Amaranthaceae	Herb
3	Azadirachta indica	Neem/Vambu	Meliaceae	Tree
4	Benincasa hispida	Pumpkin / Poosanikai	Cucurbitaceae	Vegetable
5	Boerhavia diffusa	Red spiderling/ Mukkarattai keerai	Nyctaginaceae	Herb
6	Calotropis gigantea	Yerukku	Apocynaceae	Shrub
7	Calendula officinalis	Mary gold	Calenduleae	Herb
8	Capsicum frutescens	Chilly	Solanaceae	Vegetable
9	Caryota urens	Jaggery Palm	Arecaceae	Herb
10	Ceiba pentandra	Java kapok/ Ilavam	Malvaceae	Tree
11	Cissus quadrangularis	Devil's Backbone/ Pirandai	Vitaceae	Climber
12	Citrus medica	Lemon	Rutaceae	Shrub
13	Coccinia grandis	Little gourd/ Kovai	Cucurbitaceae	Climber
14	Cocos nucifera	Coconut Palm	Arecaceae	Tree
15	Commelina benghalensis	Benghal dayflower/ Kanan Keerai	Commelinaceae	Herb
16	Coriandrum sativum	Coriander/ Kothamalli	Apiaceae	Herb
17	Croton bonplandianum	Croton/ Rail Poondur	Euphorbiaceae	Herb
18	Evolvulus alsinoides	Vishnukiranthi	Convolvulaceae	Herb
19	Eucalyptus sp.	Thailamaram	Myrtaceae	Tree
20	Helianthus annuus	Sunflower	Asteraceae	Oil seed
21	Ixora coccinea	Jungle flame/ Ittly Poo	Rubiaceae	Shrub
22	Jatropha curcas	Physic nut	Euphorbiaceae	Shrub
23	Jatropha gossypifolia	-	Euphorbiaceae	Shrub
24	Lantana camara	Wild Sage/ Unni	Verbenaceae	Shrub
25	Lawsonia inermis	Henna/ Maruthani	Lythraceae	Shrub
26	Mangifera indica	Mango/ Maa	Anacardiaceae	Tree
27	Momordica charantia	Bitter Guard/ Paval	Cucurbitaceae	Climber
28	Moringa pterygosperma	Drum Stick/ Murungai	Moringaceae	Tree
29	Murraya koenigii	Curry tree/ Karuvappli	Rutaceae	Shrub
30	Musa paradisiaca	Banana	Musaceae	Tree
31	Ocimum canum	Holy Basil/ Nai thulasi	Lamiaceae	Herb
32	Parthenium hysterophorus	Parthenium weed/ Visha Poondur	Asteraceae	Herb
33	Pennisetum americanum	Pearl millet/ Kambu	Poaceae	Pulses
34	Phyllanthus emblica	Amla/ Nelli	Phyllanthaceae	Tree
35	Portulaca oleracea	Pigweed/ Paruppu Keerai	Portulacaceae	Herb
36	Prisopis julifera	Mesquite	Fabaceae	Shrub
37	Pithecellobium dulce	Manila tamarind/ Kodukkapuli	Fabaceae	Tree
38	Solanum melongena	Brinjal	Solanaceae	Vegetable
39	Solanum nigrum	Black nightshade/ Manathakali	Solanaceae	Herb
40	Sorghum halepense	Johnson grass/ Solam	Poaceae	Pulses
41	Tamarindus indica	Tamarind/ Poliyamaram	Fabaceae	Tree
42	Tribulus terrestris	Tackweed/ Nerinji	Zygophyllaceae	Herb
43	Vigna radiata	Green Gram/ Pasi Payaru	Fabaceae	Pulses
44	Vitex negundo	Nochi	Lamiaceae	Shrub
45	Zea mays	Myze	Poaceae	Pulses

Table-2: Important pollen groups in the study area and their frequency

S.No	Family	Pollen type	S	IM	M	FO*
1	Acanthaceae	Aerva tomentosa			5	33.30
2		Alternanthera sessilis			10	66.70
3		Digera muricata			6	40.00
4	Anacardiaceae	Mangifera indica	1	3		26.60
5	Apiaceae	Coriandrum sativum		2	1	20.00
6	Apocynaceae	Calotropis gigantea		6	1	46.67
7	Asteraceae	Cocus nucifera	12	2	1	100.00
8		Helianthus annuus	6	3		60.00
9		Parthenium hysterophorus	3	2	2	46.67
10		Tridax procumbens		1	5	40.00
11	Commelinaceae	Commelina benghalensis			11	73.33
12	Convolvulaceae	Evolvulus alsinoides			5	33.30
13	Euphorbiaceae	Croton bonplandianum			3	20.00
14		Jatropha curcas		4	2	40.00
15	Fabaceae	Cajanus cajan		5	3	53.33
16		Pongamia pinnata		4	3	46.67
17		Mimosa pudica			4	26.60
18		Tamarindus indica	2	5	3	66.70
19	Lamiaceae	Vitex negundo		1	1	13.33
20	Meliaceae	Azadirachta indica	6		1	46.67
21	Myrtaceae	Eucalyptus sp.		2	2	26.60
22	Musaceae	Musa paradisiaca	7		4	73.33
23	Phyllanthaceae	Phyllanthus emblica	1	4		33.33
24	Poaceae	Pennisetum americanum			3	20.00
25		Sorghum halepense		4		26.60
26		Zea mays	7	3	2	80.00
27	Rutaceae	Citrus medica	3	1	4	53.33
28	Solanaceae	Capsicum frutescens	1	5		40.00
29		Solanum melongena		6		40.00
30	Zygophyllaceae	Tribulus terrestris		3		20.00

*Frequency observation