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First Report of Nesting Habits, Nest Architecture and Foraging Behaviour of the Stingless bee, *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) (Hymenoptera: Apidae: Meliponini) from India

Basavaraj N. Hadimani, Kumaranag K. M, Debjani Dey

Division of Entomology, ICAR- Indian Agricultural Research Institute, New Delhi, India

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Corresponding author
Debjani Dey, PhD
Professor, Division of Entomology, ICAR-Indian Agricultural Research Institute, 110012, New Delhi, India.
E-Mail: ddey@iari.res.in

Abstract

The present study aimed to understand nesting habits, nest architecture, and foraging behavior of the stingless bee, *Tetragonula* (*Tetragonula*) *ruficornis* Smith, a widespread species in the north Indian plains from Varanasi to Punjab. Among the 47 wild, unmanaged colonies studied, 27 were situated in the cavities of tree trunks and 20 in the cavities of building walls. The internal nest structure revealed that brood cells were arranged in clusters at the center of the cavity with encircling pollen and honey pots. Further, the natural nesting cavity's total length, width, and height varied from 105 to 168 mm, 129 to 216 mm, and 283 to 472 mm, respectively. The foraging activity of outgoing and incoming foragers of *T. ruficornis* varied significantly during different hours of the day, across the seasons and prevailing weather conditions. Further, activity was limited to daytime from 08:00 to 18:00 h, with peak activity of outgoing and incoming forager bees between 10:00 and 12:00 h during all seasons. Irrespective of seasons, the foraging activity of outgoing, pollen, and nectar foragers was at its maximum at 12:00 h. However, foraging ceased during December and January due to low temperatures, while peak activity was observed during April. The foraging pattern showed a significant positive correlation with mean monthly maximum and minimum temperatures and a significant negative correlation with relative humidity. The current investigation delivers crucial information on nest architecture and foraging behavior, which could be exploited for designing bee hives and utilization in directed pollination services.

Introduction

Stingless bees are highly evolved eusocial bees, widely distributed in the Afrotropical, Australasian, Indo-Malayan, and Neotropical regions of the world (Ruttner, 1988; Michener, 2000; Engel et al., 2023) and considered close relatives of honey bees and bumble bees. Unlike honey bees, stingless bees have an atrophied, vestigial stinger, which makes it impossible to sting. Body size varies from 2 mm in *Leurotrigona pusilla* Moure & Camargo to 15 mm in *Melipona fuliginosa* Lepeletier. They are maintained commercially for their honey and to meet the increasing demand for managed pollination services in a variety of agricultural and horticultural crops (Heard, 1999; Slaa et al., 2006; Jha & Dick, 2010). In total, currently, 630 species of stingless bees are described worldwide, belonging to 55 genera comprising both living and fossils (Michener, 2000; Rasmussen & Cameron, 2010; Rasmussen et al., 2017; Engel et al., 2023). A total of 22 stingless bee species described from India belong to three genera, *Tetragonula* Moure, *Lisotrigona* Moure, and *Lepidotrigona* Schwarz, and among these, *Tetragonula* is the most dominant (Rasmussen, 2013; Viraktamath & Roy, 2022).

The stingless bee, *Tetragonula* (*Tetragonula*) *ruficornis* Smith, was first described by Frederick Smith in 1870 from Varanasi, Uttar Pradesh, India (Rasmussen, 2013). This species is widely distributed across the north Indian plains, from Varanasi to Punjab. They are predominantly well-adapted to cold climates, possibly the only species occurring in Uttar

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Pradesh, Uttarakhand, Haryana, Punjab, and Delhi (Shanas & Faseeh, 2019). The survival, maintenance, and reproduction of stingless bees depend on the presence of nesting sites and floral and food resources (Batista et al., 2003; Eltz et al., 2003). They adapted to various nesting sites, from living in ant and termite nests above and below ground, cavities in trees, branches, rocks, or human constructions to largely self-built, exposed tree nests (Roubik, 2006). Entrance tubes are sometimes distinctive, even diagnostic for specific genera, subgenera, or species, and range from simple, thin tubes with a single opening to multi-opening structures (Engel et al., 2023). The chief building component of the colony is cerumen, a mixture of bee wax and plant resins (Rasmussen, 2013). The common structure of stingless bee nests consists of entrance tunnels, brood cells, food storage (honey and pollen cells), cerumen, bitumen layers, and involucrum (Michener, 2007). Brood cells are made of soft cerumen, and each cell is built to rear one individual bee. There are three main types of cell arrangements: horizontal combs, vertical combs, and clustered cells (Guiter, 2020). Centrally placed brood cells were commonly surrounded by a series of thin, concentric layers of cerumen, forming brood involucrum. The presence of an involucrum is more frequently associated with species that make brood combs, and it is commonly absent in species that make small brood clusters and irregular combs (Melo, 2020). Food storage pots are usually constructed around the area occupied by the brood cells. In common with other social bees, stingless bees are generalists in their search for flower resources, harvesting nectar and pollen from a wide range of plant species (Vit et al., 2018). Foraging is possible throughout the year in some species (e.g., *Apotrigona nebulata* (Smith) in Uganda, Kajobe & Echazarreta, 2005; *Melipona beecheii* Bennett in Mexico, Di Trani & Villanueva-Gutiérrez, 2018; *Tetragonula carbonaria* (Smith) in Australia, Heard & Hendrikz, 1993), whereas in others, foraging is reduced or ceases completely during some months, either due to low temperatures, resource scarcity, extreme drought or intense rain (Halcroft et al., 2013; Maia-Silva et al., 2015; Hrncir et al., 2019). Climatic factors like temperature and light intensity are most important for determining the flight activity of these bees (Heard & Hendrikz, 1993). Nearly all species show at least some level of seasonality in their foraging activity.

The small size of workers and foraging range of stingless bees may prove effective for the pollination of crops, especially vegetables like *Capsicum annuum* and *Cucumis sativus* under protected conditions. Nine crop species are confirmed to be effectively pollinated by stingless bees, contributing to the pollination of nearly 60 other crops (Heard, 1999). Although *Tetragonula* species are common visitors to flowering plants in the tropics, their small size allows them access to flowers whose openings are too narrow to permit penetration by other bees. A sound knowledge of their nest architecture and foraging behavior is essential to exploit the stingless bees for pollination and honey production. However, few research works have been performed on the stingless bee species *Tetragonula* (*Tetragonula*) *iridipennis* (Smith) (Danaraddi et al., 2009; Roopa et al., 2015; Vijayakumar et al., 2013; Virkar et al., 2014; Layek & Karmakar, 2018; Chauhan & Singh, 2021; Choudhary et al., 2021). Therefore, studies have yet to be made on the nest architecture and foraging behavior of *Tetragonula* (*Tetragonula*) *ruficornis* in India. Hence, the present study may provide important information for species conservation and sustained use of these species, including for crop-directed pollination.

Material and Methods

Study area

The present investigations were conducted from October 2021 to September 2022 at the Indian Agricultural Research Institute (IARI), New Delhi, which is situated in the trans-Gangetic plains of India. The campus is between latitudes 28.62° and 28.65° N and longitudes 77.15° and 77.17° E, at an average altitude of 228.61 meters above mean sea level. The climate of the campus is subtropical semiarid, characterized by dry, hot summers and cold winters. May is the hottest month, with a mean maximum and minimum temperature of 39.1°C. January, with a recorded monthly average maximum and minimum temperature, was the coldest month at 6.5 °C. The IARI campus receives around 769 mm of rainfall annually, with the major portion (550 mm) received during the monsoon season (July-September). During winter, a small amount of rainfall (about 63 mm) is received due to western disturbances (Ahmadzadeh et al., 2015). The campus's predominant vegetation comprises trees, shrubs, hedges, lawns, and other flower beds. The study area consists of plants such as *Ficus religiosa*, *Azadirecta indica*, *Alstonia scholaris*, *Morus alba*, *Syzigium cumini*, *Callistemon viminalis*, *Roystonea regia*, *Eucalyptus tereticornis*, *Polyalthia longifolia* and other trees. All other important flowering tree species includes *Cassia fistula*, *Plumeria rubra*, *Delonix regia*, *Lagerstroemia speciosa*, *Millettia peguensis*, *Callistemon viminalis* and *Thevetia peruviana*. The major field crops grown here include *Oryza sativa*, *Triticum aestivum*, *Zea mays*, *Brassica nigra*, *Helianthus annuus*, *Sesamum indicum*, and vegetables such as *Abelmoschus esculentus*, *Allium cepa* and *Coriandrum sativum*, which occupy a major portion of the total cropped area in the campus.

Natural bee colony and its pattern of nest architecture

Explorations were conducted on the campus of the Indian Agricultural Research Institute to locate wild, unmanaged colonies of *Tetragonula* (*Tetragonula*) *ruficornis* in all possible concealed places. Observations were recorded on external characteristics such as the preference for nesting sites, orientation of the nest entrance, height of the nest from the ground, and shape of the nest entrance. In order to study the internal structure of nests, three live colonies nested in dried branches of trees were selected. These dead branches were carefully separated from trees with the help of an electric saw and chisel. The colonies were shifted to experimental locations and allowed to settle and develop for 2-3 months before they were opened for studying the nest architecture. These colonies were then dissected to observe their internal nest architecture and colony structure. Observations were recorded on the arrangement of brood, pollen honey pots, etc. Measurements were taken for the length of the wax tube (entrance) and the size of the brood cells, honey pots, and pollen pots using digital vernier calipers.

Stingless bees were collected from all the wild, unmanaged colonies. Ten worker bees from each colony were mounted for morphological studies, and identification was done with the help of available literature (Sakagami, 1978; Rasmussen, 2013; Viraktamath & Roy, 2022). Dr. S. Viraktamath, Emeritus Scientist from the Department of Entomology, University of Agricultural Sciences, Bengaluru, India, confirmed the species identification. All the specimens were deposited in the National Pusa Collection (NPC), Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi, India.

Foraging activity of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith)

In order to study the foraging activity, three wild, unmanaged colonies other than those used for nest architecture studies were selected. Visual observations were made on the number of outgoing and incoming forager bees at fortnightly intervals for one year. Each sampling day was divided into two hourly intervals from 06:00 h to 18:00 h (Danaraddi et al., 2011). Outgoing and incoming forager bees with and without pollen loads were counted for 5 minutes (3 replications) for every two-hour interval with the help of a tally counter. For simultaneous observations, an observer was stationed at each colony. Bees entering the colony with a pollen load were considered pollen foragers, whereas those without were considered nectar foragers. Workers cleaning debris and guarding bees flying around the nest entrances were excluded from counting. Each colony was considered a replication. The data obtained were pooled season-wise and month-wise before statistical analysis.

Association between weather parameters and foraging activity of outgoing, pollen, and nectar foragers

To investigate the effect of ambient environmental factors on foraging activity, the data on meteorological parameters such as maximum and minimum temperature (°C), relative humidity (%), rainfall (mm), and wind speed (kmph) were collected from the Automated Weather Station on campus, located just 500 meters away from the experimental site. The systemic and functional relationship of weather parameters related to bee foraging activity was estimated.

Statistical analysis

Data regarding the internal characteristics of nests were analyzed by descriptive statistics, such as means with standard deviations (S.D.) and mode, to check the significant difference among the nests studied. The data on the foraging activity of outgoing and incoming (pollen and nectar) foragers was subjected to a two-way analysis of variance (ANOVA) to compare the significant differences across the months and timings. To know the influence of weather parameters on the foraging activity of *T. ruficornis*, we followed Karl Pearson's correlation coefficient (r) method using IBM SPSS Statistics version 25.

Results

Identification of stingless bee, *Tetragonula* (*Tetragonula*) *ruficornis* (Smith)

Stingless bee, *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) can be distinguished from other *Tetragonula* species by following taxonomic characteristics such as shiny body; Head brownish black; scape dark brown with a yellowish brown basal area; pedicel dark brown; flagellomeres dark brown on the upper side and dull brown-yellow on the lower side; erect setae on vertex light brown; Plumose hairs on clypeus and frons abundant; mandible amber yellow with a near black apical area; Inner surface of hind tibia with basal sericeous area lesser than half of the length of basitarsus; stout setae of the outer surface of hind tibia white; pubescence on metasomal T3-T4 sparsely covered; metasomal terga with light yellowish brown at basal terga and reddish brown at apical half; sterna brownish yellow. Body length 3.53 mm; head 1.23× wider than its length (1.18 mm); compound eyes 3× longer than its width (0.36 mm); upper interocular distance 1.09 mm; interocellar distance 0.41 mm; malar space length 0.07 mm; forewings 3.58 mm long, 1.39 mm wide; wing diagonal length 1.07 mm; hind tibiae 1.38 mm long, 0.53 mm wide; hind basitarsus 1.82× longer than its width (0.29 mm).

Natural bee colony and its pattern of nest architecture

A total of 47 wild, unmanaged nests of stingless bees, *Tetragonula* (*Tetragonula*) *ruficornis* (Smith), were studied for nesting behavior at the Pusa campus, New Delhi; of those, 27 were found in the cavities of tree trunks, and 20 in the cavities of human building walls (Fig 1). *T. ruficornis* preferred to construct its colonies in cavities of *Syzygium cumini* L. (Myrtaceae) (12 nests), *Cassia fistula* L. (Fabaceae) (9 nests), *Ficus religiosa* L. (Moraceae) (5 nests), and *Morus alba* L. (Moraceae) (1 nest) and corresponding to families Myrtaceae (12 nests), Fabaceae (9 nests) and Moraceae (6 nests). Nests were constructed in the crevices of building walls made of cement and bricks (15 nests) and cement and stones (5 nests). Nest entrances were found to be oriented in all directions, but several nest entrances facing towards the southeast were more numerous (12 nests), followed by southwest (9 nests).

The height of the nesting sites from the ground level varied from 52 to 1039 cm. The nest entrance of colonies found in tree cavities extended inside the nest through an internal tunnel, and its length ranged between 72 and 115 mm, with an average of 91.33 ± 21.83 mm. Colonies nested in tree cavities had cavity lengths ranging from 105 to 168 mm, with a mean of 136.67 ± 31.50 mm. The width of cavities ranged from 129 to 216 mm with an average of 171.33 ± 43.55 mm, while the height ranged from 283 to 472 mm with an average of 365.67 ± 96.70 mm. The average total volume of the cavity was 136.67 mm × 171.33 mm × 365.67 mm (Table 1). All the *T. ruficornis* nests had dark brown-colored nest entrances constructed using cerumen (a mix of wax and plant resins). However, the shape of the nest entrance varied from round and oval to irregular, with different diameters (Figs 2 a-f).

Table 1. Internal nest characteristics of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) in New Delhi, India.

Sl.no.	Characteristics	Range	Mean value ± S. D	Mode value
1	Internal tunnel length	72-115 mm	91.33 mm ± 21.83 mm	
2	Length of cavity	105-168 mm	136.67 mm ± 31.50 mm	
3	Width of cavity	129-216 mm	171.33 mm ± 43.55 mm	
4	Height of cavity	283-472 mm	365.67 mm ± 96.70 mm	
5	Location of brood cell	Centre		
6	Colour of brood cell	Light brown		
7	Shape of brood cell	Oval		
8	Length of brood cell	3.05-3.94 mm	3.48 mm ± 0.08 mm	3.38 mm
9	Width of brood cell	2.18-2.61 mm	2.38 mm ± 0.09 mm	2.48 mm
10	Density (cells/cubic inch) of brood cell	35-57	46.38 ± 0.35	53
11	Location of pollen pot	Periphery		
12	Colour of pollen pot	Dark brown		
13	Shape of pollen pot	Spherical-polygonal		
14	Length of pollen pot	7.19-9.25 mm	8.10 mm ± 0.14 mm	8.69 mm
15	Width of pollen pot	4.79-8.07 mm	6.73 mm ± 0.73 mm	7.23 mm
16	Density (cells/cubic inch) of pollen pot	7-16	11.53 ± 0.32	13
17	Location of honeypot	Periphery		
18	Colour of honey pot	Dark brown		
19	Shape of honey pot	Spherical to polygonal		
20	Length of honey pot	6.72-9.45 mm	8.52 mm ± 0.46 mm	8.45 mm
21	Width of honey pot	5.47-8.03 mm	7.36 mm ± 0.56 mm	7.29 mm
22	Density (cells/cubic inch) of honey pot	6-14	10.3 ± 0.36	12

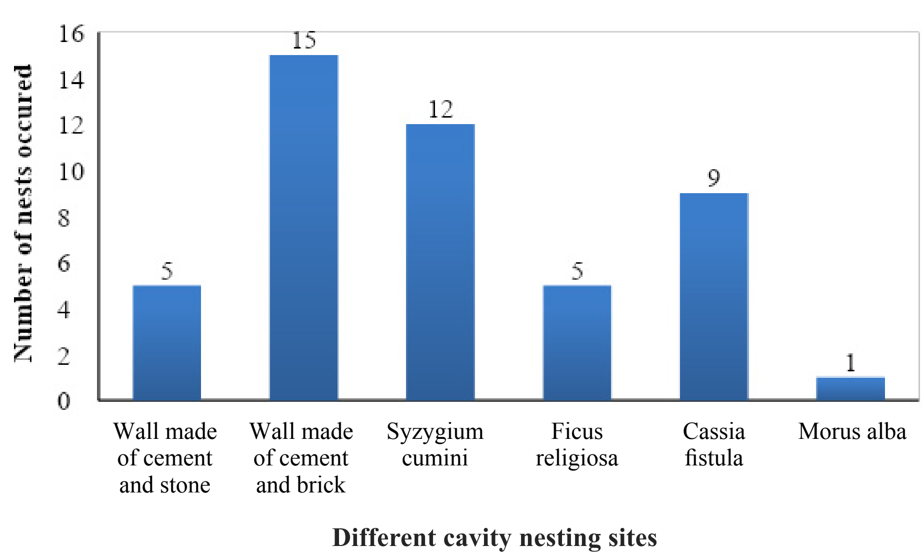

Fig 1. Preferred nesting habitat of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith)in New Delhi, India, during 2021-2022.

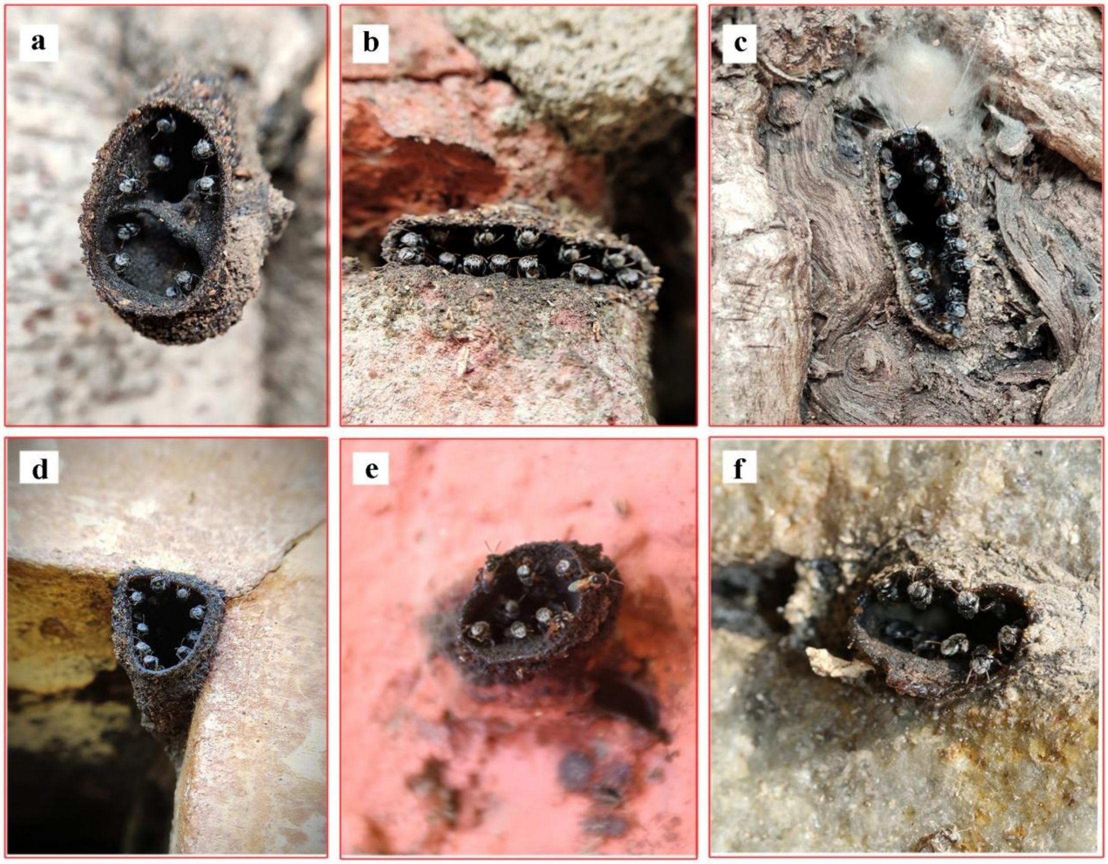

Fig 2. Variation in shape of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) nest entrances in New Delhi, India. **a-** oval shaped entrance; **b** and **c**-slit-like entrance; **d-** triangular entrance; **e**-circular entrance; **f**-irregular shaped entrance.

All the observed colonies comprised distinctive food storage and brood zones arranged internally in a definite geometrical fashion. The food storage zone was divided into pollen and honey pots arranged in clusters. The amorphously clustered brood cells were situated in the center of the nest with some spaces, which enabled easy movement of bees within the cluster of brood cells, while the pollen and honey pots were located on the periphery, but these pots were often intermixed (Fig 3). The brood cells were not covered with an involucrum. Mature brood (larval) cells were light brown and look similar to small beads in shape and size, arranged in a network of narrow vertical pillars with horizontal connectives, and oval-shaped with an average length of 3.48 ± 0.08 mm and a width of 2.48 ± 0.09 mm. Immature (egg and early instar larvae) cells were brownish, while pupal cells were creamy white (Table 1).

The pollen pots were dark brown, spherical to polygonal-shaped, with an average length of 8.10 ± 0.14 mm and a width of 6.73 ± 0.73 mm, and made up of soft cerumen. These pots were tightly packed with pollen pellets and sealed. Only a few open pots were used to store incoming pollen. The pollen was slightly moist and had a bitter taste. In most cases, the pollen zone was larger than the honey zone, indicating active brood rearing and the availability of abundant pollen resources in the surrounding areas. Once the pollen pots were filled, they were sealed. Newly constructed pots surrounded these, and occasionally they were intermixed. Honey pots were slightly larger than pollen pots, dark brown, spherical to polygonal-shaped with an average length of 8.52 ± 0.46 mm and width of 7.36 ± 0.56 mm, and located at the inner periphery. Like pollen pots, honey pots were also sealed after the ripening of honey. The average density (number of cells or pots per cubic inch) of brood cells was 46.83 ± 0.35, 11.53 ± 0.32 for pollen pots, and 10.30 ± 0.36 for honey pots (Table 1).

Foraging activity of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith)

Foraging activity for outgoing and incoming forager bees (pollen and nectar foragers) was confined between 08:00 and 18:00 h. Irrespective of the seasons, initiation of the foraging activity was observed at 08:00 h, which increased gradually, and it peaked at 12:00 h for the number of average outgoing bees (60.11 bees/5 min.), pollen (17.07 bees/5 min.) and nectar foragers (61.94 bees/5 min). The lowest foraging activity with a minimum number of outgoing (33.39 bees/5 min.), incoming pollen (6.29 bees/5 min.), and nectar foragers (44.24 bees/5 min.) was recorded at 18:00 h before the cessation of the foraging activity. Month-wise pooled average for foraging activity showed that maximum activity of outgoing and incoming (pollen and nectar) foragers was observed in April, followed by March, whereas lowest in February. Pooled average foraging activity over the seasons

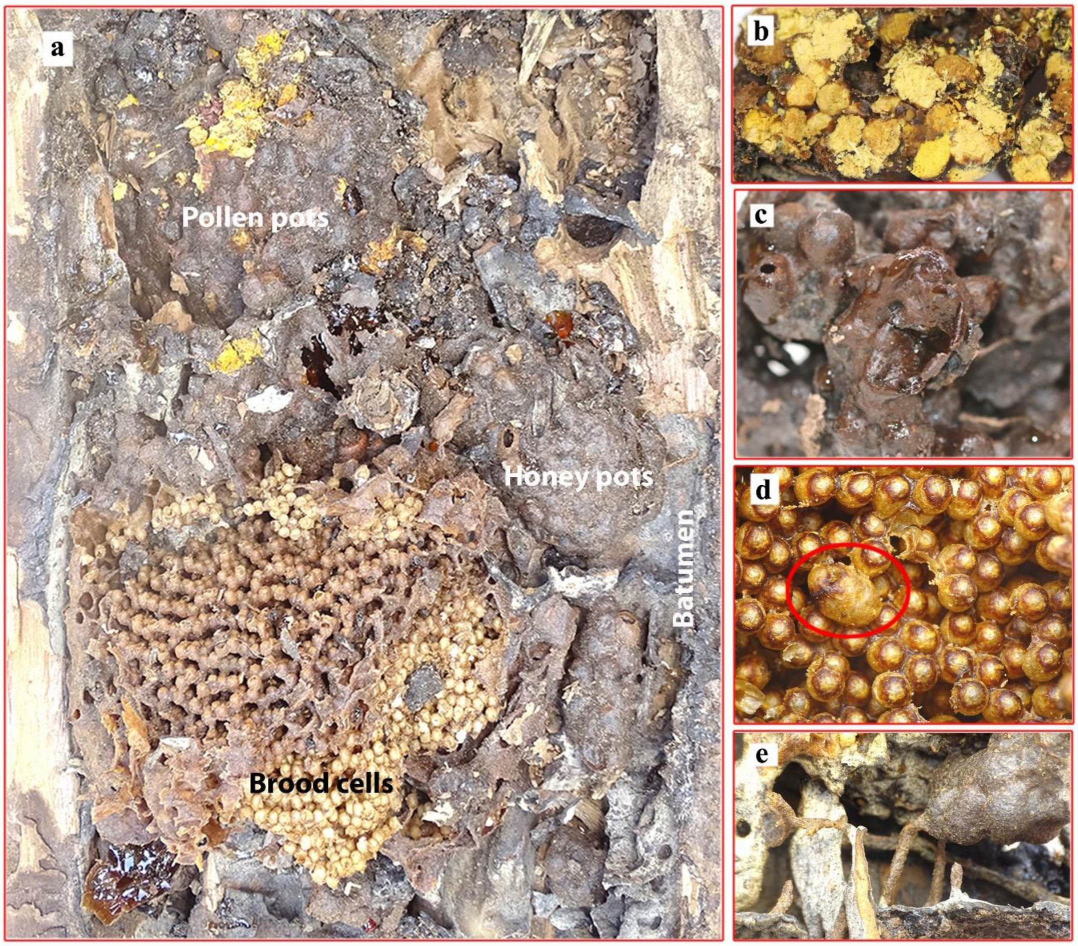

Fig 3. Internal structure of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) nest in New Delhi, India. **a-** vertical section of whole nest; **b-** pollen pots; **c-** honey pots; **d-** brood cells with queen cell; **e-** pillars connecting different cell.

revealed that summer was the most active season with a maximum number of outgoing bees, incoming pollen, and nectar foragers (monthly minimum temperature ranged from 19.8 °C to 24.5 °C). Average foraging activity was low during the winter season, with complete cessation of the foraging activity during December and January (monthly minimum temperatures 7.3°C and 7.49°C, respectively) (Table 2, 3, and 4).

Table 2. Activity of outgoing foragers of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) irrespective of season during 2021-22 in New Delhi, India.

	6.00	8.00	10.00	12.00	14.00	16.00	18.00	Mean
June	0.00	63.17	74.33	99.50	91.33	74.67	72.17	67.88 (2.88)^{ab}
July	0.00	68.67	59.83	59.33	49.00	42.50	30.00	44.19 (2.61)^{c}
August	0.00	57.00	66.00	66.00	57.83	49.83	40.17	48.12 (2.68)^{c}
September	0.00	57.83	76.33	80.00	84.33	68.00	35.67	57.45 (2.77)^{bc}
October	0.00	28.50	52.17	62.00	62.33	56.33	25.00	40.91 (2.58)^{c}
November	0.00	0.00	12.50	49.83	46.33	11.00	0.00	17.10 (1.95)^{d}
December	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (1.31)^{e}
January	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (1.31)^{e}
February	0.00	0.00	0.00	22.50	44.00	25.00	0.00	13.07 (1.82)^{d}
March	0.00	71.83	95.17	101.50	97.17	93.83	59.67	74.17 (2.91)^{ab}
April	0.00	78.67	105.83	111.50	100.33	91.17	77.17	80.67 (2.99)^{a}
May	0.00	41.67	56.50	69.17	62.50	75.17	60.83	52.26 (2.72)^{bc}
Mean	0.00 (1.31)^{d}	38.94 (2.37)^{c}	49.89 (2.55)^{b}	60.11 (2.74)^{a}	57.93 (2.74)^{a}	48.96 (2.61)^{ab}	33.39 (2.31)^{c}	
		Factors	SE(d)	C.D.	SE(m)			
		Month(M)	0.10	0.05	0.03			
		Time (T)	0.07	0.04	0.03			
		M X T	0.25	0.13	0.09			

Note: Values in the parentheses are √x+0.5 transferred values.

Table 3. Activity of pollen foragers of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) irrespective of season during 2021-22 in New Delhi, India.

	6.00	8.00	10.00	12.00	14.00	16.00	18.00	Mean
June	0.00	11.00	10.83	8.50	9.17	7.17	5.33	7.43 (1.85)^{c}
July	0.00	20.33	15.67	17.83	13.33	9.33	6.33	11.83 (2.01)^{c}
August	0.00	30.50	32.83	38.33	20.67	17.83	13.50	21.95 (2.27)^{ab}
September	0.00	18.83	21.33	29.00	22.17	16.67	8.50	16.64 (2.17)^{b}
October	0.00	10.33	16.00	16.00	13.83	11.00	5.50	10.38 (2)^{c}
November	0.00	0.00	1.67	8.67	7.33	2.50	0.00	2.88 (1.58)^{d}
December	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (1.31)^{e}
January	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (1.31)^{e}
February	0.00	0.00	0.00	1.67	4.00	1.17	0.00	0.98 (1.43)^{e}
March	0.00	23.17	45.00	32.50	30.50	24.50	12.83	24.07 (2.32)^{ab}
April	0.00	43.67	46.17	40.33	24.33	21.17	20.50	28.02 (2.38)^{a}
May	0.00	7.00	11.50	12.00	11.83	8.17	3.00	7.64 (1.89)^{c}
Mean	0.00 (1.31)^{d}	13.74 (1.96^{bc})	16.75 (2.05)^{ab}	17.07 (2.10)^{a}	13.10 (2.04)^{abc}	9.96 (1.92)^{c}	6.29 (1.76)^{d}	
		Factors	SE(d)	C.D.	SE(m)			
		Month (M)	0.09	0.05	0.03			
		Time (T)	0.07	0.04	0.03			
		M X T	0.24	0.12	0.09			

Note: Values in the parentheses are √x+0.5 transferred values.

Table 4. Activity of nectar foragers of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) irrespective of season during 2021-22 in New Delhi, India.

	6.00	8.00	10.00	12.00	14.00	16.00	18.00	Mean
June	0.00	63.33	97.83	104.17	101.50	98.50	92.33	79.67 (2.98)^{a}
July	0.00	55.67	58.33	60.33	54.67	56.67	47.67	47.62 (2.67)^{b}
August	0.00	50.33	57.17	62.33	58.50	51.67	44.00	46.29 (2.66)^{b}
September	0.00	43.50	56.50	87.67	74.67	88.83	70.83	60.29 (2.8)^{ab}
October	0.00	30.50	54.33	66.33	63.50	60.67	37.00	44.62 (2.63)^{b}
November	0.00	0.00	13.00	52.17	49.17	29.67	0.00	20.57 (2.02)^{c}
December	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (1.31)^{d}
January	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (1.31)^{d}
February	0.00	0.00	0.00	18.17	44.33	33.17	0.00	13.67 (1.82)^{c}
March	0.00	52.50	70.33	100.00	88.00	100.33	64.17	67.91 (2.81)^{ab}
April	0.00	73.00	91.67	106.83	110.83	96.67	92.67	81.67 (3)^{a}
May	0.00	42.00	66.00	85.33	79.17	82.67	82.17	62.48 (2.81)^{ab}
Mean	0.00 (1.31)^{d}	34.24 (2.31)^{c}	47.10 (2.53)^{b}	61.94 (2.76)^{a}	60.36 (2.76)^{a}	58.24 (2.71)^{a}	44.24 (2.43)^{bc}	
		Factors	SE(d)	C.D.	SE(m)			
		Month (M)	0.12	0.06	0.04			
		Time (T)	0.09	0.05	0.03			
		M X T	0.32	0.16	0.11			

Note: Values in the parentheses are √x+0.5 transferred values.

When the foraging activity was considered season-wise, during the monsoon (June to September), the foraging activity of outgoing, pollen, and nectar foragers was maximum during June, August, and June month (67.88, 21.95, and 79.67 bees/ 5 min., respectively). Further, the minimum foraging activity of outgoing, pollen, and nectar foragers was observed during July, July, and August (44.19, 11.83, and 46.29 bees/5 min., respectively) (Fig 4A-C). In the winter season (October to January), the foraging activity of outgoing, pollen, and nectar foragers was observed maximum during October (40.91, 10.38, and 44.62 bees/5 min. respectively), while the lowest activity during November (17.10, 2.88, and 20.57 bees/5 min., respectively) (Fig 4D-F). In summer (February to May), the foraging activity of outgoing, pollen, and nectar foragers was observed at its maximum during April (80.67, 28.02, and 81.67 bees/5 min, respectively), whereas minimum foraging activity was recorded during February (13.07, 0.98 and 13.67 bees/5 min, respectively) (Fig 4G-I).

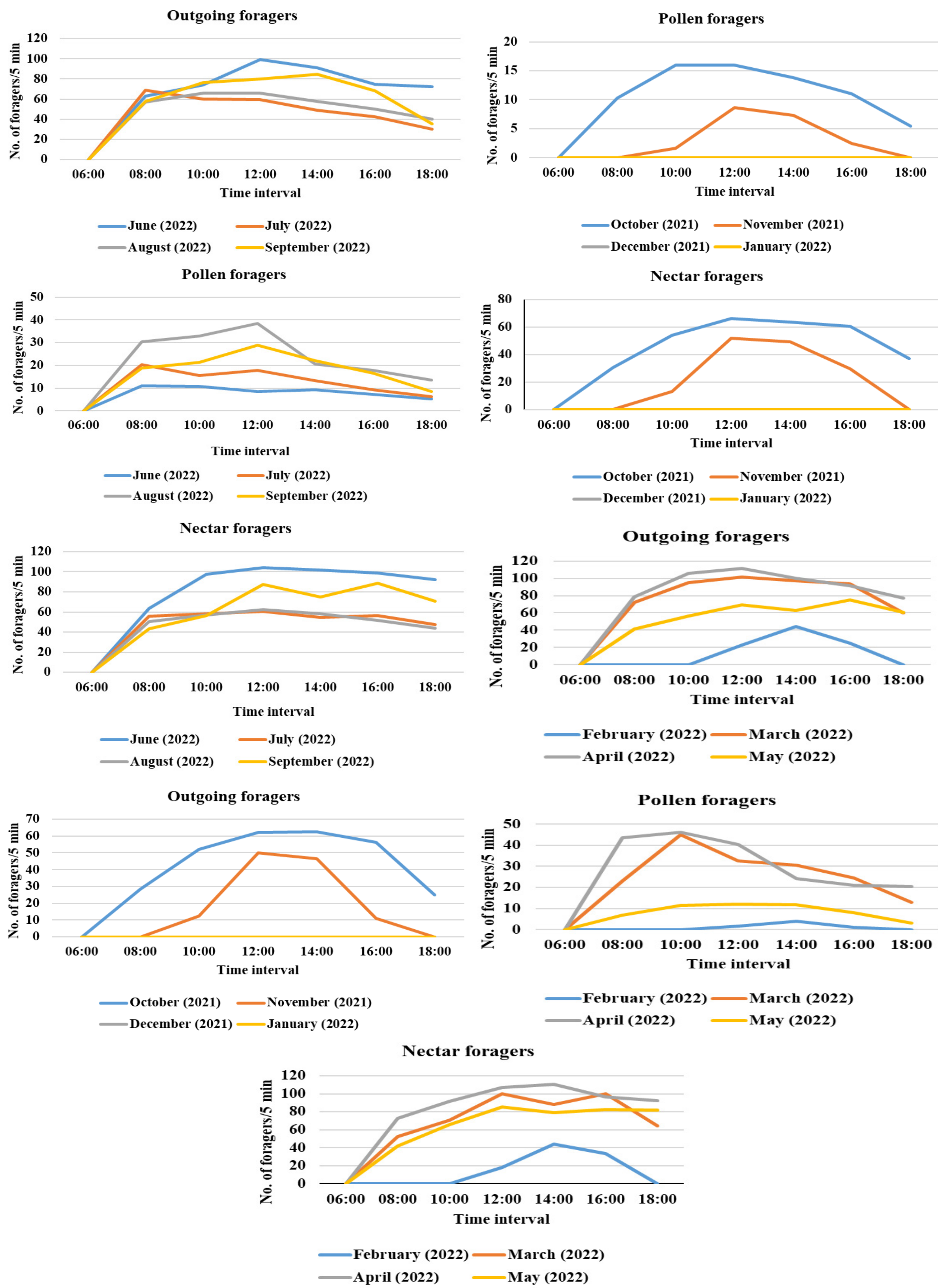

Fig 4. Foraging activity of outgoing, pollen and nectar foragers of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) across the different seasons in New Delhi, India, during 2021-2022. (**A to C-** Monsoon; **D to F-** Winter; **G to I-** Summer).

Association between weather parameters and foraging activity of outgoing, pollen, and nectar foragers

The number of outgoing foragers showed significant and positive association with maximum temperature (r = 0.897**), minimum temperature (r = 0.747**), and wind speed (r = 0.625*), while significant and negative association with average relative humidity (r = -0.733**) (Table 5). Further, a significant and positive association was found between pollen foragers and maximum temperature (r = 0.645*) (Table 5).

Table 5. Correlation between foraging activity of *Tetragonula* (*Tetragonula*) *ruficornis* (Smith) and climatic variables during 2021-22in New Delhi, India.

Weather parameters	Pearson correlation Coefficient (r)		
	No. of Outgoing foragers/5 min	No. of Pollen foragers/5 min	No. of Nectar foragers/5 min
Maximum temperature (ºC)	0.897**	0.645*	0.944**
Minimum temperature (ºC)	0.747**	0.550	0.797**
Average R.H. (%)	-0.733**	-0.478	-0.763**
Rainfall (mm)	-0.024	-0.038	-0.008
Wind speed (kmph)	0.625*	0.346	0.676*

*and **indicates correlation coefficients significance at p = 0.05 and 0.01, respectively.

Nectar foragers showed significant and positive association with the maximum temperature (r = 0.944**), minimum temperature (r = 0.797**), and windspeed (r = 0.676*), while significant and negative association with average relative humidity (r = -0.763**) (Table 5). However, rainfall exhibited a negative and non-significant association with outgoing (r = -0.024), pollen foragers (r = -0.038), and nectar foragers (r = -0.008) (Table 5).

Discussion

Stingless bees have evolved diverse nesting and feeding behaviors that allow them to adapt to various nesting sites (Roubik, 2006; Engel et al., 2023). The stingless bee, *Tetragonula* (*Tetragonula*) *ruficornis* (Smith), builds its colonies predominantly in tree cavities rather than wall cavities. This result did not agree with earlier studies by Danaraddi et al. (2009) and Gajanan et al. (2005), who stated that wall cavities made suitable nesting sites for *Tetragonula* (*Tetragonula*) *iridipennis* (Smith). This is probably due to the greater availability of natural cavities in tree trunks suitable for this species nesting where the study was performed, especially the availability of trees from the families Myrtaceae, Moraceae, and Fabaceae. Studies by Layek and Karmakar (2018) in West Bengal indicated similar nesting behavior for *T. iridipennis*, where most nesting substrates were *Butea monosperma* (Lam.) Taub. (Fabaceae), *Ficus benghalensis* L. (Moraceae), and *Ficus religiosa* L. (Moraceae), in addition to some artificial substrates such as wall cavities, window rims, or ridges of agricultural fields.

The nesting sites of *Tetragonula* (*Tetragonula*) *fuscobalteata* (Cameron) were found in stone, brick wall, wooden wall, bamboo, and iron cavities, *T. biroi* (Friese) in the wooden wall, stone, and brick wall cavities, *T. sapiens* (Cockerell) in stone cavities, while *T. laeviceps* (Smith) was found nesting in wooden walls (Suriawanto et al., 2017). The importance of *Syzygium cumini* and *Cassia fistula* as major preferred trees for nesting *T. ruficornis* was observed here by us from New Delhi, India. This may be due to the high frequency of old aged trees of the particular plant taxon in the studied area and the availability of space in the stem with water and termite resistance. Very fine heartwood of the plant taxon might have attracted this bee species for easy colonization. Although the directions of nest opening varied, there was a particular preference for the southeast (12 nests), followed by the southwest (9 nests) and eastward (7 nests). Preference for southward direction was also documented by Layek and Karmakar (2018) for stingless bee nests studied in West Bengal and northward by Nayak et al. (2013) from Karnataka. The direction of the nest entrance may be guided by the microclimate of the nesting site, which influences the thermoregulation of the nest. Stingless bee nest entrance characteristics are influenced by several variables, such as nest age, microclimate, foraging activity, and defense (Roubik, 2006). For instance, the eastward direction of *the Liotrigona bottegoi* (Magretti) nest entrance probably exposed the nest to the early morning sunlight, resulting in higher temperature and the beginning of foraging flight for this little bee species (Kajobe, 2007). As with other small stingless bee species, the deposition of a sticky resin surrounding the bee species' entrance may help guide foraging bees to recognize the nest and protect the colony from natural enemies (Barbosa et al., 2013). The entrance serves as a crucial point for communication between the nest and its resources, the outside world, and its threats. As a result, the size and other features of the external nest entrance are crucial to the colony's survival inside the nest (Couvillon et al., 2008). According to Barbosa et al. (2013), the nest entrance of *Geniotrigona subterranea* (Friese) was circular. However, Kelly et al. (2014) reported that the nest entrance of stingless bees varied in shape, length, and color in *Heterotrigona itama* (Cockerell) and *G. thoracica* (Smith).

The length of the entrance tube observed in the current study corresponds with the reports of Danaraddi et al. (2009) and Gajanan et al. (2005), who observed similar variations in the entrance tube of the bee species (*T. iridipennis*) in Karnataka. However, in a study by Pooley and Michener (1969), the entrance tube was shorter (6 – 25 mm) in the case of the *Hypotrigona gribodoi* (Magretti) nest. The length of the internal tunnel appears to be a species-specific characteristic and may also depend on the nesting site type and the used cavity size (Michener, 2007). The stingless bees that nest in tree trunks maintained a constant microclimate because of the shade and insulation offered by the tree. Additionally, the ability of the trunk wood to act as a thermal and moisture insulator is determined by the structure of the wood. In contrast, ground-nesting bees are believed to nest at a sufficient depth to ensure a stable nest environment even when the outside temperature is high (Moritz & Crewe, 1988).

Most stingless bees are cavity nesters and arrange their brood cells in combs or clusters (Michener, 2007). Our results indicate that the cluster type of cell arrangement was present in *T. ruficornis*, while a similar type of cell arrangement was found in *Liotrigona bottegoi* (Magretti) from Ethiopia (Hora et al., 2023). Cluster cell arrangement is found in several groups of small to minute stingless bees (Melo & Costa, 2009). It seems an adaptive feature of small bees nesting in small and irregular cavities (Gonzalez et al., 2018). Similar to that of *T. iridipennis,* which has been described by Danaraddi et al. (2009) and Roopa et al. (2015), the centrally placed oval-shaped brood cells of *T. ruficornis* were considerably smaller than peripheral food pots.

In contrast to *Tetragonula* (*Tetragonula*) *gressitti* (Sakagami), involucrum was absent in the *T. ruficornis* colony, which built its brood cells in a cluster (Chauhan & Singh, 2021). This result is consistent with the finding that some species of stingless bees, like *Trigona fulviventris* Guérin-Méneville, do not build involucrum around their brood (Barbosa et al., 2013). It suggests that involucrum may be an optional structure in stingless bee nests and its construction is related to external temperature conditions. The shape of food pots slightly deviated from the reported oval shape by Danaraddi et al. (2009) because we found spherical to polygonal shapes in addition to oval shapes. However, Layek and Karmakar (2018) reported similar shapes of food pots. The density of brood cells was higher than that of pollen and honey pots. These results support the findings of Danaraddi et al. (2009) and Gajanan et al. (2005).

The foraging time of the bee species varied according to plant species as well as to seasons due to temporal fluctuations in resource availability and weather conditions. The foraging activity of *T. ruficornis* started at 08:00 h during the monsoon and summer seasons when average monthly temperatures varied between 29.10 °C and 38.24 °C. However, it was delayed until 10:00 h during the cool winter months when average monthly temperatures varied between 12.5 °C and 18.9 °C. They also observed visiting flowers later on a foggy, chilly, or cloudy day compared to bright sunny days. The initiation of the foraging activity is comparatively later in this species compared to previously recorded foraging activity of *T. iridipennis,* starting at 06:00 h (Danaraddi et al., 2009) and 07:00 h (Devanesan et al., 2002). The foraging continued up to 18:00 h during the monsoon and summer, whereas in the winter, the activity ceased in advance, at or before 16:00 h.

Further, only one peak of outgoing and incoming forager bees' (pollen and nectar foragers) activity was observed between 10:00 and 12:00 during all the seasons. The highest foraging activity in the morning (8:00 to 11:00 h) was also documented by Azmi et al. (2015) for stingless bee *Lepidotrigona terminata* (Smith). When the foraging activity was considered irrespective of seasons, only one peak of outgoing, pollen, and nectar foragers was observed at 12:00 h. The foraging activity of the bee species (*T. iridipennis*) having a single peak was also obtained by Danaraddi et al. (2011) in research performed in Karnataka. However, Devanesan et al. (2002) recorded the two-peak activity of *T. iridipennis* at 12:00 and 15:00 h in Kerala, and Layek and Karmakar (2018) obtained two distinct peaks of activity between 09:00 to 11:00 h and 15:00 to 16:00 h in research performed in West Bengal. The probable causes of these changes in peak activity findings include temperature variations during different day hours, the availability of floral resources during different periods, and variations in the altitudes of the places where the research was performed. However, less activity occurred in the early morning and late evening, probably due to low temperature and a reduced intensity of sunshine at the present study site. Contrarily, Jaapar et al. (2018) observed that the mean outgoing and incoming rate of stingless bee *H. itama* was maximum during morning hours (08:00 to 10:00 h) and minimum from 4:00 to 6:00 p.m. h. When considering foraging activity seasonally, the peak activity was seen in early summer, probably due to greater resource availability and favorable weather conditions, and also due to a reduction of food reserves because of less foraging activity during the winter season due to unfavorable weather conditions (low temperatures and overwintering) as well as lower resource availability. Choudhary et al. (2021) reported that the foraging activity of *T. iridipennis* ceased from the second week of December to the second week of February, and successful overwintering was also observed during the winter season. These results vary widely due to the variation in these locations' colony conditions, forage sources, and climatic conditions.

Foraging activity increased proportionally with the increase in temperature up to 39 °C. Similar observations were reported by Burrill and Dietz in 1981. The present findings had strong support from the observations of Dustmann and Ohe (1988), who found a direct effect of maximum daytime temperature on the foraging activity of honeybees, whereas the cold snap restricted foraging activity and disrupted pollen intake in Germany. Further, relative humidity was negatively correlated with all foragers, which is not in line with Joshi and Joshi (2010), who reported that relative humidity showed less effect on the foraging activities of *Apis* species. Presumably, there was a negative correlation between rainfall and all foraging activities, which is supported by the observations of Abou-Shaara et al. (2012) that foraging activity dropped significantly during rain and that both *Apis cerana indica* and *A. mellifera* preferred to stay inside their hives. In the present study, wind speed showed no significant effect on pollen foragers, which is consistent with the findings of Reddy et al. (2015). This is probably due to the slight variation in wind speed (average wind speed 1.5 to 5.33 kmph) in the places where the studies were conducted.

Conclusion

The present study successfully described the nesting habits, internal nest characteristics, and foraging behavior of *T. ruficornis*. The availability of natural nesting sites such as trees and suitable nesting substrates is essential for the natural multiplication of this species in the study area. Foraging activity is greatly influenced by ambient environmental conditions such as temperature, rainfall, and relative humidity. The information generated from this study on nest architecture and foraging behavior would aid in understanding foraging activity during different seasons. It would also be an important starting point for designing artificial hives for their scientific manipulation in meliponiculture and their utilization in directed pollination services. There is a need for further studies on the availability of floral resources, foraging preferences, and food stores required for the successful overwintering of colonies to prepare a suitable conservation plan for this species.

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Authors' Contributions

B.N.H.: Investigation, data curation, formal analysis, and writing-original draft.
D.D.: Conceptualization and writing-reviewing and editing draft.
K.K.M.: Data curation and writing, review, and editing draft.

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