

## Nesting site selection and architectural adaptations of the stingless bee *Tetragonula iridipennis* Smith in Jaipur, Rajasthan

Vikas Kumar Meena, Santosh Kumar Charan\*

Department of Zoology, Bee Biodiversity Research lab, University of Rajasthan, Jaipur, Rajasthan, India

**Corresponding Author:** Santosh Kumar Charan

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### Abstract

This research paper investigates the nesting site characteristics of the stingless bee, *Tetragonula iridipennis* Smith, within the University of Rajasthan campus in Jaipur, Rajasthan. The study focuses on various nesting attributes, including preferred locations, nesting sites, colony locations, nest orientation, nest color, size and shape of nest openings, and materials used for nest enclosures. The findings indicate that the bees predominantly select educational buildings for nesting, with stone walls being the most common nesting site. Colonies are primarily situated within the interior parts of buildings, exhibiting a preference for south-facing orientations. The nests typically display a black coloration, with medium-sized, oval-shaped openings. Materials utilized for nest enclosures include resin, wax, wooden pieces, sand, mud, tar, blue paint, pollen, leaf fragments, stones, cow dung, and animal feces. The study also measures various nest dimensions, such as height from the ground, length, and breadth, and observes worker traffic at the entrance. This research provides valuable insights into the nesting behavior of *Tetragonula iridipennis* and contributes to a better understanding of their ecological preferences and habitat requirements. The significance of this study lies in its contribution to the understanding of the nesting behavior of *Tetragonula iridipennis*, which can inform conservation efforts and habitat management practices. By identifying the preferred nesting sites and materials, researchers and conservationists can design environments that support the sustainability and proliferation of these bees.

**Keywords:** Stingless bee, *Tetragonula iridipennis*, nesting sites, nest orientation, nesting behavior, ecological preferences, habitat requirements, conservation strategies

### Introduction

Stingless bees belonging to the tribe Meliponini within the family Apidae, are highly eusocial insects that play a pivotal role in pollination, significantly contributing to biodiversity conservation, agricultural productivity, and ecosystem stability (Roubik, 2006; Michener, 2007) [4, 14]. As keystone pollinators, they are crucial in sustaining floral diversity and ensuring the reproductive success of various plant species, particularly in tropical and subtropical ecosystems (Slaa *et al.*, 2006; Cortopassi-Laurino *et al.*, 2006) [4, 27]. Unlike honeybees (*Apis* spp.), stingless bees lack a functional sting and instead employ alternative defense mechanisms, including collective aggression, nest architecture modifications, and resin-based entrance barriers to deter intruders (Michener, 2007; Grüter, 2020) [14].

*T. iridipennis* is widely distributed across diverse habitats and exhibits remarkable adaptability by nesting in both natural and artificial cavities, such as tree trunks, termite mounds, stone walls, mud walls abandoned pipes, and building crevices (Choudhary *et al.*, 2021; Charan *et al.*, 2023 [2, 3]; Saaivignesh & Manickavasagam, 2024; Saranya *et al.*, 2024; Meena *et al.*, 2025; Meena *et al.*, 2025) [11, 24]. The nest comprises of entrance, brood pots, honey and pollen storage pots, waste and resin dumps and nest envelope like involucrum and batumen (Pooley & Michener, 1969; Mohan & Devanesan, 1999; Alves *et al.*, 2018) [1, 15, 17]. Nesting site selection is a crucial aspect of stingless bee ecology, as it directly affects colony survival, thermoregulation, and foraging efficiency. Various biotic and abiotic factors influence the suitability of a nesting site, including substrate availability, cavity size, microclimatic

conditions, predation pressure, interspecific competition, and proximity to floral resources (Eltz *et al.*, 2002; Roubik, 2006; Michener, 2007) [4, 6, 14]. Studies have demonstrated that *T. iridipennis* strongly prefers enclosed cavities that provide structural stability and insulation from environmental fluctuations, which are essential for colony growth and brood development (Wille & Michener, 1973; Roopa *et al.*, 2015 [20, 32]; Grüter, 2020; Hadimani *et al.*, 2020) [8]. Nest orientation also significantly optimizes temperature regulation and maximizes early morning sunlight exposure, which is critical for colony activity and foraging efficiency (Leonhardt *et al.*, 2011; Nayak *et al.*, 2013; Vollet-Neto *et al.*, 2015 [10, 16, 30]; Grüter, 2020). For example, Wille & Michener (1973) [32] highlight the importance of enclosed cavities in maintaining stable internal conditions, while Leonhardt *et al.* (2011) and Vollet-Neto *et al.* (2015) [10, 30] emphasize the role of resin use and behavioral adaptations in preventing nest overheating and ensuring proper thermoregulation.

Amid rapid urbanization and habitat fragmentation, stingless bees are increasingly adapting to human-modified landscapes, often utilizing artificial structures for nesting. This transition underscores their ecological resilience and behavioral plasticity but also raises concerns about habitat loss, resource competition, and long-term population sustainability (Cortopassi-Laurino *et al.*, 2006; Slaa *et al.*, 2006) [4, 27]. Understanding the nesting biology of *T. iridipennis* is essential for developing evidence-based conservation strategies, supporting sustainable meliponiculture, and ensuring their continued role in

pollination services in both natural and urban ecosystems (Rasmussen & Camargo, 2008; Jaffé *et al.*, 2015) [9, 19].

This study aims to investigate the nesting site preferences, architectural characteristics, and environmental adaptability of *T. iridipennis* within the University of Rajasthan campus, Jaipur. By systematically examining the nesting ecology of *T. iridipennis* in an urbanized environment, this research provides valuable insights into their behavioral adaptations, habitat selection criteria, and the broader ecological implications of their nesting strategies. The findings will contribute to a comprehensive understanding of stingless bee nesting ecology and inform strategies for conservation, habitat restoration, and urban pollinator management.

## Materials and Methods

### Study Area

The study was conducted at the University of Rajasthan campus, Jaipur, Rajasthan, which covers approximately 285.29 hectares (705.0 acres) along Jawaharlal Nehru Marg, also known as the central spine of Jaipur. Jaipur has a semi-arid climate with extreme seasonal variations, summer temperatures exceeding 45°C (113°F), winter lows reaching 5°C (41°F), and annual precipitation averaging 500–700 mm. The campus encompasses a diverse landscape, including urban, semi-urban, and natural environments. It

features a mix of educational buildings, residential quarters, hostels, offices, roadsides, parks, banks and a post office, providing a variety of structural substrates suitable for stingless bee nesting.

The survey covered multiple habitat types within the campus to document the nesting preferences of *Tetragonula iridipennis*. Anthropogenic structures, including stone walls, brick walls, pillars, electric boxes, and cement-plastered surfaces, were examined, as these structures provide cavities that may serve as potential nesting sites. Additionally, natural substrates, such as tree trunks and mud walls, were included to assess the species' nesting flexibility in both artificial and natural environments. The vegetation within the study area consisted of ornamental plants, roadside trees, and patches of greenery, which likely play a role in providing floral resources for foraging bees.

This location was selected due to its high human activity, diverse nesting substrates, and variation in microclimatic conditions, making it an ideal setting to evaluate the nesting site preferences and adaptability of *T. iridipennis* in an urbanized landscape. The study provides valuable insights into how stingless bees utilize available nesting resources in an environment influenced by both natural and human-made elements.

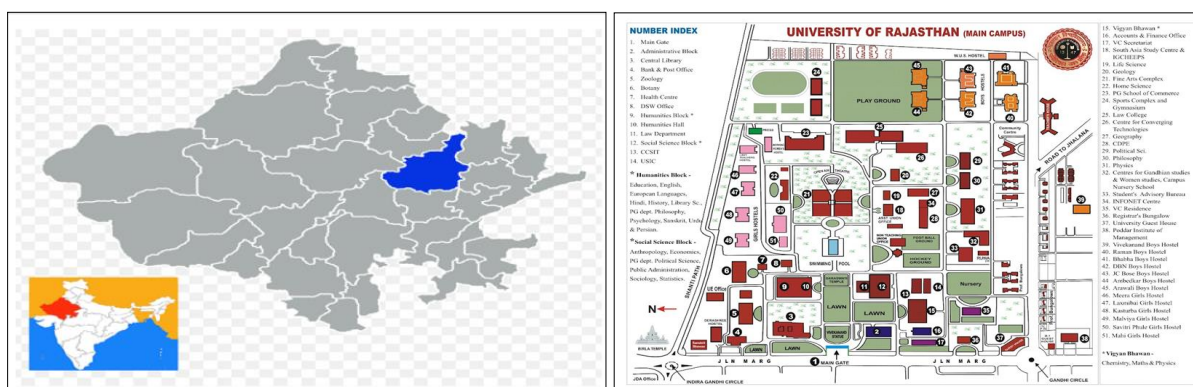


Fig: Map of study area

### Survey and Data Collection

A comprehensive field survey was conducted over 10 months (November 2024– August 2025) to document the nesting behavior of *Tetragonula iridipennis*. Observations were carried out weekly between 09:00 and 17:00 hours, employing an all-out search method to locate nests systematically. The presence of colonies was primarily identified by tracking worker bee flight activity near potential nesting sites. Data on nesting site attributes, elevation, orientation, and nest longevity were recorded following standardized methodologies outlined by Sheetal and Basavarajappa (2009) [28]. Measurements of nest components were taken using a centimeter-scale measuring tape, ensuring precision and consistency in data collection. The selection of nesting sites was analyzed based on structural stability, microclimatic conditions, and

accessibility, as these factors play a crucial role in colony establishment and persistence. All recorded nests were photographed using a Canon digital camera for documentation and future comparative analysis.

### Data Analysis

The collected data were analyzed using descriptive statistics to identify the most frequently preferred nesting sites, orientations, and environmental conditions. The results were compared with previous studies on *T. iridipennis* and other stingless bee species to assess ecological trends and nesting adaptability. Data compilation and statistical analysis were performed using SPSS software (Version 17).

### Different Nesting attributes of the *Tetragonula iridipennis* at Rajasthan University campus, Jaipur

S. No	Attributes	Observation	Most preferred
1.	Places visited	Residential quarters, educational buildings, hostels, offices, roadside, parks, post office,	Educational buildings
2.	Nesting site	Stone wall, brick wall, pillars, Electric box, Cement plastering, Tree trunk, Mud wall	Stone wall
3.	Location of colony	Interior, exterior (from the roadside)	Interior
4.	Nest orientation	North, south, east, west, northeast, northwest, southeast, southwest	South

5.	Nest colour	Black, grey, brown, light green, orange, yellow	Black
6.	Size of nest opening	Small, medium, large	Medium
7.	Shape of nest opening	Circular, oval, irregular	Oval
8.	Nest enclosure materials	Resin, wax, wooden pieces, sand, mud, tar, blue paint, pollen, leaf bits, stones, cow dung, animal faces.	Resin, wax
9.	The worker traffic at the entrance	Low- <10 individuals Moderate- 10-20 individuals High- >20 individuals	Moderate- 10-20 individuals
10.	Nest Surface	Smooth, Rough	Rough
11.	Vegetation nearby nest	Low Moderate High	Moderate
12.	Sunlight incidence	Low Moderate High	Moderate
Nest measurements			
13.	Nest height from the ground	0-12 ft.	
14.	Nest Length	1.83 ± 0.72 cm	
15.	Nest breadth	1.26 ± 0.42 cm	
16.	Length of external entrance tube	0.76 ± 0.67 cm	

## Results and discussion

### Nesting Locations and Substrate Preferences

The nesting site preferences of *Tetragonula iridipennis* at the University of Rajasthan campus, Jaipur, were systematically analyzed to assess their ecological adaptability in an urban environment. The findings reveal a pronounced inclination toward anthropogenic structures, with the majority of colonies established in stone walls (42.86%), followed by cement-plastered surfaces (23.81%) and brick walls (14.29%). These nesting choices underscore the species' preference for solid, durable substrates that provide enhanced structural stability, insulation, and protection from environmental fluctuations and potential predators. In contrast, a smaller proportion of colonies were found in natural substrates, including tree trunks (9.52%) and mud walls (7.14%). The limited selection of natural nesting sites suggests either a reduced availability of suitable tree cavities or a strong adaptive shift favoring human-made environments. Additionally, electric boxes accounted for the lowest percentage of nest locations (2.38%), indicating that such sites may be less favorable. A Chi-Square test ( $\chi^2 = 27.43$ ,  $p = 4.71 \times 10^{-5}$ ) indicated a statistically significant deviation from an expected uniform distribution, confirming that *T. iridipennis* does not randomly select nesting sites but exhibits clear preferences for specific substrates. This non-random selection highlights the species' ability to exploit urban structures for colony establishment, contributing to their ecological success in densely populated regions.

A significant proportion of colonies (71.43%) were established in interior spaces of buildings, while the remaining colonies (28.57%) were found on external roadside structures. This distribution pattern highlights the species' preference for shaded, protected environments that mitigate direct exposure to climatic stressors such as extreme heat, heavy rainfall, and strong winds. Interior nesting may also reduce the risk of predation and anthropogenic disturbances, while providing stable microclimatic conditions that enhance the longevity of nest structures.

Nesting habitat preference	No of colonies
Stone walls	18
Brick walls	06
Cement plastering	10
Tree trunk	04
Electric Box	01
Mud wall	03

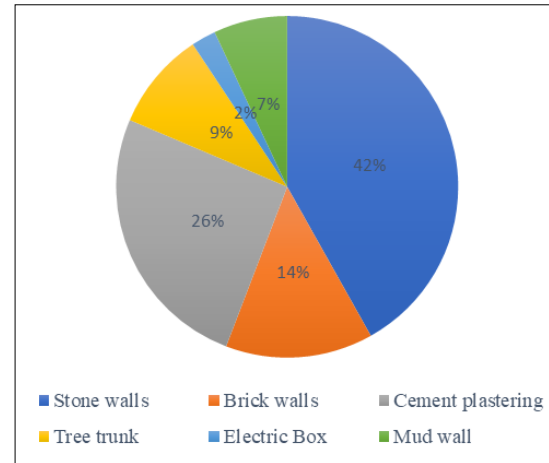


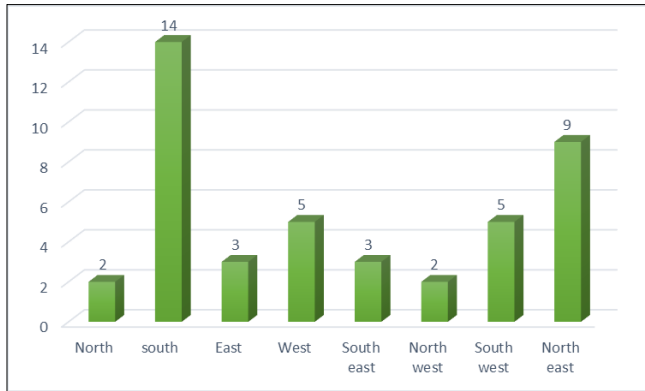
Fig: Nesting habitat preference

### Nest Orientation and Structural Characteristics

Nest orientation in *Tetragonula iridipennis* revealed a distinct preference for south-facing nest entrances, followed by northeast and east orientations. This pattern suggests a non-random selection of nest direction, likely influenced by environmental factors specific to the region. The predominance of southern orientation indicates a potential adaptation to local climatic conditions, ensuring optimal thermal regulation and protection from environmental stressors. The occurrence of northeast and east-oriented nests further suggests a degree of variability in nesting preferences, possibly reflecting microhabitat conditions or site-specific structural influences. These findings highlight the species' ability to adjust its nesting orientation to local environmental conditions, facilitating colony establishment and survival in both natural and anthropogenic nesting sites. A Chi-Square test ( $\chi^2 = 22.67$ ,  $p = 0.00194$ ) revealed a statistically significant preference for certain orientations over others. The results suggest that nest direction is not random and that *T. iridipennis* actively selects orientations likely based on environmental factors such as sunlight exposure and temperature regulation.

Table: Nest orientation

Nest orientation	No of colonies
North	02
South	14
East	03
West	05
South east	03
North west	02
South west	05
North east	09



**Fig:** Nest orientation

### Nest colour

The nest colour of *Tetragonula iridipennis* exhibited noticeable variation, with black being the most frequently observed, followed by shades of grey, brown, light green, orange, and yellow. This diversity in nest coloration may be attributed to the incorporation of different nesting materials such as resin, wax, mud, and plant-derived substances, which contribute to structural integrity and camouflage. The predominance of black-colored nests suggests a potential adaptive advantage, possibly aiding in thermal regulation by absorbing heat, which could be particularly beneficial in moderating internal nest temperatures in varying environmental conditions. The presence of lighter shades, though less frequent, indicates that nest composition may be influenced by the availability of construction materials within the immediate habitat.

### Nest surface texture

The nest surface texture of *Tetragonula iridipennis* exhibited variability, with rough surfaces being the most commonly observed. This rough texture is likely a result of the diverse nesting materials used, including resin, wax, mud, sand, and plant fragments, which contribute to the structural stability and protective characteristics of the nest. The irregular surface may enhance adherence to nesting substrates, providing additional resilience against external disturbances and environmental fluctuations. The presence of smooth nest surfaces, though less frequent, suggests that certain colony locations or material compositions may facilitate a more compact and uniform nest structure. This variation in surface texture reflects the species' adaptive ability to construct nests using locally available resources, optimizing nest durability and environmental suitability.

### Nest Architecture and Material Composition

#### Nest entrance size and shape

The nest entrances of *Tetragonula iridipennis* were predominantly medium-sized, with smaller and larger entrances occurring less frequently. In terms of shape, oval entrances were the most common, followed by circular and irregular forms. The consistent preference for medium-sized and oval-shaped entrances indicates a recurring pattern in nest architecture. Variations in entrance size and shape were observed across different nesting sites, suggesting some degree of structural flexibility that depends on the nesting substrate.

#### Nest enclosure materials

The nest enclosures of *Tetragonula iridipennis* comprised a diverse range of materials, including resin, wax, wooden

pieces, sand, mud, tar, blue paint, pollen, leaf bits, stones, cow dung, and animal feces. Resin and wax were the most commonly observed materials, forming the primary structural components, while mud and plant-derived substances were frequently incorporated. Nests located in natural substrates primarily utilized organic materials, whereas those in anthropogenic structures often included synthetic elements such as tar and blue paint. The occasional presence of cow dung and animal feces suggests opportunistic material usage based on local availability. The variation in nest enclosure materials across different nesting sites indicates a degree of flexibility in material selection, with colonies utilizing both natural and human-associated resources.

### Nest Entrance Activity and Environmental Factors

**Worker traffic at the entrance:** The worker traffic at the nest entrance of *Tetragonula iridipennis* varied across colonies, with observations categorized into three levels: low (<10 individuals), moderate (10–20 individuals), and high (>20 individuals). Moderate worker traffic was the most commonly recorded, followed by high traffic, while low worker activity was the least frequent. Variations in worker traffic were observed across different nesting sites, suggesting potential influences of colony size, resource availability, and environmental factors. Colonies with high worker traffic were typically located in well-established nesting sites, whereas nests with lower activity may indicate smaller colony sizes or less foraging activity at the time of observation. These findings highlight differences in foraging dynamics and nest activity among *T. iridipennis* colonies in diverse environments.

### Vegetation density near nests

The vegetation density surrounding the nests of *Tetragonula iridipennis* varied across nesting sites and was categorized into three levels: low, moderate, and high. Moderate vegetation density was the most commonly observed, followed by low vegetation, while nests surrounded by high vegetation were the least frequent. Variations in vegetation density near nests suggest potential influences on colony establishment, with moderate vegetation possibly providing an optimal balance of foraging resources, microclimatic stability, and protection. Nests in areas with low vegetation density were often located in anthropogenic structures, while those in areas with high vegetation were primarily associated with natural substrates. These observations indicate that *T. iridipennis* can establish nests across different habitat conditions, with a tendency to select locations offering moderate vegetation cover.

### Sunlight exposure

The sunlight exposure at the nest sites of *Tetragonula iridipennis* varied and was categorized into three levels: low, moderate, and high. Moderate sunlight exposure was the most frequently recorded, followed by high exposure, while nests receiving low sunlight were the least common. Variations in sunlight exposure across different nesting sites suggest potential influences on nest placement, with moderate exposure likely providing optimal thermal conditions for colony activity. Nests in high-exposure areas were primarily found in open or external structures, while those with low sunlight exposure were often located in shaded or enclosed environments. These findings indicate

that *T. iridipennis* exhibits flexibility in nest site selection, with a general preference for locations receiving moderate sunlight.

### Nest Dimensional Characteristics and Structural Metrics

#### Nest height

The nest height of *Tetragonula iridipennis* varied across different nesting sites, ranging from ground level up to 12 feet. The most frequently observed nest height was between 6–8 feet, followed by nests located at lower and higher elevations. Nests positioned at moderate heights were commonly found in anthropogenic structures such as stone and brick walls, while those at lower heights were often associated with mud walls or tree trunks. Higher nests were primarily located in elevated structures like building facades or tall tree cavities. The variation in nest height suggests flexibility in site selection, with colonies utilizing available substrates at different elevations based on structural stability and environmental suitability.

#### Nest dimensions

The structural dimensions of *Tetragonula iridipennis* nests were systematically measured, revealing an average nest length of  $1.83 \pm 0.72$  cm, nest breadth of  $1.26 \pm 0.42$  cm, and an external entrance tube length of  $0.76 \pm 0.67$  cm. Nest size varied across different nesting sites, influenced by the substrate and available space. Medium-sized nests were the most frequently recorded, with smaller and larger nests occurring less frequently. Nests in anthropogenic structures, such as stone and brick walls, were generally more compact and integrated into crevices, whereas those in natural substrates like tree trunks displayed greater dimensional variability.

### Discussion

The present study provides significant insights into the nesting behavior and site preferences of *Tetragonula iridipennis* in the University of Rajasthan campus, Jaipur. Our findings indicate a strong inclination toward anthropogenic structures, particularly stone walls, cement-plastered surfaces, and brick walls, which aligns with previous research conducted in different regions of India and Southeast Asia. Choudhary *et al.* (2021)<sup>[3]</sup> reported that *T. iridipennis* predominantly nests in brick walls (86.67%), with a smaller proportion occupying hollow tree trunks (13.33%), a trend similar to our observations. The preference for artificial substrates suggests a remarkable ability of these bees to adapt to human-modified environments, likely due to the stability and protection offered by such structures (Choudhary *et al.*, 2021)<sup>[3]</sup>.

Our study revealed that nest entrance orientation was primarily south-facing, followed by northeast and east orientations. This contrasts with findings from Karnataka and Tamil Nadu, where colonies exhibited a preference for east and northeast orientations, presumably to maximize exposure to morning sunlight and regulate nest temperature (Saaivignesh & Manickavasagam, 2024)<sup>[24]</sup>. The deviation observed in Jaipur may be influenced by regional climatic factors, such as higher diurnal temperature variations, prevailing wind directions, and seasonal solar radiation patterns, which could necessitate greater thermal buffering and nest insulation (Nayak *et al.*, 2013)<sup>[16]</sup>.

The structural composition of nests, including entrance dimensions and enclosure materials, exhibited notable

diversity. The average entrance tube dimensions in our study were comparable to those reported by Saranya *et al.* (2024)<sup>[26]</sup> in Assam, with a predominant use of resin, wax, mud, and plant-derived materials. Interestingly, our findings also documented the incorporation of synthetic elements like tar and paint in urban settings, reinforcing the species' adaptability to available nesting resources (Saaivignesh & Manickavasagam, 2024). This aligns with Roubik's (2006)<sup>[4, 24]</sup> observations that stingless bees exhibit considerable variation in nest construction based on local material availability (Roubik, 2006)<sup>[4]</sup>.

Worker traffic at nest entrances varied significantly among colonies, with moderate activity being the most frequent. Similar patterns were reported by Divya *et al.* (2015)<sup>[5]</sup> in Kerala, where the number of guard bees at nest entrances was higher in midland colonies compared to upland colonies. This suggests that foraging activity and colony defense mechanisms may be influenced by factors such as resource availability, predation pressure, and environmental conditions (Roopa *et al.*, 2015)<sup>[20]</sup>. Moreover, our findings on vegetation density and sunlight exposure near nests indicate that moderate levels of both factors were most preferred, providing an optimal balance of microclimatic stability and resource accessibility (Suriawanto *et al.*, 2017)<sup>[29]</sup>.

Nest height and dimensions exhibited considerable variation, with most colonies established at moderate elevations (6–8 feet), consistent with findings from North-Western India and Karnataka (Nayak *et al.*, 2013; Choudhary *et al.*, 2021)<sup>[3, 16]</sup>. The structural dimensions of nests, including an average length of  $1.83 \pm 0.72$  cm and breadth of  $1.26 \pm 0.42$  cm, align with values reported by Saaivignesh & Manickavasagam (2024)<sup>[24]</sup>, further emphasizing the consistency in nest architecture across different regions. The observed variability in nest dimensions and placement may be influenced by site-specific conditions, including substrate availability, structural integrity, and climatic factors (Choudhary *et al.*, 2021)<sup>[3]</sup>.

The ecological and evolutionary implications of these findings highlight the resilience and adaptability of *T. iridipennis* in urbanized environments. The ability to exploit diverse nesting materials, flexible site selection, and orientation strategies suggests an evolutionary advantage in persisting across varied habitats (Roubik, 2006)<sup>[4]</sup>. Additionally, the preference for moderate environmental conditions may indicate an adaptive response to balance resource availability and microclimatic stability (Suriawanto *et al.*, 2017)<sup>[29]</sup>. Future research should explore the influence of urbanization on nesting success, colony health, and long-term population dynamics of *T. iridipennis*. Understanding these aspects will be crucial for developing conservation strategies and promoting sustainable meliponiculture practices to enhance pollination services in both natural and agricultural landscapes (Roopa *et al.*, 2015)<sup>[20]</sup>.

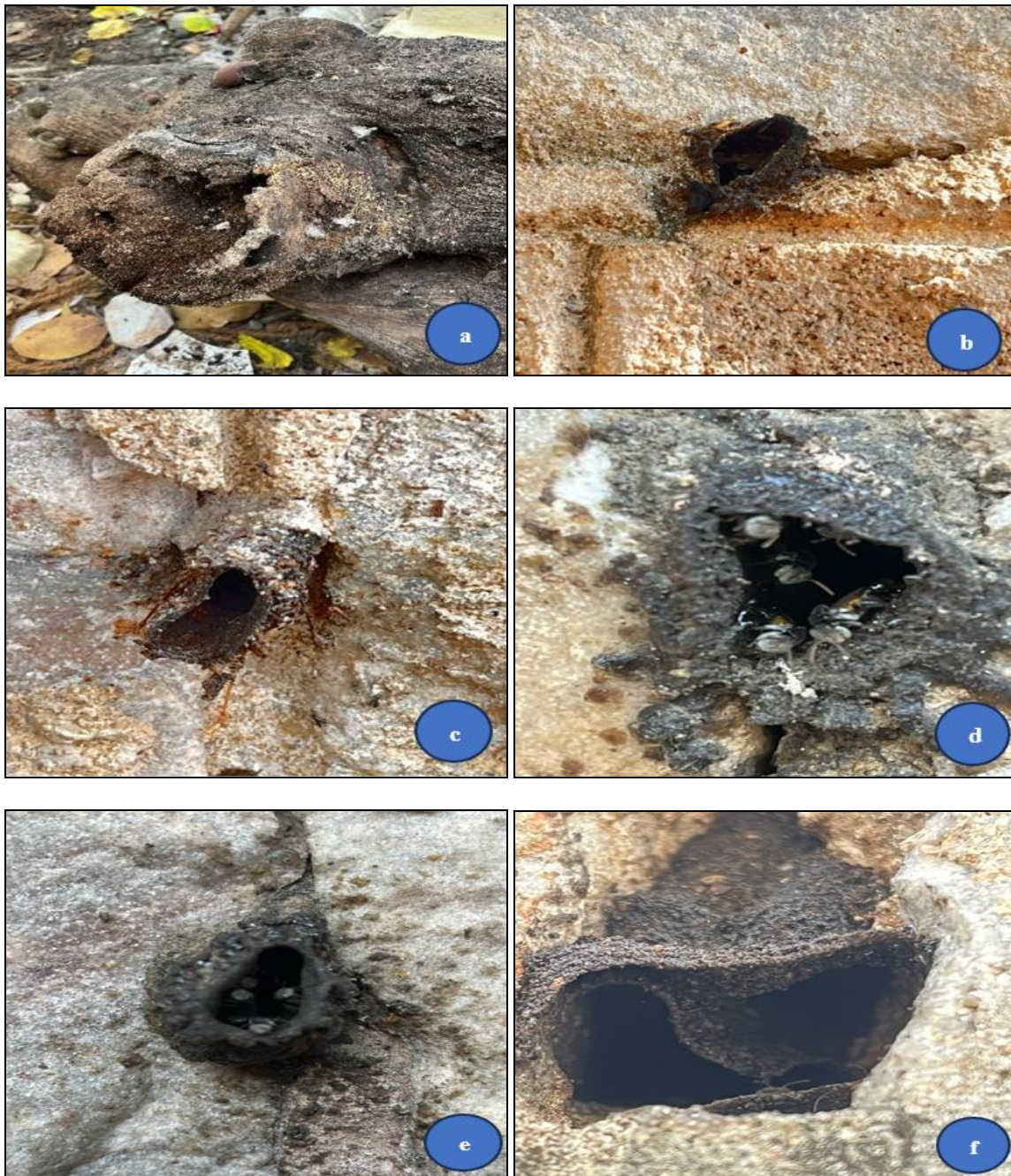
### Conclusion

The present study provides a comprehensive analysis of the nesting site preferences and architectural attributes of *Tetragonula iridipennis* in the University of Rajasthan campus, Jaipur. Our findings indicate a strong preference for anthropogenic structures, particularly stone walls, cement-plastered surfaces, and brick walls, demonstrating the species' remarkable adaptability to urban environments.

Nest orientation showed a predominant inclination towards the south, suggesting a possible response to local climatic conditions for optimal thermal regulation. The use of diverse enclosure materials, including resin, wax, mud, and even synthetic substances, highlights the species' ability to exploit available resources for nest construction.

Variability in nest entrance size and shape, as well as nest height and dimensions, suggests flexibility in site selection, influenced by environmental and structural factors. Worker traffic at the nest entrance, along with vegetation density and sunlight exposure, revealed a preference for moderate environmental conditions that balance foraging efficiency and microclimatic stability. These nesting attributes collectively contribute to the ecological success of *T. iridipennis* in both natural and modified landscapes.

The findings of this study contribute valuable baseline data for understanding the nesting biology of *T. iridipennis* and have significant implications for conservation strategies. Given the increasing urbanization and habitat fragmentation, future research should explore the impact of environmental changes on nesting success and colony health. Additionally, further studies on the role of nest architecture in colony defense and thermoregulation would enhance our understanding of the adaptive strategies of stingless bees. Promoting sustainable meliponiculture practices and ensuring the conservation of natural and semi-natural nesting habitats will be crucial for maintaining *T. iridipennis* populations and their vital pollination services in ecosystems.



**Fig a-f:** Various types of Nest entrances of *Tetragonula iridipennis* at Rajasthan University Campus. Guard bees are present at the entrance of some nests

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