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NOT / NOTE

ETHOLOGY OF STINGLESS BEES (*Tetragonula "iridipennis"* sp. group) IN HABITANCY

Kendi Bölgesinde İğnesiz Arıların (Tetragonula "iridipennis" sp. grubu) Davranışları

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ABSTRACT

Most primitive stingless bee species in Asia are *Tetragonula "iridipennis"* sp. group, which are highly eusocial and corbiculate. The aim of the study is to explore thestingless bee behaviour around their habitat, as they have constructed their nest in cracks and crevices. General behaviour of stingless bee, inside the hive and their habitat was studied through periodical observation of colony throughout the research period 2020 to 2021. Colony behaviour showed that the bees (i) resort to switching of entrance (during morning and night), (ii) construct false nest and vestibule chamber to prevent intruders by trapping and mummification, (iii) follow mass provisioning (time and number of workers involved in provisioning per brood pot) and ovipositioning process (time taken for egg laying by queen and sealing of brood pots by workers after oviposition), (iv) resort to cleaning process through removal of waste as a debris ball, (v) resort to mess and soil manner of foraging (burglary of floral rewards), (vi) forage on resin substances (like natural milky latex from plant and petroleum product-grease) and (vii) peak forage time was between 10.00 and 01.00 hours.

Keywords: *Tetragonula*sp., Behaviour, Defence, Mass provision and oviposition, Foraging

ÖZ

Asya'daki en ilkel iğnesiz arı türü, oldukça eusosyal ve korbikülat olan Tetragonula "iridipennis sp. grubudur. Çalışmanın amacı, yuvalarını çatlak ve yarıklara inşa ettikleri için yaşam alanlarının etrafındaki iğnesiz arı davranışlarını keşfetmektir. Arıların kovan içindeki ve yaşam alanlarındaki genel davranışları, 2020-2021 araştırma dönemi boyunca kolonilerin periyodik olarak gözlemlenmesi yoluyla incelenmiştir. Koloni davranışları, arıların (i) giriş değiştirmeye (sabah ve gece boyunca) başvurduklarını, (ii) tuzak ve mumyalama yoluyla davetsiz misafirleri önlemek için sahte yuva ve giriş odası inşa ettiklerini, (iii) toplu tedariki (kuluçka kabı başına tedarikte yer alan işçi sayısı ve süresi) ve yumurtlama sürecini (kraliçe tarafından yumurtlama için geçen süre ve yumurtlamadan sonra işçiler tarafından kuluçka kaplarının kapatılması) takip ettiklerini göstermiştir, (iv) atıkların bir döküntü yumağı olarak uzaklaştırılması yoluyla temizleme işlemine başvurma, (v) dağınıklık ve toprak arama yöntemine başvurma (çiçek ödüllerinin çalınması), (vi) reçine maddeleri (bitkiden elde edilen doğal

sütlü lateks ve petrol ürünü-gres gibi) üzerinde yemleme ve (vii) en yoğun yemleme zamanı 10.00 ile 01.00 saatleri arasında gözlenmiştir.

Anahtar Kelimeler: *Tetragonula* sp., Davranış, Savunma, Toplu tedarik ve Yumurtlama, Yiyecek arama

GENİŞLETİLMİŞ ÖZET

Çalışmanın amacı: Bu çalışma, Apis spp. türlerinden önemli ölçüde farklı olan iğnesiz arının (Tetragonula sp.) biyolojisi, yuvalama davranışı, besin sağlama ve yumurtlama gibi davranışlarını ve kovan ve habitatlarındaki yiyecek arama davranışlarını keşfetmek için yapılmıştır.

Gereç ve Yöntem: Yabani iğnesiz arı kolonileri Salem (11048'14 "N 770 51'28 "E), Dharmapuri (120 19'04 "N 780 06'48 "E), Cuddalore (110 27'44 "N 790 19'57 "E) ve Puducherry (120 00'42 "N 790 44'30 "E) gibi Tamil Nadu'daki çeşitli yerlerden yakalanmış ve davranış çalışmaları için kovanlarda evcilleştirilmiştir. Yuvalama davranışı çalışmaları için, koloniler evcilleştirildikten hemen sonra, evcilleştirmeden sonraki üç gün içinde sabah (06.00 ila 10.00 saat) ve akşam (18.00 ila 23.00 saat) boyunca giriş ve giriş tüpü yapımını kapatmak için görsel olarak gözlemlenmiştir. Kuluçka kabı yapımı, tedarikte yer alan arı sayısı, ana arı yumurtlaması, atık yönetimi ve yiyecek arama gibi ek davranışlar haftada iki kez görsel olarak gözlemlenmiş, kuluçka kabı başına toplu tedarik ve mühürleme için geçen süre ve reçinenin boşaltılması bir kronometre kullanılarak kaydedilmiştir.

Bulgular: Gece zararlılarından veya avcılardan kaçınmak için giriş kapatma davranışı gerçekleştirilmiştir. İşçi arılar 20.00-23.00 saatleri arasında girişe az miktarda çamur bırakarak girişi kapatmakta ve 06.00-09.00 saatleri arasında açmaktadır. Sahte yuva ve giriş odası inşa edilmekte ve koruyucu yuvalama için arılar tarafından yuva giriş tüplerine çamur parçacıkları ve yağ gibi mevcut malzemeler biriktirilmektedir. Kağıt yaban arısı ve buğday biti gibi arı düşmanları reçine tükürülerek tuzağa düşürülmekte ve çamur kullanılarak mumyalanmaktadır. İşçi arıların toplu beslenmesini teşvik etmek için ana arı kontrol eder, kanatlarını çırpar ve antenleriyle inşa edilen kuluçka kabının yakınındaki işçilere dokunur. Kitlesel sağlama için 4-6 işçi arı, larva besinini bir kuluçka kabının dörtte üçüne kusarak larva besinini doldurmaya katılmıştır. Kitlesel sağlama ve yumurtlamadan sonra, tek bir işçi kuluçkayı 04-55 saniye içinde mühürlemektedir. Toplu yemleme, yumurtlama ve bir kuluçka kabının kapatılması için geçen toplam süre 1,0 ila 3,25 dakika arasında değişmişmektedir. Temizleme işleminde, döküntü topu çene kemiği ile tutularak taşınmış, düşürmek için 2-3 metre uzağa uçmaktadır. Yiyecek arama faaliyeti, sabah erken ve akşam geç saatlere kıyasla gün ışığı saatlerinde zirve yapılmaktadır. Tecoma çiçeklerinde dağınık ve toprakta yiyecek arama şekli gözlenmişve ayrıca çiçekleri ziyaret etmekten ziyade ekstra çiçek nektarı toplamayı da içerdiği gözlenmiştir. Çamur için, işçi arılar Euphorbia antiquorum'dan sütlü lateks ve gres yağını benzersiz bir şekilde toplamaktadır. Tek bir işçi arının korbikulayı gres yağı ile doldurması 3,5 dakika sürmektedir.

Sonuç: Yukarıda bahsedilen bu benzersiz davranışlar, kişniş, havuç vb. gibi küçük çiçeklerle tarımsal ve bahçecilik ürünlerini tozlaştırmak veya ballarını ve balmumu yerine kullandıkları çamur insan hastalıklarını iyileştirmek için kullanmak amacıyla iğnesiz arıları evcilleştirmede başarılı olmamıza yardımcı olabilir.

INTRODUCTION

Stingless bees differ from *Apis* species in biology and foraging behaviour. The process of feeding the larvae is very different from that of the *Apis*. The system of larval feeding in stingless bees is called mass provisioning whereas, in *Apis* sp*p*., the larvae are fed progressively. Development of a queen takes several weeks, while in honeybees it takes 15-16 days (Roubik, 1992). Periodically they construct queen cells even under queen right condition (Mythri *et al*., 2018). During foraging, dammer bees show a preference for different plants. Their small size allows them to have access to many kinds of flowers whose openings are too narrow to permit penetration by other bees and they are common visitors to flowering plants in the tropics (Heard, 1999).They prefer to live in perennial colonies and have a different mechanism of caste determination and queen rearing when compared to other social bees (Engels and Imperatriz-Fonseca, 1990and Leonhardt *et al*., 2007) and some species have biting and spitting as their defense mechanism attack that causes irritation (Rahman *et al*., 2015). Sticky secretions act effectively against arthropod

predators as a mechanical barrier. They trap the predator by gluing their mouthparts and locomotory organs or apply it near the entrance tube to immobilize their enemies (Pasteels *et al*., 1983 and Roubik, 2006). Stingless bees are polylectic (collect pollen from various floral species), they are a manageable pollinator as they have ability to adopt extreme conditions and various nesting habits (George, 1934 and Solomon Raju *et al*., 1999). *Tetragonula iridipennis* Smith is the most abundant stingless bee in India. This bee forages in diverse flora with a high degree of floral fidelity (Layek and Karmakar, 2018). The objective of this study is to explore the behavioural habits of stingless bee in their habitat, due to lack of information on behavioural aspectsas they are constructed the nest in enclosed surfaces like cracks and crevices, which will be helpful in domestication and proliferation of colonies.

MATERIALS AND METHODS

This study was mainly carried out in Frisch Bee Garden of the Department of Entomology, Faculty of Agriculture, Annamalai University, Chidambaram, Cuddalore, Tamil Nadu, India that is located at $11^0$23'$53^0$49" N and $79^0$41'$43^0$28" E.

Behavioural studies were carried out in domesticated colonies which are recovered from different localities in Tamil Nadu province *viz*., Salem (Mettur), Dharmapuri (Pennagaram and Morappur), and Cuddalore (Vridhachalam), and one place in Puducherry (Vanoor) (Table 1). Soon after domestication and before the construction of the entrance tube, switching of nest entrance behaviour in the hive was visually observed during the morning (06.00 to 10.00 hours) and evening (18.00 to 23.00) within the first 3 days of domestication.

Other behaviours, such as brood pot construction, number of bees involved in mass provisioning per brood, queen oviposition, waste management, and foraging were visually observed twice a week through a glass top hive, and time taken for mass provisioning and sealing per brood pot and unloading of resin & pollen were recorded using a stopwatch.

Table 1. Collection sites of feral stingless bee colonies for behavioural studies.

S.No	Location	Collection site	Geographical position	No.of colonies domesticated
1.	Mettur (Salem)	Kunjandiyur	$11^0$48'14"N 77^0 51'28"E	5
2.	Pennagaram (Dharmapuri)	Anumandhapuram	12^0 19'04"N 78^0 06'48"E	7
3.	Morappur (Dharmapuri)	Dhodamapatti	12^0 03'22"N 78^0 29'56"E	13
4.	Vridhachalam (Cuddalore)	Aanandhakudi	11^0 27'44"N 79^0 19'57"E	15
5.	Vanoor (Pondicherry)	Aurobindo Ashram	12^0 00'42"N 79^0 44'30"E	2

RESULTS

Switching off the entrance

To avoid nocturnal pests or predators, workers closed the entrance during the night and opened it in the morning. Workers closed the entrance between 20.00 and 23.00 hrs and opened it between 06.00 and 09.00 hrs. Workers deposited a small quantity of cerumen near the entrance and liquified it to close the entrance by plastering on the entrance hole (Fig. 1).

Fig 1. Switching of entrance (soft rim of cerumen near the entrance for closing).

Nest defence

The protective building behaviour such as false nest (entrance tube) and vestibule chamber (empty storage pots) construction was visually observed in the domesticated colony (Fig. 2). Enemies like paper wasp and weevil (*Alcidodes* sp.) got stuck in the spitting of resin and were mummified by the workers using the cerumen within the nest (Fig. 3). Deposition of materials such as mud particles and grease on the entrance tube of some hives to avoid predators were also observed visually.

Fig 2. False nest (entrance tube) for nest defence

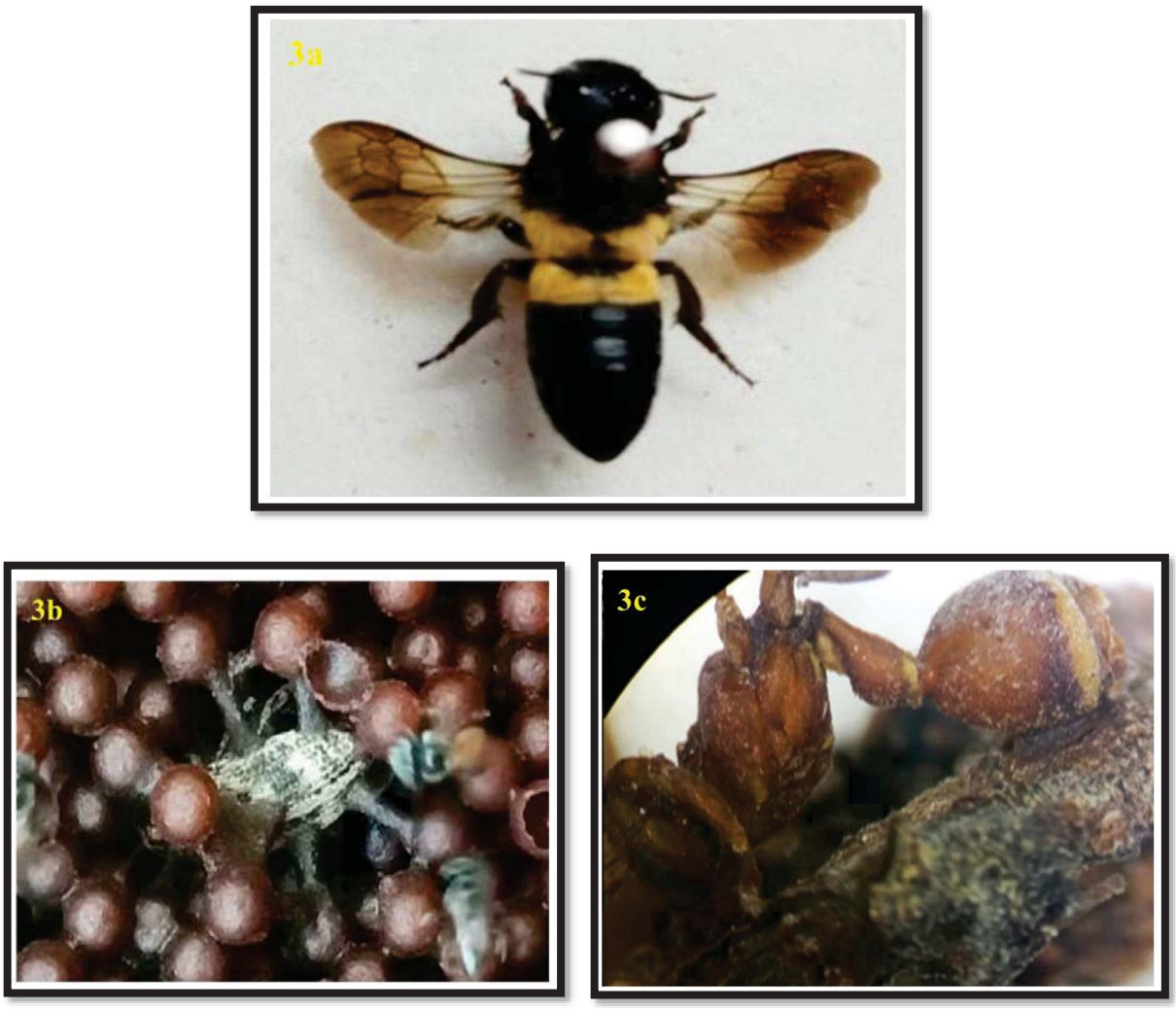

Fig 3. Pests of *Tetragonula* colony. (a) Resin bee (*Megachile* sp.), (b) Mummification of Weevil (*Alcidodes* sp.), (c) Mummification of Paper wasp

Mass provisioning and egg laying

Queen bee inspected the constructed pots and fluttered her wings and tapped the worker near the constructed brood pot with her antennae, which stimulated the workers for provisioning. For mass provisioning a single brood pot, 4-6 workers regurgitated the larval food to fill three fourth of the pot (Fig. 4). Queen laid a single egg in each pot on the provisioned brood food. After egg laying, a single worker sealed the brood pot within 4-55 s. The entire time taken from mass provisioning, egg laying and sealing the brood pot ranged from 1.0 to 3.25 min.

Fig 4. Provisioning and Ovipositioning (POP) process. (a) Mass provisioning of worker and queen inspection, (b) Egg laying by queen in provisioned brood pot

Waste management

Worker bees were involved in cleaning the waste or debris material from the hive. Bees carried out the waste ball and flew to drop the waste ball outside the nest entrance at least 0.61 to 0.91 meters away. Spherical shaped debris ball with projected structure aided in easy holding with the mandible (Fig. 5). In some cases, instead of making debris balls, workers removed the (emerged out brood pot) cocoon as such from the hive through the entrance.

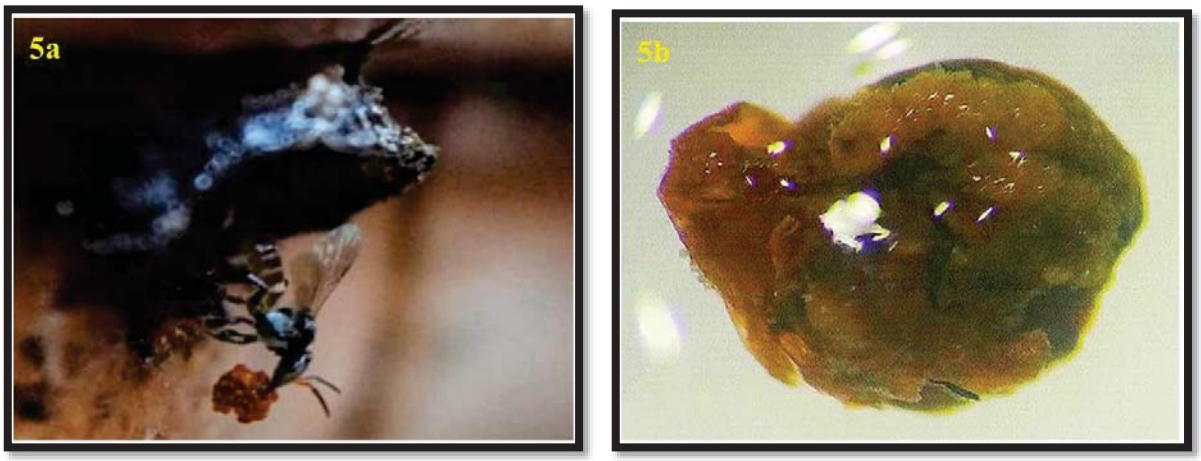

Fig 5. Waste management by *Tetragonula* workers. (a) Waste material carried by workers, (b) Microscopic view of debris ball

Foraging behaviour

Foraging by bees was at its peak at high sunshine hours than in the early morning and late evening. Workers resorted to messing and soil manner of foraging in *Tecoma* flowers, where they robbed the floral rewards (nectar, pollen and resin) by damaging the corolla tube from outside without entering through the open central tube (Fig. 6). Foragers collected extrafloral nectar (EFN) from plant parts rather than visiting flowers. *Tetragonula* beeshave been observed to forage the milky latex from the sadhurakalli tree (*Euphorbia antiquorum*). The main source of forage by workers was pollen which enhanced the colony growth during the honey flow period and helped to manage the dearth period. Among the storage pots, the number of pollen pots was more than honey pots.

A single worker took 3.5 min to load the corbicula with grease (Fig. 7). Resin foragers unloaded the raw resin pellets by using their middle leg to push and by sticking the load onto the resin dump or hive substratum (Fig. 8). The aroma and flavour of honey not only depends on the plants which they foraged but also the plants from which the resin was foraged.

Fig 6. Robbing floral rewards by damaging corolla tube of *Tecoma stans.* (a) damaging corolla tube by biting, (b) Damaged corolla tube after floral larency

Fig 7. Foraging on grease (Petroleum product)

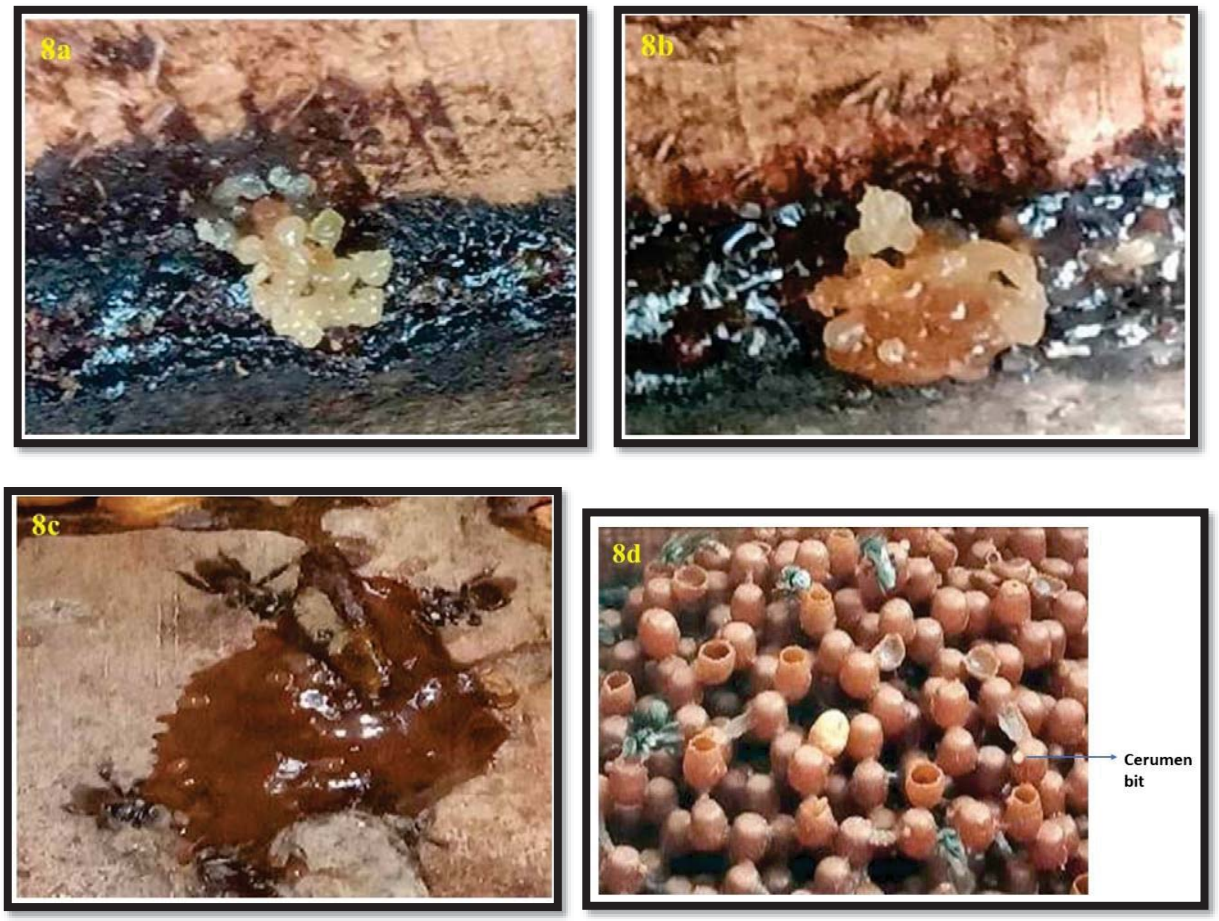

Fig 8. Storage of resin loads to resin dumps (cerumen) for construction**. (a)** Raw resin pellets, **(b)** Resin to cerumen conversion, **(c)** Cerumen or Resin dumps, **(d)** Cerumen bits on top corner of brood pots for construction

DISCUSSION

Switching off the entrance

Workers closed and opened the entrance at night and morning between 20.00-23.00 h and 06.00-09.00 h respectively. This finding is in accordance with Wille and Michener (1973) and Gruter *et al.* (2011). The entrance opening time varied with respect to the available flora in the surrounding. Process of entrance closing in *Tetragonula* sp. happened from 20.00 to 23.00 h by depositing cerumen bits, liquefying and plastering in the entrance hole. It was dissimilar to the results of Roubik (1992) who reported that in *Lestrimelitta* sp., the workers close the entrance within seconds by pulling the soft rim inwards.

Nest defence

In *Tetragonula* sp., construction of false nest and vestibule chamber to protect the colony from predators are in accordance with Camargo and Pedro (2003), who reported in *Partamona* sp. that enemies like paper wasp and weevil (*Alcidodes* sp.) were trapped by spitting of resin followed by mummification within the nest. This is again similar to the reports of Camargo and Pedro (2003) and Alves *et al.* (2018), who reported that sticky substances are effective against bees, wasps and ants.

Mass provisioning and ovipositioning

Before egg laying, queen fluttered her wings and tapped the worker near the constructed brood pot with her antennae, which stimulated the workers for provisioning. These observations were similar to the

findings of Sakagami and Zucchi (1974) and Sakagami (1982) who observed that in *Melipona quinquefasciata* and *Trigonisca duckei,* after queen inspected the brood pot, approximately 4-6 workers regurgitated the larval food to fill three fourth of brood pot. It is contradictory to the findings of Sakagami (1982) and Roopa *et al*. (2017) who reported that 2-19 and 6-8 worker bees were respectively involved in mass provisioning. After egg laying, a single worker sealed the brood pot within 4-55 s and the total time taken for mass provisioning, egg laying and sealing of the brood pot ranged between 1.0 and 3.25 min. These findings were dissimilar with the results of Roopa *et al.* (2017) and Kishirsagar and Chauhan (1977), who stated that the time taken for sealing the brood pot after egg laying was two minutes and sixteen seconds and two minutes and forty seconds respectively.These dissimilarities might be due to availability of cerumen in the nest component and also by the worker efficiency in sealing the brood pot.

Waste management

Bees carrying the waste ball and flying to drop the waste ball outside the nest entrance at least 0.61 to 0.91 meters away was contradictory to the findings of Darchen (1969) and Sakagami and Zucchi (1974) who stated that *Melipona* sp*.* fly to drop the waste ball at a range of 50m from the entrance whereas, *Leurotrigona muelleri* dropped it near the edge of the entrance.These contradictions might be due to the differentiation from genus and species level and hygienic behaviour of stingless bee colonies. In a few cases, instead of making debris balls, workers removed the cocoon from the hive through the entrance. This might be due to rainy days or other unknown causes.

Foraging behaviour

Peak foraging during high sunshine hours than in the early morning and the late evening was in accordance with the findings of Danaradi *et al.* (2011) who reported that the foraging activities (outgoing bees and incoming bees with and without pollen) were at their peak from 10.00 to 12.00 during all the seasons.

Workers of *Tetragonula* sp*.* robbed the floral rewards (nectar, pollen and resin) by damaging the base of the corolla tube in *Tecoma* before flower opening to reduce the competition between other pollinators. This finding is in accordance with Noll *et al.* (1996) that *Trigona* aids in robbing pollen or nectar by damaging the floral resources without pollinating the plant. Foragers visited the nodal region of plants such as castor, papaya and cucurbits to collect EFN rather than visiting flowers. This is in accordance with the findings of Noll *et al.* (1996) that *Trigona hypogea* may not visit the flowers for nectar but collect sugar-rich liquids from EFN. The behaviour that a single worker took 3.5 min to load the corbicula with grease and forage the milky latex from the sadhurakalli tree (*Euphorbia antiquorum*) was similar to the findings of Absy and Kerr (1977), Roubik (1992) and Pereira and Tannus-Nelo (2009) who reported that *Melipona, Trigona* and *Scaptotrigona* have been observed to collect milky latex.

Conclusion

The general behaviour of stingless bees, *Tetragonula "iridipennis"* sp*.* group such as switching off the entrance to avoid natural enemies, forming a false net and vestibule chamber for nest defense, mass provisioning for the brood, waste management by debris ball, foraging activity peak at high sunshine hours, collection of EFNs, high amount of pollen in storage pots when compared to honey were unique and different when compared to other honey bees of Apidae family. These above said behaviours help us to be successful in domesticating the stingless bees for the purpose of either pollinating the agricultural and horticultural crops with tiny flowers such as coriander, carrot, etc., or use of their honey and cerumen for curing human ailments. The other behaviours like queen pot construction, number of worker bees involved in filling the honey and pollen pots and variety of substrates that are foraged by bees are yet to be explored in future.

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Author contribution: BSV: performed the collection and domestication of feral colonies and observation on behavioural studies; drafting the manuscript, **SM**: as the advisor for the research work and for drafting the manuscript, **BA**: performed the manuscript drafting. Hence, the authors equally contributed towards the experiments. The authors read and approved the final manuscript.

NOT / NOTE

Conflict of interest: The authors have no conflict of interest to declare.

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Ethical issues: Not applicable

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