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A sustainable technique for colony multiplication by eduction of wild nests of the stingless bee *Tetragonula iridipennis* Smith

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Abstract

Colony multiplication of stingless bees, *Tetragonula iridipennis*, largely relies on the eduction of wild colonies from their natural nesting sites in India. During the hiving of wild colonies, colonies were destroyed with the loss of robust wild foragers and built-in storage reserves over the years. The present study was conducted to devise a technique to sustainably multiply the colonies of stingless bees from the wild colony and the colony establishment and development during the eduction process. The annexure hives provided for eduction were accepted in a shorter time (3.25 ± 1.18 days), with the construction of storage pots observed at 7.75 ± 1.59 days after hive acceptance by the bees. The movement of foragers between the wild colony and the annexure hives was noticed for 13.80 ± 4.20 days. The foragers settled in the annexure hives and started foraging after 18.20 ± 2.49 days. The advancing fronts were observed at 26.67 ± 2.58 days after the addition of the laying queen in the established annexure hives. There was a significant increase in the number of inhive workers after the queen seeding in the annexure hives. This technique is the easiest and most sustainable non-destructive way of multiplication of stingless bee colonies without loss in viability of the perennial wild colony.

Introduction

Stingless bees, *Tetragonula iridipennis* (Apidae: Hymenoptera), are eusocial corbiculate bees and are reported to be the essential pollinators of crops like coconut (Gadhiya & Pastagia, 2015), sunflower (Hemanth Kumar et al., 2020), gourds (Kishan et al., 2017), greenhouse cultivated sweet pepper (Cruz et al., 2005). They construct perennial colonies (Slaa et al., 2006) in the cavities of old walls of abandoned buildings, tree cavities, termite mounds, and concealed places (Roubik, 2006; Danaraddi, 2007). Identification of wild colonies is vital to conserving the natural nesting sites of *T. iridipennis* from destruction. Wild colonies of bees will contain inherent desirable qualities, viz., efficient foragers with an ability to forage at a relatively longer distance, unlike the domesticated colonies of bees (Oliver, 2014). Wild colonies of bees were also reported to be a reservoir of genetic diversity (Oleksa et al., 2013), and protection of their natural nesting sites is of paramount importance in the current era of bee decline globally (Jaffé et al., 2010). The domesticated colonies of stingless bees have the advantage of a year-round sustained source of rich foraging plants to ensure the pollen/nectar supply for the foragers because the food sources for the domesticated colonies depend on the beekeeper's food management. At the same time, the wild colonies thrive in human habitats and urban settlements with limited resource availability for foraging and maintenance of their colonies.

Stingless bees live in perennial colonies with much lower absconding/swarming behavior, unlike honeybees (Quezada-Euán, 2018). In addition, colony availability for beekeepers is very seasonal, and the development of stingless bee nurseries is still in the initial stage in India. Commercial beekeepers highly rely upon wild colonies of stingless bees to increase the number of colonies in addition to standard

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methods of colony division (Oliveira et al. 2013). The common practice of hiving a wild colony of stingless bees involves the complete destruction of the hive structure and transferring the hive components, viz., brood cells, storage pots, and foragers, inside an artificial box hive. This might result in the complete loss of perennial wild colonies constructed by the bees over the years, and many wild colonies get lost yearly (Heard, 1988). Vijayakumar et al. (2013) reported the hiving of a wild colony of *T. iridipennis* using the eduction method wherein an annexure hive was connected to the hive entrance of the wild colony and studied the colony established in the annexure hive. Systematic published reports on sustainable hiving of wild colonies of stingless bees for multiplication and colony development in India are very scarce. Moreover, there is a vital need to educate the stingless beekeepers for sustainable harvest/division of wild colonies rather than the complete dismantling of perennial colonies during the process of hiving. The current study investigated the sustainable hiving of a colony in box hives from the wild colonies of stingless bees without damaging the natural colony and studied the colony establishment and development parameters.

Materials and Methods

Identification of wild colonies of stingless bees

The rural and urban areas (household settlements, agricultural fields) were regularly surveyed weekly during the year 2019-2020 for the presence of wild colonies of stingless bees. We confirmed The presence of colonies by the occurrence of a hollow/elliptical nest entrance with guard bees at the entrance. We recorded the number of colonies at each site, the shape of the nest entrance, the diameter of the nest entrance, the number of guard bees at the nest entrance, height of the nest from the ground level. The sighting of foragers entering any tunnel-shaped entrance coated with a blackish resin layer was also considered an indication of an active, live colony of stingless bees.

Eduction of wild colonies

The eduction of eight wild colonies was conducted during 2019-2021 only from the mud walls as the site was relatively safe from stray animals. After locating the wild colony of stingless bees, we gently removed the nest entrance of the colony using a fine needle. A transparent polyethylene tube of inner diameter (1.0 cm) was taken, and one end of the tube was gently inserted into the nest entrance for more straight forward observation (Fig 1). The tube fitted at the nest entrance was tightly fastened with tape to prevent its further movement. A rectangular wooden hive as an annexure hive (18x12x8 cm) was taken with a small hole made at the bottom portion in the side (1.0 cm) (Singh, 2016). A transparent polyethylene tube (1.0 cm dia.) was inserted into the hole in the rectangular hive to serve as an entry/exit for foragers. The hole on the hive's other side was applied with resin collected from the wild colonies. This hive fitted to the wild colony was retained at the study site for three months. Eight such colonies as replicates were maintained. The number of days of active movement of foragers between the wild colony and rectangular hive was recorded.

The moving foragers carrying any resource from the wild parent colony were carefully observed through the connecting polyethylene transparent tube. The rectangular box hives were inspected at weekly intervals to ensure the settlement of foragers inside the hive. The movement of the foragers into and out of the hive was also monitored. The formation of new brood cells (if any) in the annexure hives was observed and recorded. After three months, the established colony in the annexure hives was shifted to the meliponiary at ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India.

Colony establishment and development parameters

The number of days taken for acceptance of the hive was recorded. The active foragers' movement in and out of

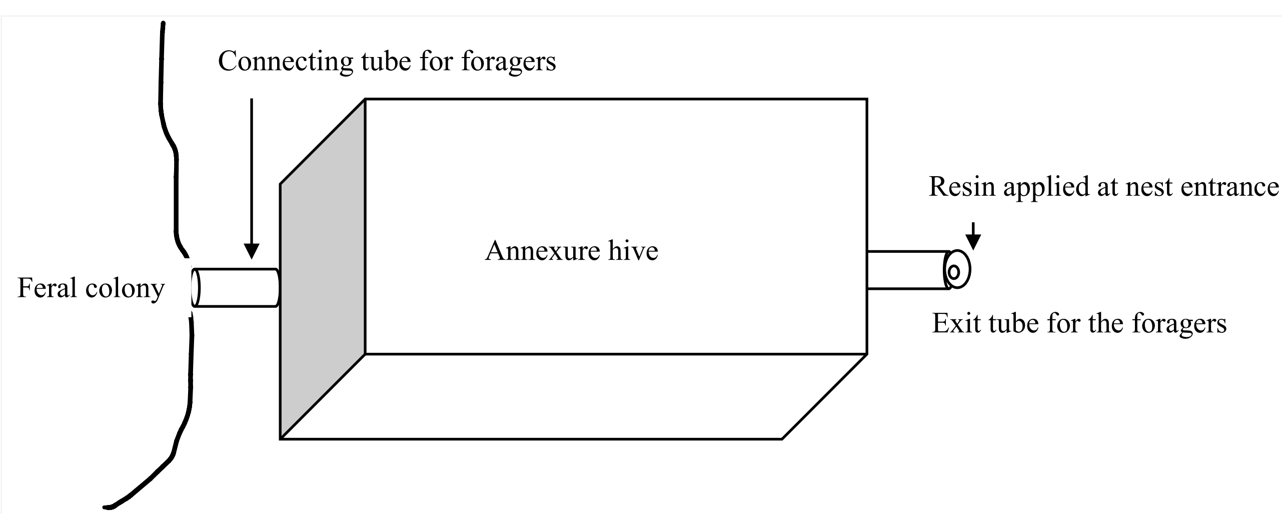

Fig 1. Eduction set up to hive the wild colony.

the hive was considered the presence of an active colony. The number of days wherein the active movement of foragers was seen through the polyethylene transparent connecting tube from the wild colony to the annexure hive was recorded. The established colony in the annexure hives was observed for the number of honey and pollen pots built per hive during the study period.

Addition of laying queen and queen cells in the established colonies

In most of the established annexure hives, only active forager movement with the construction of storage pots was observed. An experiment was conducted to seed the established hives with queen cells / introduce the laying queen to ensure the faster establishment of a colony. From a strong colony maintained in the meliponiary of the ICAR-National Bureau of Agricultural Insect Resources Yelahanka campus, Bengaluru, Karnataka, India, mature queen cells and a laying queen were carefully collected using a fine camel hair brush and kept inside a rearing dish (9x4 cm) fitted with a mesh and added to the annexure hives maintained at the eduction site. Two treatments were maintained, viz., adding a mature queen cell and laying queen with four replicates. Two hives per replicate were maintained. The acceptance of queen cells with brood by the in-hive workers of the established colony was recorded. The hives seeded with the queen cells, and brood were observed for the emergence of a queen and new bees. After the emergence of the new bees from the seeded colonies, the colonies were shifted to the meliponiary of ICAR-National Bureau of Agricultural Insect Resources Yelahanka campus, Bengaluru, Karnataka, India, during the late dusk hours of the day by closing the hive entrance with a thin sheet of paper. The number of days taken to form advancing fronts in hives added with mature queen cells and laying queen was recorded. The number of in-hive worker bees after adding queen cells and laying queen in the hives was recorded and the correlation was worked out. The hives were maintained in the meliponiary and observed for the hive traffic of foragers daily.

Survival of wild colonies after eduction

The wild colonies were monitored regularly to investigate the effect of education on the survival of the original wild colony. Since the wild colonies are perennial in nature, observations of broods and in-hive components to determine the colony's viability might need a destructive sampling of the colony which may disturb the eduction setup. Hence, the traffic of foragers into the hive and the presence of guard bees at the wild colony nest entrance were taken as factors of indication of a viable colony in the original site of the wild colony. The movement of foragers per hour in the wild colonies and the number of guard bees at the entrance were recorded before the eduction. The movement of foragers per hour was recorded for ten days once in 3,6,9 and 12 months after placing the eduction set up. The number of guard bees at the nest entrance per day was observed for five consecutive days at 3, 6, 9, and 12 months after eduction.

Statistical analysis

The colony characters, viz., mean ± SD of the number of guard bees at the nest entrance, entrance diameter (mm), and colony height from the ground level (m), were calculated. Analysis of variance (GLM in SAS 9.3; SAS Institute, Cary, NC) was used to compare the time taken for hive acceptance, time taken for guard bee activity, initiation of forager's activity, time taken for construction of storage pots across different years of study and the time taken for the formation of advancing fronts and incoming foragers in the colonies added with queen cells and laying queen. Where a significant difference was detected, treatment means were separated using Tukey's HSD Test (0.5%).

Results

Location of wild colonies

The wild colonies of *T. iridipennis* were identified in the rural/semi-urban areas of Hessaraghatta region of Bengaluru (13° 8' 18.8088'' N, 77° 28' 40.4040'' E), Karnataka, India, during 2019-2020. Around 32 live colonies were observed in various places, viz., Plastic drainage pipe (Fig 2), mud wall (Fig 3), stone wall, and electric switch box (Fig 4). The hive entrance varied in shapes, viz., circular, roughly circular, and irregular in shape. The nest entrance was coated with black-colored resin, often sighted by the guard bees (Fig 5). The nest entrance diameter varied between 9.33 ± 2.31 mm to 11.00 ± 1.30 mm, respectively. The wild colonies were located at different heights viz., 1.57 ± 0.6 m from ground level in the plastic drainage pipe, 3.51 ± 0.41 m from ground level in the mud walls, 1.97 ± 0.84 m from ground level in the stone wall and 1.15 ± 0.36 m from the ground level in the electronic switch box. The mean number of guard bees at the nest entrance ranged from 2.83 ± 1.47 bees/colony to 4.85 ± 1.78 bees/colony (Table 1).

Fig 3. Colony of *T. iridipennis* in mud wall.

Fig 4. Colony of *T. iridipennis* in electric switch box.

Fig 5. Nest entrance with guard bees.

Establishment of a colony in the eduction site

Across the study period, there was a significant difference in the acceptance of annexure hives by the bees during the eduction (F value = 7.59; P value <0.0001) (Table 2). The bees accepted the hives at 3.50 ± 0.74, 3.25 ± 1.18, and 5.01 ± 1.24 days during 2019-2021 after the placement of eduction set up with the wild colony. Guard bees were observed at the nest entrance at 8.13 ± 0.83, 7.63 ± 1.06, and 8.50 ± 0.93 days after the acceptance of the annexure hive connected to the wild colony. There was an active movement of foragers between the wild colony and the Annexure hive after accepting the hive for a certain period. There was a significant difference in the number of days for active movement of foragers between the wild colony to the annexure hive through the connected polyethylene tube across the three years of study (F value = 9.38; P value <0.0001). The number of days of movement of foragers between the wild colony to the rectangular hive was 20.40 ± 1.67, 14.20 ± 2.86, and 13.80 ± 4.20 days during 2019, 2020, and 2021 respectively. The settlement of foragers inside the rectangular box hives was observed at 25.60 ± 3.51, 21.80 ± 7.19, and 18.20 ± 2.49 days from 2019-2021. After the cessation of forager movement from the wild colony to the hive, the foragers were observed to be repairing the hive entrance. During the movement of foragers from the wild colony to the hive, they were observed to carry pollen resources into the hive. The initiation of storage pot construction was observed at 7.75 ± 1.59 days, 9.50 ± 2.93 days, and 11.0 ± 1.91 days during 2019-2022 after the settlement of foragers in the hive. After the construction of storage pots, a few worker bees were

Table 1. Eduction of wild colonies of *Tetragonula iridipennis.*

Nest substrate	Number of colonies	Nest entrance height above ground (m)	Shape of nest entrance opening	Nest entrance diameter (mm)	No of guard bees at nest entrance
Plastic drainage pipe	7	1.57 ± 0.36	Circular	11.0 ± 1.30	4.85 ± 1.78
Mud wall	13	3.51 ± 0.41	Irregular	10.28 ± 1.38	4.72 ± 0.82
Stone wall	4	1.97 ± 0.84	Roughly circular	9.83 ± 1.47	4.00 ± 2.09
Switch box	8	1.15 ± 0.36	Irregular	9.33 ± 2.31	2.83 ± 1.47

Table 2. Colony establishment in the hives post eduction from wild colonies.

Queen seeding	Time span for formation of advancing fronts (days)	Number of incoming foragers/hours
Colonies added with mature queen cell	39.10 ± 6.51[a]	19.33 ± 3.20
Colonies added with laying queen	26.67 ± 2.58[b]	24.01 ± 7.45
F value	4.85	NS
P value	P < 0.0001	-

observed to fly out for resource collection. The sampling of hive-returning bees revealed nectar-filled in their crop. Based on the observations, it was clear that the foragers initially used pollen from the wild colony and collected nectar from outside flora to fill the constructed storage pots. There was no significant difference in the time taken for sighting the guard bees at the nest entrance of the established hives across the three years of study. There was a significant difference (F value = 24.48; P value <0.0001) with a steady decline observed in the number of honey pots per hive built across the study period. The number of honey pots per hive observed during 2019, 2020, and 2021 was 227.60 ± 63.21, 184.67 ± 13.61 and 97.33 ± 29.09. The number of pollen pots observed across the study period differed significantly (F value = 8.11; P value <0.0001). The number of pollen pots per hive was 126.33 ± 35.72, 85.67 ± 39.10, and 43.33 ± 24.54 during 2019, 2020, and 2021 (Fig 6).

The brood cell construction was noticed only in one established hive, wherein the queen bee was sighted after 73 days. The brood cell formation was observed at 90 days in the colony sighted with a queen.

Development of established colony

There was a significant difference in the number of days taken for the formation of advancing fronts in the hives due to the addition of mature queen cells and laying queen (F value = 4.85; P <0.0001) (Table 3). The advancing fronts were constructed at 39.10 ± 6.51 days and 26.67 ± 2.58 days in the hives added with a mature and laying queen cell, respectively. The number of incoming and outgoing foragers per hour in the hives added with a mature queen cell and laying queen were 19.33 ± 3.20 and 27.01 ± 7.46, respectively. There was a positive correlation ($y = 10.9x + 36.5$; $R^2 = 0.9759$) (Fig 7)

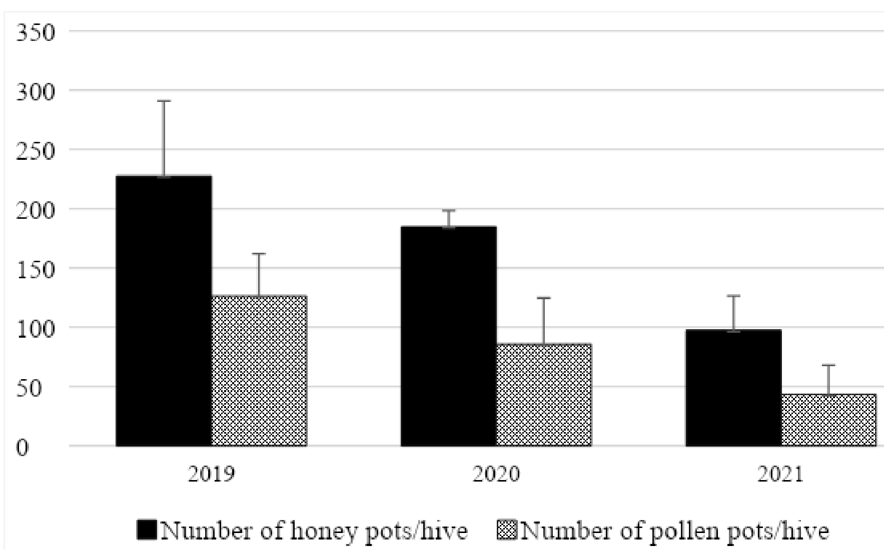

Fig 6. Number of storage pots per hive during the study period.

Table 3. Colony development in the hives.

Year of study	Time span for hive acceptance (days)	Time span for guard bee activity at nest entrance (days)	Time span for forager movement from feral colony to hive (days)	Time span for initiation construction of storage pots(days)	Time span for initiation of foraging activity after settling of foragers in hive
2019	3.50 ± 0.74[b]	8.13 ± 0.83	20.40 ± 1.67[a]	7.75 ± 1.59	25.60 ± 3.51
2020	3.25 ± 1.18[b]	7.63 ± 1.06	14.20 ± 2.86[b]	9.50 ± 2.92	21.80 ± 7.19
2021	5.01 ± 1.24[a]	8.50 ± 0.93	13.80 ± 4.20[b]	11.01 ± 1.90	18.20 ± 2.49
F value	7.59	NS	9.38	NS	NS
P value	P <0.0001	-	P <0.0001	-	-

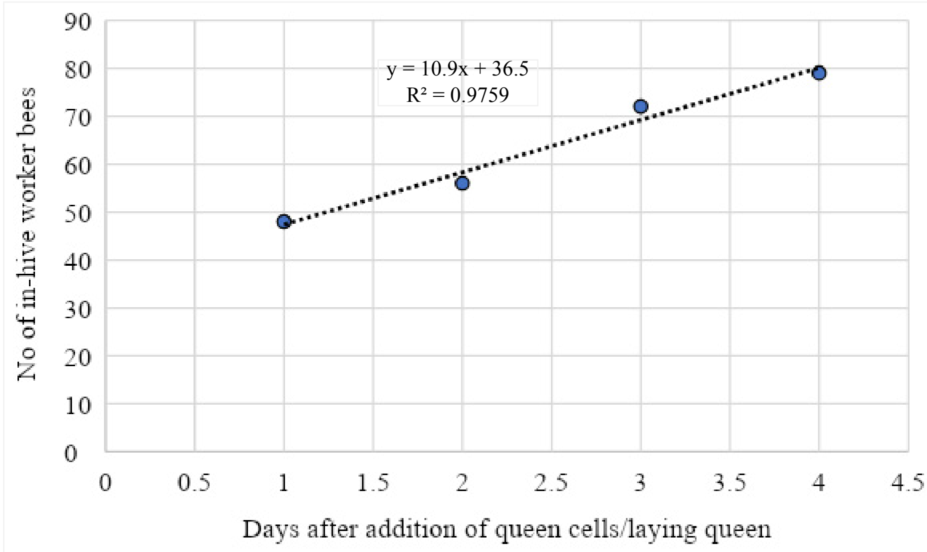

Fig 7. Relation between numbers of addition of queen cells/laying queen on the number of in-hive workers.

between the increase in the number of in-hive workers with the number of days after the addition of queen cells/laying queen.

Survival of wild colony after eduction

There was a significant difference in the movement of foragers per hour in the wild colonies at 3, 6, 9, and 12 months after eduction from the wild colony (F value = 7.59; P <0.0001) (Fig 8). The movement of foragers in the wild colony before eduction was 39.40 foragers per hour, which were on par with foragers' movement recorded at three months (37.20 foragers per hour), six months (34.20 foragers per hour), and nine months (28.80 foragers per hour) after eduction. There was a significant reduction in the forager movement at twelve months after eduction (16.40 foragers per hour). There was no significant reduction in the number of guard bees at the nest entrance before and even after eduction at 3,6,9 and 12 months. The number of guard bees at the nest entrance ranged between 1.41 to 2.23 bees per colony per day across observation periods.

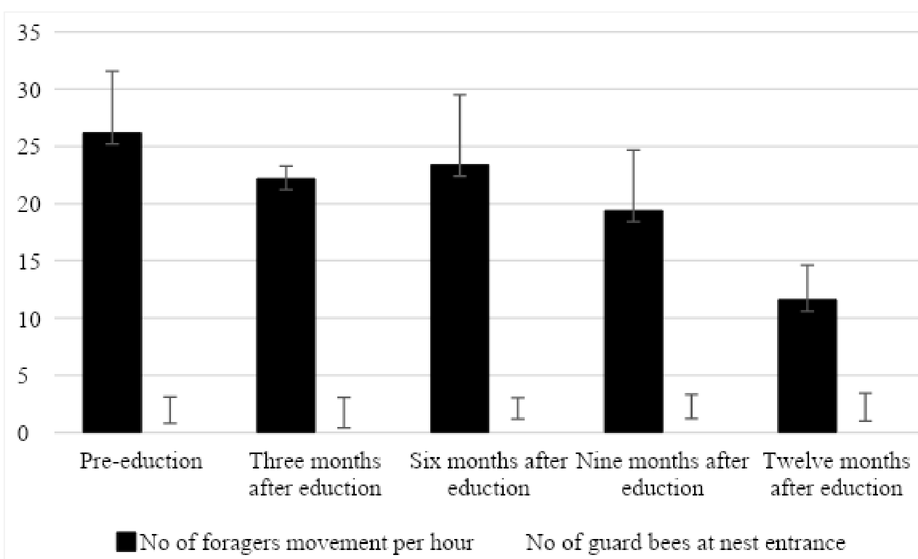

Fig 8. Foragers and guard bees' activity in wild colonies pre and post eduction.

Discussion

In the present study, the natural wild colonies of *T. iridipennis* were primarily located in semi-urban households. Pavithra et al., 2013 reported the natural nests of *T. iridipennis* in brick walls, rock crevices, pillars, metallic sheaths, and water pipes in Karnataka, India. Our findings confirmed the sustainable harvest of foragers from the wild colony with minimum damage to its natural nesting site. Though guard bees exhibited defense activity within a few days after hive acceptance, there was no activity of outgoing foragers for resource collection. Resource-carrying foragers were observed during the initial period of colony establishment inside the box hive. The active movement of foragers between the wild colony to the box hive was observed during the initial periods of hive acceptance. As the building of storage pots was observed within a few days after settling of foragers inside the box hive, we presume that the foragers might have utilized the initial building material from the parent wild colony. The active movement of foragers between the wild colony to the rectangular box hive ceased after 20 days. Such movement cessation might be due to the settlement of foragers inside the new hive with many storage pots. We noticed a steady decline in the number of storage pots per hive across the study period. The pollen stored in the cerumen pots serves as base food material for bee bread preparation for feeding the broods by the nurse bees. Since there was no brood cell formation for a long period, i.e., three months after colony establishment, the quantum of pollen pots per hive might have diminished over time. The presence of young broods greatly influencing the pollen foraging activity in bees was reported by many workers (Eckert et al., 1994; Dreller et al., 1999). The decline in the number of honey pots per hive might be due to hampering the task allocation for foragers toward nectar collection. The resource inflow (pollen and nectar) into colonies and unutilized forage reserves without brood cell construction might be factors for the decline in the number of storage pots.

The construction of brood cells took longer (≈ 3 months) in only one established hive. The queen bee was observed in a single colony, and acquiring/movement of such a queen into the hived colony is a definite 'chance factor.' Hence, seeding the laying queen or adding new queen cells in the established colony is a viable method to hasten brood production and colony multiplication.

There was an increase in the number of in-hive workers after the emergence of the new brood. The increase in in-hive workers is important for colony growth and development. The in-hive workers attend to several duties of colony maintenance, like the construction of the nest entrance (Nogueira-Neto, 1948) and waste dumping of dead foragers (if any) inside the hive (Medina et al., 2014). A more significant number of in-hive workers is also a sign of expanding the colony.

There was a reduction in the foragers' movement in the wild colony twelve months after eduction. We never observed the complete loss of the colony during this period. Since eduction was conducted twice at six months with the settling of foragers inside the rectangular box hives, the number of foragers might have faced a dip in the parental colony. Discontinuing the eduction from such wild sites for a few months might help the parental colony revive the foragers' activity and development as storage pots are intact inside the colony.

The destruction of wild colonies might also expose the colony components to invaders like pests and predators. Rather than for colony, the wild colonies of stingless bees were also destroyed to harvest the honey (Eardley, 2004), which also resulted in the loss of perennial colonies in nature. The invasion by pests and predators might discourage the hive occupancy of homeless, deserted foragers of the wild colony during the process of destruction (Jaffe et al., 2015). Locating stingless bees' perennial colonies will also help conserve their natural nesting sites in urban habitats (Mogho et al., 2018). The availability of colonies of stingless bees is a seasonal factor, unlike honeybee colonies under Indian conditions. The results of the current study will provide new insights into the sustainable multiplication of colonies with the slightest disturbance to wild colonies. The results of this study could be used to train and educate the beekeepers for sustainable eduction of the wild colony coupled with continuous multiplication of stingless bee colonies.

Authors' Contributions

AU: conceptualization, methodology, investigation, writing-original draft, writing-review & editing.
VHS: data analysis.
CKGR: investigation.
TMS: writing: review & editing.
ANS: investigation.
KS: writing: review & editing.
SNS: writing: review & editing.

Competing Interests

The authors declare no financial or non-financial interests directly or indirectly related to the work submitted for publication.

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