

**BIO-ECOLOGY AND MANAGEMENT OF  
STINGLESS BEES (Apidae : Meliponinae)**

**By**

**RAAKHEE MOHAN**

**THESIS**

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**DEPARTMENT OF AGRICULTURAL ENTOMOLOGY  
COLLEGE OF AGRICULTURE  
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THIRUVANANTHAPURAM**

**2000**

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I hereby declare that this thesis entitled "Bio-ecology and management of stingless bees (Apidae : Meliponinae)" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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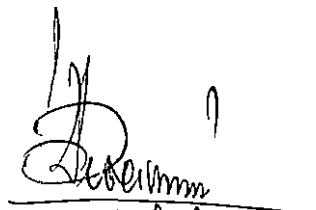
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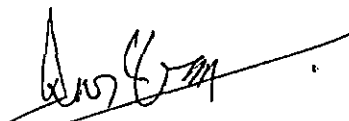
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## CONTENTS

|                                    | <b>Page No.</b> |
|------------------------------------|-----------------|
| <b>INTRODUCTION</b> .....          | 1 - 3           |
| <b>REVIEW OF LITERATURE</b> .....  | 4 - 19          |
| <b>MATERIALS AND METHODS</b> ..... | 20 - 30         |
| <b>RESULTS</b> .....               | 31 - 52         |
| <b>DISCUSSION</b> .....            | 53 - 64         |
| <b>SUMMARY</b> .....               | 65 - 68         |
| <b>REFERENCES</b> .....            | i - xi          |
| <b>ABSTRACT</b> .....              |                 |

## LIST OF TABLES

| Sl. No. | Title  | Page No. |
|---------|--|----------|
| 1       | Average dimensions of brood cells of <i>T. iridipennis</i>                                     | 32       |
| 2       | Average dimensions of pollen pots  | 35       |
| 3       | Average dimensions of honey pots   | 35       |
| 4       | The mean brood development of <i>Trigona iridipennis</i> in different hives (cm <sup>3</sup> ) | 37       |
| 5       | Honey yield of <i>Trigona iridipennis</i> maintained in different hives                        | 37       |
| 6       | Duration of developmental stages of workers of <i>Trigona iridipennis</i>                      | 43       |
| 7       | Morphometric characters of the queen of <i>Trigona iridipennis</i>                             | 45       |
| 8       | Morphometric characters of the worker of <i>Trigona iridipennis</i>                            | 46       |
| 9       | Diurnal variations in the foraging activity (nectar and pollen) of <i>Trigona iridipennis</i>  | 48       |
| 10      | Flora visited by <i>Trigona iridipennis</i>  | 51       |



## LIST OF FIGURES

| Sl. No. | Title   | Page No. |
|---------|---|----------|
| 1       | Mean brood development of stingless bees in different hives                       | 38       |
| 2       | Foraging activity of <i>Trigona iridipennis</i> during different hours of the day | 49       |

## LIST OF PLATES

| Sl. No. | Title  | Between pages |
|---------|--|---------------|
| 1       | Wooden hive for keeping <i>T. iridipennis</i>                | 21 - 22       |
| 2       | Bamboo hive for keeping <i>T. iridipennis</i>                | 21 - 22       |
| 3.      | Earthen pot for keeping <i>T. iridipennis</i>                | 21 - 22       |
| 4.      | Feral colony of <i>T. iridipennis</i> in rock wall           | 21 - 22       |
| 5.      | Hive entrance guarded by bees                                | 32 - 33       |
| 6.      | Nest cavity coated with resin                                | 32 - 33       |
| 7.      | Queen cells and worker brood cells                           | 33 - 34       |
| 8.      | Pollen pots  | 33 - 34       |
| 9.      | Honey pots   | 33 - 34       |
| 10.     | Pillars of wax acting as a foundation for brood cells        | 40 - 41       |
| 11 a.   | Brood cells <i>T. iridipennis</i> constructed in clusters    | 40 - 41       |
| 11 b.   | Wax connectives between brood cells of <i>T. iridipennis</i> | 40 - 41       |
| 12.     | Brood cells at various stages of construction                | 40 - 41       |
| 13.     | Waste dumps found in a colony of <i>T. iridipennis</i>       | 43 - 44       |
| 14.     | Eggs of <i>T. iridipennis</i>                                | 43 - 44       |
| 15.     | Brood cells ready for the emergence of adult bees            | 46 - 47       |
| 16.     | Stingless bee queen over the brood cells                     | 46 - 47       |
| 17.     | Antenna of <i>T. iridipennis</i> worker                      | 49 - 50       |
| 18.     | Mandible of <i>T. iridipennis</i> worker                     | 49 - 50       |
| 19.     | Fore wing of <i>T. iridipennis</i> worker                    | 49 - 50       |
| 20.     | Hind wing of <i>T. iridipennis</i> worker                    | 49 - 50       |
| 21.     | Fore leg of <i>T. iridipennis</i> worker                     | 49 - 50       |

| Sl. No. | Title   | Between pages |
|---------|---|---------------|
| 22.     | Middle leg of <i>T. iridipennis</i> worker                                      | 49 - 50       |
| 23.     | Hind leg of <i>T. iridipennis</i> worker  | 49 - 50       |
| 24.     | Hind leg and metatarsus of <i>T. iridipennis</i> showing <i>Pencillium</i>      | 49 - 50       |
| 25.     | Bee flora - Cannon ball tree - <i>Cauropita guinensis</i>                       | 51 - 52       |
| 26.     | Bee flora - Coral creeper - <i>Antigonon leptopus</i>                           | 51 - 52       |
| 27.     | Bee flora - Drum stick - <i>Muringa oleifera</i>                                | 51 - 52       |
| 28.     | Bee flora - Birds cherry - <i>Mundingia calabura</i>                            | 51 - 52       |
| 29.     | Bee flora - Sponge gourd - <i>Luffa cylindrica</i>                              | 51 - 52       |
| 30.     | Bee flora - Bajra - <i>Pennisetum typhoides</i>                                 | 51 - 52       |
| 31.     | Bee flora - Cinnamon - <i>Cinnamomum zeylanicum</i>                             | 51 - 52       |
| 32.     | Bee flora - Ixora - <i>Ixora</i> sp.  | 51 - 52       |
| 33.     | Bee flora - Sunflower - <i>Helianthus annus</i>                                 | 51 - 52       |
| 34.     | Bee flora - Balsam - <i>Impatiens balsaminae</i>                                | 51 - 52       |
| 35.     | Bee flora - Hamelia - <i>Hamelia patens</i>                                     | 51 - 52       |
| 36.     | Bee flora - Marigold - <i>Tagetes erecta</i>                                    | 51 - 52       |
| 37.     | Spider - Predator on <i>T. iridipennis</i> (unidentified) waiting over the hive | 51 - 52       |
| 38.     | A spider predating a stingless bee in the field                                 | 51 - 52       |

# **INTRODUCTION**

## 1. INTRODUCTION

Apiculture is an ideal agro-based enterprise providing supplementary income to the people in rural areas. Now a days this enterprise is developing as a major industry and many entrepreneurs are taking it on commercial basis. Besides providing honey, beeswax and royal jelly, honeybees enrich agro-horticultural crops by its unique pollination services. In India over 80 per cent of crops *viz.*, oilseeds, pulses, vegetables, fruits and commercial crops are benefited by bees.

Beekeeping was practiced in erstwhile Travancore in a primitive way which got revolutionised by the introduction of movable frame hives in 1924. It made rapid strides in Kerala during the second half of the twentieth century. Detection of rubber (*Hevea brasiliensis* Muell. Arg.) as a rich source of extrafloral nectar during seventies gave a big boost to the beekeeping industry in the state. Migratory beekeeping with Indian bees, *Apis cerana indica* Fab. forms the basis of the apiculture industry of the state. Kerala was contributing 70 per cent of the annual production of honey in India till 1991 (Jacob *et al.*, 1992). But the beekeeping industry in the state got a set back during 1991-1992 due to the severe outbreak of Thai Sacbrood Virus (TSBV) disease. European bee, *Apis mellifera*, which is resistant to (TSBV) was introduced into the state to rebuild the declining beekeeping industry.

More than 90 per cent of the available literature on beekeeping in the world pertains to the two domesticated species *viz.*, *A. mellifera* and *A. cerana* (Michener, 1964 and Crane, 1992). *A. dorsata* and *A. florea* are wild in nature and their domestication have been met with only partial success.

*A. dorsata* is ferocious in nature while *A. florea* is highly migratory. India is blessed with four species of true honeybees viz, Rock bee, *Apis dorsata* Fab., Asian honeybee *Apis cerana indica* Fab., European bee, *Apis mellifera* Linn., Little bee *Apis florea* Fab. and the stingless bee *Trigona iridipennis* Smith.

The stingless bees belonging to the subfamily Meliponinae, with more than 500 species exhibit larger biodiversity than honeybees (Crane, 1992). They have the potential to be incorporated as one of the components in the apiculture industry of Kerala. Along with true honeybees they form an excellent example of social evolution in bees. They are highly eusocial sharing several specialized features; large perennial colonies, extreme caste differentiation, inability of queens to form solitary nests; elaborated nest architecture, communication systems, storage of large quantities of food and highly effective thermoregulation. However because of their tropically confined distribution, the biology of stingless bees has been far less explored than that of other honey bees (Sakagami, 1982).

Stingless bee products are used worldwide as food and medicine. Despite the small amount of honey produced per hive, the honey is in great demand and fetches comparatively higher prices in the market due to its medicinal value. The present production is unable to meet the demand. Moreover stingless bees are ecologically important because of their role in pollination of tropical flora. Their abundance combined with their biodiversity make stingless bee play a key role in pollination of natural tropical plants and crops. Over 130 species of stingless bees world over have been identified as potential pollinators of crops and can be managed for this purpose

(Roubik,1995). Meliponiculture (Stingless beekeeping) is suitable for women because it does not involve heavy physical work and provides an additional means of income to the rural women. Stingless bees are common in Kerala. Stingless bee keeping can modestly contribute to the economy of peasant households, as a single component or integrated in beekeeping with other honeybees. However, lack of systematic studies about the species and its management practices is a limiting factor in popularising meliponiculture. The present studies were hence taken up with the following objectives.

1. Identification of species present in Kerala
2. Study of biology, behaviour and management of the identified species
3. Documentation of stingless bee flora in Kerala
4. Composition of stingless bee honey

# **REVIEW OF LITERATURE**



## 2. REVIEW OF LITERATURE

### 2.1 CLASSIFICATION AND DISTRIBUTION

The taxonomic status of stingless bee as explained by Winston and Michener (1977) and Willie (1979) is summarised below.

|                |             |
|----------------|-------------|
| Super family : | Apoidea     |
| Family :       | Apidae      |
| Subfamily :    | Meliponinae |
| Tribes :       | Meliponini  |
|                | Trigonini   |

Over 500 species of stingless bees are known and the majority are in South America. Relatively few species occur in Africa, Asia and Australia (Crane, 1992). The tribe *Meliponini* is restricted to neotropics. All Asian and African stingless bee species belong to the tribe *Trigonini*. Genera in this tribe include *Trigona*, *Plebeia*, *Tetragona* and *Nannotrigona* (Michener, 1974). *Trigona* species occur in every continent except Europe where there is no tropical region whereas the *Melipona* does not occur outside the Americas (Crane, 1992).

### 2.2 BIOLOGY AND BEHAVIOUR

#### 2.2.1 Nesting habit

Nesting habit of stingless bees were studied by Willie and Michener (1973) and illustrated by Michener (1974). Nesting sites were closed in stingless bees. Most stingless bees built nests in tree hollows and some species built nests in abandoned or still occupied nests of termites, above ground or

subterranean (Sakagami, 1982). Still others used man made cavities such as those in masonry walls (Bruijin, 1996). Most parts of the nest were built with a mixture of wax and propolis known as cerumen, although some parts were of wax only. The nest cavity was sealed from the outside world except for its flight entrance. The flight entrance was lined with propolis or cerumen and some species built a long protruding entrance tube which may be closed each night (Crane, 1992).

### 2.2.2 Brood cells

The arrangement of brood cells differed among species or higher taxa. Many species built multilayered combs, each expanding concentrically and horizontally. Some species were cluster builders or build imperfect combs (Sakagami, 1982; Crane, 1992).

According to Friese (1914), the bees removed wax from the cells as soon as cocoon was spun by the larvae occupying the cell. Traces of wax remained on cocoons which were yellowish white. The uneconomical usage of cells was compensated by the efficient application of removed cerumen for various purposes (Darchan, 1972) as quoted by Sakagami (1982). Bruijin (1993) found that during the development of a bee, the cerumen from which the brood cells was built was gradually removed from the top of the cell by other workers and they recycled most of the cerumen and used it again to build another brood cell, storage pot or other nest structures.

Kshirsagar and Chauhan (1977) reported that in the colony of *Trigona iridipennis* brood and food storage pots were connected by columns of wax.

The height and diameter of brood cells were reported to be 4.086 mm and 3.12 mm respectively. Newly constructed brood cells were brownish and became straw coloured with the advancement of age. According to Sakagami (1982) and Bruijin (1993) brood cells were used only once in stingless bees. George (1934) opined that the queen cells of *Trigona iridipennis* were larger than that of worker cells.

#### 2.4.4 Storage pots

The food chamber of both *Melipona* and *Trigona* lay outside the brood nest and consisted of pollen and honey pots which were many times larger than the brood cells (Lindauer, 1957). Kshirsagar and Chauhan (1977) reported that in *Trigona iridipennis*, the storage pots were seen very close to brood cells actually touching them or separated from brood cells. The height and diameter of the honey and pollen cells were recorded as 6.638 and 6.78 mm and 6.64 and 6.64 mm respectively. According to Crane (1992), in stingless bees, stored honey and pollen were kept in irregularly built honey pots of, soft cerumen, either separate or intermixed.

#### 2.4.5 Castes

Sakagami (1982) reported that in stingless bee queens, hind tibiae were not corbiculated, abdominal terga were devoid of wax glands and the sting was functionless, but less degenerated. The gravid stingless bee queens were far more physogastric than honeybee queens, but the number of ovarioles per ovary was four as in the case of workers and never as numerous as honey bee queens.

Instead, each ovariole of the stingless bee queen was extremely lengthened and coiled within the abdomen. Reduced wing venation was also reported. Willie (1983) and Prentice (1991) pointed out that the presence of pencillium (a brush of recurved hairs on the outer anterior corner of hind tibia) was a character of *Meliponinae*. Crane (1992) reported that *Trigona* bees had a body length from 2 mm upwards.

#### 2.4.6 Provisioning and Oviposition

George (1934) reported that egg was forced into the food by the queen, with one end slightly protruding and in a perpendicular position. According to Lindauer (1957) and Crane (1992), there was no continuous feeding of the brood, as observed in *Apis*. However, soon after the egg was laid, the cell was filled with requisite food and closed straight away.

Sakagami (1982) observed that in stingless bees, about 2 hr was required to build one cell and 1 - 2 minutes for provisioning. About 10 minutes was required for provisioning, oviposition and cell closure.

According to Bentham *et al.* (1995), cell building and provisioning were the activities of a small group of workers in *Plebeia remota*. Provisioning and operculation of all cells occurred simultaneously and 4 - 9 workers were involved in provisioning. Operculation was done by a single worker that was not involved in previous stages. Drumond *et al.* (1996) studied the oviposition behaviour of *Plebeia emerina* and *Plebeia remota* and observed that brood cells typically formed a comb. Eggs were laid in batches, brood cells constructed synchronously and provisioned.

#### 2.4.7 Waste and resin dumps

Bruijin (1993) observed that the stingless bees void their excreta in the hive on the top of the waste heap, in contrast to *Apis* colonies in which workers defecate outside the nest. Friese (1914) recorded that *Trigona iridipennis* placed scattered droplets of sticky matter in the hive and fresh resinous matter at the mouth of the entrance tube. Further the bees engaged in removing the pupae from the cells that had been damaged, dropped these pupae outside the nest. He also observed that the workers removed small pellets of yellowish green substances which he interpreted as the excrement. Lindauer (1957) observed that in front of the colony entrance they laid irregular rows of pointed lumps of resin that helped to defend against ants.

#### 2.4.8 Defence

George (1934) reported that in *Trigona iridipennis* the poison glands were present even though the sting had become non-functional. According to Lindauer (1957) the stingless bees were by no means defenceless. The bees entangled themselves in the intruders' hair and buried their powerful mandibles in his flesh. Willie (1983) reported that stingless bees possessed efficient means of defence. But all the species were not aggressive. Some species used unpleasant odours as a means of defence. The bees *Trigona (Oxytrigona) tataira* and its relatives had enlarged mandibular glands capable of producing enough caustic fluid to cause blisters when they bite. Crane (1992) observed that defence mechanisms against an intruder included biting, ejecting a caustic fluid and irritating by crawling into eyes and ears.

#### 2.4.9 Life cycle

George (1934) assigned a period of 21 to 25 days for the completion of transition from egg to adult in the case of *Trigona iridipennis*. According to Salmah *et al.* (1996), the time from oviposition to adult emergence for workers was 46.5 days (egg 4.2 days, larva 10.4 days, pupa 31.8 days) in *Trigona itama*. The length of incubation was twice than that of *Apis mellifera*.

#### 2.4.10 Swarming and reproduction

Friese (1914) opined that *T. iridipennis* gave swarms in August or September. Schwarz (1948) suggested that among stingless bees who left the mother nest, there should be a new daughter queen rather than old mother as in honeybees. According to Nogueira-Neto (1954) swarming was preceded by the production of new queens who either left the mother colonies for finding new ones (swarming) or remained in the former succeeding the mother (supersedure). Crane (1992) suggested that, reproductive swarming took place when colonies were populous and drones were present. Before the issue of a swarm, workers in the parent colony reared new queens and selected a nest site for the swarm. They also took pollen mixed with honey in their honey sacs. In the parent colony workers treated the young virgin queen as a queen and the mother queen heading the colony tolerated them. An issuing swarm consisted of one of these virgin queens and many young workers. When the swarm arrived at the new nest a congregation of drones waited near by and the queen got mated and started laying eggs in brood cells.

#### 2.4.11 Flight range

Lindauer (1957) reported that in *Trigona iridipennis* communication ceased at 100 m and flight ceased at 120 m. Kerr (1959) and Willie (1983) investigated the flight ranges of stingless bees. According to Willie, small bees like those of genus *Plebeia* (3-4 mm) had a flight range of about 300 m. Medium sized bees as in subgenus *Triogona* (5 mm) had a flight range of about 800 m. Very large bees (13 - 15 mm) had a flight range of about 2000 m.

### 2.5 FLORA AND FORAGING BEHAVIOUR

Peckolt (1894) found that in Brazil, the stingless bees *T. jatay*, *T. bipunctata* and *T. mosquito* started foraging activity at 0700 h, 0700 h and between 0530 h and 0600 h respectively during good weather. Chandran and Suryanarayana (1970) observed honeybees (*Apis cerana indica*) and *Trigona* spp. collecting pollen from flowers of parasitic *Dendrophthoe falcata*. Gilbert (1973) noticed that foraging activity of *Trigona fluviventris* began before light, declined in intensity throughout the morning hours to a stable level maintained during mid day and increased to a second peak before dark. Goel and Kumar (1981) observed *Trigona iridipennis* in considerable abundance on sunflower (*Helianthus annuus* L.). The bee was reported as major pollinator of sunflower crop owing to their large numbers during the blooming phase. Most preferred timing for the visit of the bee to the crop was 10.00 h followed by 13.00 h. Of the bee species *T. iridipennis* was the most frequent visitor to the flower heads (Rao *et al.*, 1995; Sharma and Bichoo, 1996).

Anderson *et al.* (1982) observed that the most efficient pollinators in pollen transfer experiments in mango were wasps and native bees (*Trigona* sp.) Schneider (1982) observed that the pollen foraging *Trigona* sp. were found to slip into the stigmatic fluid effecting the pollination of *Nymphoea gigantea*. According to Hawkeswood (1983), *Trigona carbonaria* was the main pollinator effecting self pollination of *Cupaniopsis anacardiodes*. Reddi *et al.* (1983) found that the flowers of *Sapindus emarginatus* Vahl. (Sapindaceae) were foraged for pollen and / or nectar by 39 species of insects of which *Apis cerana*, *A. florea* and *Trigona* spp. were the commonest. Lack and Kevan (1984) reported that the canopy tree *Syzigium syzygiodes* (Myrtaceae) was visited by *Trigona* sp. Ormond *et al.* (1984) observed that the main pollinator of *Jatropha gossypifolia* was the stingless bee *Plebia mosquito*. *Trigona spinipes* also contributed to pollination.

Armbruster (1985) found that *Trigona* spp. collected resin from the resin glands which were close to the stigmas and anthers in the flowers of *Dalechampia scandens* (Euphorbiaceae) and the bees were effective pollinators. Pollination studies of *Socratea exorrhiza* and *Iriartea ventricosa* revealed that pollination was effected by stingless bee (*Trigona* sp.) which collected pollen and possibly trichomes (Henderson, 1985). Pande and Bandyopadhyay (1985) reported that *Apis cerana*, *A. dorsata* F. and *Trigona* sp. foraged on pigeon pea and helped in its pollination. They foraged mostly for nectar. Among the foragers, *Trigona* population was highest but the activity was low compared to *A. cerana*. In *Trigona*, flight activity started a little earlier and stopped correspondingly later. Time spent by *T. iridipennis*



on a flower was  $5.33 \pm 0.8$  seconds and the peak period of activity was 10.00 h.

According to Sihag (1985), the most important pollinators of onion crop included *Apis florea*, *A. cerana*, *Trigona iridipennis* and *Andrena leaena*. Silva *et al.* (1986) found a species of *Trigona* to be a frequent visitor of the African oil palm (*Elaeis guineensis* Jacq.) and the American oil palm (*Elaeis oleifera* (H. B. L.) Lortés) (Arecaceae). Utami (1986) found that Sago palm (*Metroxylon sagu*) was visited by the bees *Apis cerana* and *Trigona iridipennis* and played an important role in fruit setting. Burquez *et al.* (1987) reported that in the rain forest palm, *Astrocaryum mexicanum*, two *Trigona* spp. were the commonest visitors. They collected pollen only from the male flowers and did not crawl inside the inflorescence of female flowers. Rao and Suryanarayana (1988) observed that *Trigona iridipennis* visited the onion crop during its blooming period and they mostly foraged for pollen. The bee visited 1.28 umbels per minute during pollen foraging. It spent about 5 seconds on each floret, while *A. cerana* and *A. florea* spent 1.0 and 1.5 seconds respectively. Peak population of *A. cerana* and *T. iridipennis* were observed at 1300 h.

Ramaltio *et al.* (1989) observed that plants belonging to Melostomataceae, Myritaceae, Solanaceae and Leguminaceae were the main pollen and nectar sources of *Meliponine* sp. Utami (1989) reported that *Trigona iridipennis* played an important role in the fruit set of Pinang yaki (*Areca vestiana* Giseke). Kalpana and Ramanujan (1990) and Ramanujan and Kalpana (1991) highlighted the importance of *Prosopis juliflora* as a major nectar and pollen source for *Trigona*. Aagren and Schemske (1991) observed

that *Trigona grandipennis* was the most frequent flower visitor of *Begonia inolucrata* and the bees showed a strong preference for male-phase inflorescence.

Adams *et al.* (1992) reported that Trigona bees were confirmed pollinators of five Australian sp. of *Dendrobium*, two sp. of *Cymbidium* and *Caladenia carnea* and probable pollinators of other dendrobiums. Studies by Adegas and Couto (1992) revealed that the most frequent flower visitors to rape (*Brassica napus* L. var. *oleifera*) flowers were *Apis mellifera* (80.6 per cent), *Trigona spinipes* (12.8 per cent) and *Dialictus* sp. (6.6 per cent). Husband and Barrett (1992) reported that *Trigona* sp. collected pollen from several populations of *Eichhornia paniculata*. The bees visited a lower proportion of long styled inflorescences than expected and tended to visit more mid and short styled inflorescence in succession. Sedgley *et al.* (1992) found that in *Acacia mangium* and *A. auriculiformis* bees were the most common visitors which included *Trigona* spp., *Apis mellifera* and bees belonging to the family collitidae.

Trigona bees (*T. hockingsi* and *T. carbonaria*) were found to pollinate the orchids, *Cymbidim canaliculatum*, *C. madidum*, *C. suave*, *Dendrobium lichenastrum*, *D. monophyllum* and *D. toressae* (Bartareau *et al.*, 1993). Beismejjer (1993) reported that the bee, *Trigona biroi* foraged from 0615 h to 1850 h. The foraging and pollination efficiency study of *Trigona minangkabau* and *Apis mellifera* revealed that stingless bees could efficiently pollinate strawberry (Kakutani *et al.*, 1993). Listabarth (1993) showed that Meliponine bees collected pollen from the inflorescence of *Genoma macrostachys* and helped in its pollination.

Mustaers (1993) observed that the bees that visited the flowers of Banana and Plantain included *A. mellifera*, *Trigona* spp. and *Dactylurina staudingeri* and they were observed from 0630 h to 1830 h. Among the insect visitors of *Kerianthera preclara* (Rubiaceae), *Trigona williana* was the only flower visitor observed to collect both pollen and nectar (Souza *et al.*, 1993). Analysis of honey and pollen loads collected from a colony of dammar bees, *Trigona iridipennis* by Ramanujan *et al.* (1993) revealed that *Prosopis juliflora* was the chief nectar source and *Peltophorum pterocarpum* and *Prosopis juliflora* were the major pollen sources. *Cocos nucifera*, *Rotrala densiflora*, *Eucalyptus globulus* and *Loranthus longiflorus* acted both as nectar and pollen source. *Bombax malabaricum*, *Antigonon leptopus*, *Tamarindus indica*, *Delonix regia*, *Azadirachta indica*, *Lawsonia alba*, *Ageratum conyzoides* were the other pollen sources. *Cyanotis* sp. and *Leucanea leucocephala* served as nectar sources.

A survey of the insects visiting the flowers of two cultivars (white and purple) of *Bahunia variegata* showed that the most frequently occurring species were *Apis mellifera* (26.9 and 6.6 per cent) and *Trigona spinipes* (25.48 and 20.1 per cent) (Santos *et al.*, 1993). The weedy mint *Leonotis nepetaefolia* have facultatively autogamous mating systems and produce completely viable seeds from both large and small flowers in the complete absence or presence of pollinators such as sunbirds (*Nectarina* spp.) and a stingless bee, *Trigona* sp. (Aluri and Reddi, 1994). Vitali (1994) reported that among the insects that visited the flowers of *Murraya exotica* L. (Rutacea), the most effective pollinators were *A. mellifera* and *Trigona spinipes*.

In *Cymbidium madidum* Lindley and *C. suave*, only the bees *Trigona carbonaria* and *T. hockingsi* transferred pollen between flowers (Bartareau, 1995).

Tenzuka and Maeta (1995) reported pollen robbing in leguminous plants by stingless bee, *Trigona itama* and *Nannotrigona testaceicornis*, through the holes made in the keel petals of *Lespedeza thunbergii*, *Medicago sativa* and *Astragalus sinicus*, grown in green house. Maues and Venturieri (1995) found that *Apis mellifera* and some *Trigona* sp. collected pollen from Anatto (*Bixa orellana*) in a manner that achieved little pollination.

According to Mesquita and Franciscon (1995), bees that visited *Clusia nemorosa* belonged to Euglossinae and Meliponinae. Pollen was collected from staminate flowers and resin was collected from both staminate and pistillate flowers. Ratsirarson (1995) observed that the hermaphrodite and protandrous flowers of *Aloe divaricata* were visited by Souimanga sunbirds and stingless bees (*Trigona* sp.), but stingless bees did not seem to play any role in pollination.

Bittrich and Amaral (1996) noted that *Trigona* bees partly destroyed the flower tube to rob nectar in *Symphonia globulifera* (Clusiaceae). Bogh (1996) reported that *Trigona* bees were the most important pollinating agents of *Calamus longisetus*, *C. peregrinus*, *C. rudentum* and *Calamus* sp. Pollen samples collected from the corbiculae of *Trigona williana* workers were analysed and the plant families to which pollen grains belonged were Arecaceae, Melostomataceae, Myrtaceae, Caricaceae, Moraceae and Malpighiaceae while dominant plant species were *Cocos nucifera*, *Bellucia grassularioides*, *Artocarpus incisa* and *Stachytarpheta cayennensis* (Souza *et al.*, 1996).

Viera *et al.* (1996) reported that the tree *Mabea fistulifera* had a broad spectrum of pollinators and among them *Trigona spinipes*, *T. hyalinata* and *Partamona cupria* were the diurnal pollinators. Woo *et al.* (1996) observed that the stingless bees (*Trigona* sp.) were potential pollinators of green house crops.

Field studies on cowpea revealed that *Apis mellifera* (59.01 per cent), *Trigona* sp. (9.83 per cent) and *Xylocopa* sub. sp. (11.97 per cent) were the important pollinators. Seed weight / pod and seed weight / plant were significantly superior in covered rows associated with the incidence of *Trigona* sp. and *Leptoglossus* sp. (Piccirillo and Higuera, 1997). Sanchez *et al.* (1997) studied the diversity of pollen sources of three stingless bees and the important pollen types identified were *Bursera simaruba*, *Sapindus saponaria* and a type of Moraceae. Thapa and Wongsiri (1997) reported that *Eupatorium odoratum* was visited for nectar by honeybees (*Apis* spp.), stingless bees (*Trigona* spp.) and bumble bees (*Bombus* spp.). The bee *Trigona fulviventris* accounted for 95 per cent of all visits to *Begonia tonduzii* and visited male flowers 15.4 times as often as female flowers (Corff *et al.*, 1998). *Apis mellifera*, *Tetragonisca angustula*, *Trigona spinipes* and *Nannotrigona testaceicornis* were the most frequent flower visitors to *Caesalpinia peltophoroides* Benth (Leguminosae) and visiting of flowers by insects peaked between 9.00 and 13.00 h (Lazari *et al.*, 1998). According to Raju *et al.* (1999) among the flower visitors of the marking-nut tree, *Semecarpus anacardium* L.F. (*Anacardiaceae*) the bees included *Apis cerana indica* F., *A. florea* F. and *Trigona* sp.

## 2.6 PESTS

According to George (1934) a species of *Megachile* sometimes visited the nest of *Trigona iridipennis* and snatched wax from the entrance. Simoes *et al.* (1980) reported that the endoparasitic phorid fly, *Melalonctia sinistra* attacked the bee *Nannotrigona (Scaptotrigona) postica*. The larvae of the phorid fly were found inside the abdomen of the worker bees of *N. postica* and were not found in *Apis mellifera* nor in several other Meliponine species. Anderson and Gibbs (1982) isolated Kashmir Bee Virus from Australian honey bees and injected into the pupae of *Trigona* bees and suggested that *Trigona* was not a source of Kashmir Bee Virus. Baker and Baker (1985) described a new species of mite, *Neocypholaelaps phooni* in Malayasia in the nests of *Trigona nritami* and *T. thorasica*. The mites were pollen feeders and used bees as a means of dispersal. Fain and Flechtman (1985) described a phoretic deutonymph (hypopus), *Meliponopus palpifer* from a bee *Melipona seminigra*. Baker and Baker (1988) observed the incidence of two mites belonging to the genus *Eumellipitis* in the nests of *Trigona*. Delfinado-Baker *et al.* (1989) described a new species of mite *Neocypholaelaps malayensis* found in the nests of *Trigona itama* and *T. iridipennis*. The species was restricted to stingless bees and was closely associated with their hosts in all stages. They were obligatory commensals.

Ants, phorid and other flies, termites and parasitic stingless bees were some of the natural enemies of stingless bees. Larger animals like tamanduas, tayras, honey badgers and bears, civets, primates, chinipanzees were also important enemies (Roubik, 1995).

## 2.7 HONEY

George (1934) reported that the honey season of *T. iridipennis* was from March to June. Phadke (1968) asserted that Trigona honey had ash, acidity and protein in higher quantity. The honey also showed positive polarization, high L/D ratio and high dextrin contents. According to Wakhle and Desai (1983), Trigona honey contained high invertase and low diastase, catalase and glucose oxidase values compared to *A. mellifera* honey. Trigona honey had the least value for diastase, invertase and catalase but it had the highest glucose oxidase activity. Glucose oxidase was the most susceptible enzyme to heating. The enzyme values got reduced in honeys that were kept after heating.

Cortopassi and Gelli (1991) analysed honey samples against seven strains of bacteria. Honeys from stingless bees had stranger inhibition capacity than *Apis* honeys. Honey from tribe Trigonini were found to be better antibacterial agents than those of Meliponini. Wakhle and Desai (1991) tested the honeys of *A. cerana*, *A. dorsata*, *A. florea* and *T. iridipennis* for antibacterial activity. Inhibine number ranged from 0 - 5 and hydrogen peroxide accumulation varied from 2 to 380  $\mu\text{g g}^{-1}$  of honey. Due to heating there was 78-100 per cent loss of peroxide accumulation. Crane (1992) reported that honey from stingless bees were generally more acidic and contained more water than *A. mellifera* honey. They were fairly resistant to spoilage by unwanted fermentation. Ramanujan *et al.* (1993) recorded 43.4 per cent fructose and 32.189 per cent glucose in the honey of *T. iridipennis*.

A comparison of honey of *Melipona* and *Trigona* sp. conducted by Bogdanov and Kilchenmann (1996) showed that the main sugars of *Melipona* honeys were fructose and glucose with an average of 36.7 g / 100 g. The *Trigona* honeys had a completely different sugar spectrum. The principal sugar was a disaccharide, maltose with an average content of 32.3 g / 100 g while fructose and glucose were present in smaller concentrations. *Melipona* honeys had small quantities of maltose and only traces of other oligosaccharides while *Trigona* honeys had small but measurable amounts of turanose, trehalose and erlose.



# **MATERIALS AND METHODS**

### 3. MATERIALS AND METHODS

The present studies on Bio-ecology and management of stingless bees (Apidae : Meliponinae) was carried out in the Department of Entomology, College of Agriculture, Vellayani.

#### 3.1 Identification of the species of stingless bees

A random survey was conducted in different districts of Kerala for collecting stingless bees. Five locations were selected in each district and twenty five bees were collected from each location, from the hive entrance. The bees were killed with chloroform. The samples taken from each district were pooled and kept in a screw-capped glass vial containing 70 per cent alcohol, to form a composite sample. Ten bees were taken from each composite sample and sent to Dr. Roubik, Smithsonian Tropical Research Institute, Apartado, 2082, Balboa, Republic of Panama, U.S.A. for identification of the species.

#### 3.2 General description of the colony

A general description of the colony was prepared by observing various colonies. From the area covered with pollen and honey cells, individual cells having pollen and honey were separated and its size (diameter and height) were measured. The total number of cells per unit area were also recorded. Using a syringe, the quantity of honey stored in each honey cell was measured. The weight of pollen grains in each storage pot was assessed by emptying the pots on a cellophane strip and weighing on an electronic balance.

### 3.3 Hiving of feral colonies

A preliminary study was conducted to assess the acceptance of artificial hives by bees collected from feral colonies. Three types of hives as described below were utilised.

(1) Wooden hive (Plate 1). Size of 30 cm (length) x 14 cm (breadth) x 15 cm (height) made of anjili was used. A lid was provided at the top and an entrance hole at one end of the box near the bottom plank. No frames or other structures were provided. (2) Bamboo hive (Plate 2). One internode of bamboo stem (about 47 cm long and 12 cm diameter) were split into two equal semicircular halves and an entrance hole was made towards the bottom of one end. (3) Earthen pot (Plate 3). New Earthen pot with 20.5 cm diameter and height 18 cm with a small hole made on one side served as the bee entrance. A wooden lid was used for closing the pot. The hives were kept suspended in a thatched shed available in the apiary.

The feral colonies of stingless bees found in rock walls were used for the study (Plate 4). The walls were opened with the help of a crowbar and the entire brood, pollen and honey were carefully removed and put in each hive. In the case of bamboo hives, the brood, pollen and honey were placed in one split of the hive and closed with the other half and the two halves were fastened securely with iron wire. The colonies were observed first, one week in the first instance and later on at biweekly intervals. At the time of each observation, the presence of the queen, eggs and brood development were checked. Care was taken to protect the colony from the attack of ants.

**Plate 1** Wooden hive for keeping *T. iridipennis*

**Plate 2** Bamboo hive for keeping *T. iridipennis*

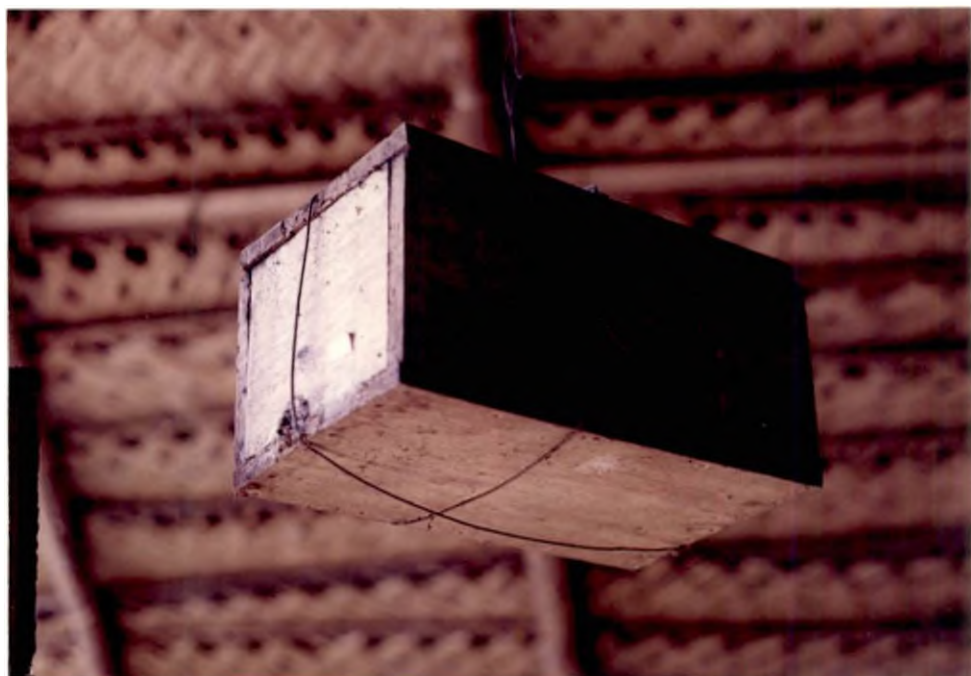


Plate 1



Plate 2

**Plate 3** Earthen pot for keeping *T. iridipennis*

**Plate 4** Feral colony of *T. iridipennis* in rock wall



Plate 3



Plate 4

### **3.4 Management of colonies**

#### **3.4.1 Division**

For this purpose, healthy and disease free stingless bee colonies maintained in wooden hives, bamboo hives and earthen pots procured from beekeepers during September 1998 and maintained in the apiary as described earlier, were used. From these, three colonies each, showing the presence of queen cells were selected for division in November 1998. To each new hive a queen cell along with half the quantity of brood (old and new), some pollen and honey pots were transferred. The volume of brood transferred to each hive was recorded. This was calculated by measuring the length, breadth and height of brood cluster. These daughter colonies were maintained in the original site while the mother colonies with the queen were shifted to a new site.

#### **3.4.2 Brood development**

Divided colonies used in the division studies earlier, were used for the assessment of brood development and population build up. Volume of brood transferred was reckoned to be the starting point. Further brood development was recorded at monthly intervals to assess the population build up.

#### **3.4.3 Method of extraction of honey**

A preliminary trial (employing three methods) was conducted to find an easy and suitable method of extraction of honey.

1. The storage pots were cut and squeezed.



2. Storage pots were cut and kept in a tray and exposed to sunlight, in a slanting manner and the honey drained at the lower end of the tray was collected.
3. Syringe was used to extract the honey from each pot.

#### **3.4.4 Assessment of honey yield**

The 18 colonies employed in studies on division were utilized for assessment of yield of honey. As found out in the preliminary study honey was extracted from each hive by cutting the storage pots and keeping in sunlight, in a tray.

#### **3.5 Behaviour and biology of stingless bees**

The biology and behaviour of stingless bees were studied by maintaining the bees in specially made observation hives. These hives were made of wood with a size of 30 x 14 x 15 cm. Glass windows were provided at the top, lateral sides and on the side opposite to the entrance hole, for easy observation. The glass pieces were covered with black paper to keep the inside of the hive dark. Five colonies were hived in this way.

##### **3.5.1 Behaviour of stingless bees**

The following observations were made, viz., (1) mode of construction of cells, (2) provisioning of cells (3) ovipositioning behaviour of the queen, (4) capping of the cells, (5) waste and resin dumps and (6) defence.

### 3.5.2 Biology

Five colonies maintained in observation hives were used in studies on the lifecycle of *Trigona iridipennis*. The egg laying by the queen was followed by visual observation and the cells in which oviposition was noticed were marked with Indian ink in the colonies and it was recorded as the first day. On every other day one such marked cell from each hive was opened and observed for the change taken place inside the cell. This procedure was repeated until the egg hatched.

The cells with one day old larvae were selected in five colonies and they were marked for observing the larval changes inside the cell. One such cell from each colony was opened every day to observe the changes till the remaining larvae pupated.

One day old pupae were selected, marked and observed in the five colonies for determining the pupal duration.

### 3.5.3 Morphological features

Laying queens were collected and measurements on length of body, antennae, wings and legs were recorded. The measurements of body, head, wings, legs, proboscis and mandibles were recorded in worker bees. All the parts to be measured were carefully dissected and placed in a drop of water on a clean slide. Measurements were taken with the aid of an ocular micrometer.

### 3.5.4 Foraging behaviour

Five colonies were selected for these studies. The number of bees

leaving and entering the hive were recorded for five minutes at one hour intervals from 0600 h to 1900 h.

### **3.6 Flora visited by the bees**

The flora visited by the bees were observed regularly in and around the Instrucitonal Farm, College of Agriculture, Vellayani and documented. Plants visited by the bees either for pollen or nectar or for both were reckoned.

### **3.7 Natural enemies and diseases**

The experimental colonies maintained were observed at periodical intervals to record the incidence of natural enemies and diseases if any.

### **3.8 Characteristics of honey**

Honey was harvested from the hives and a composite sample was prepared. The specific gravity, moisture content, acidity, pH, protein and ash was determined by the method of A.O.A.C. (1975) and fructose and glucose was estimated by the method of Roe (1934), suitably modified.

#### **3.8.1 Specific gravity**

A specific gravity bottle was cleaned thoroughly, dried and weighed. It was filled up to mark with distilled water and weighed again. Subsequently, water was removed and the bottle was dried and filled with honey and weighed. Specific gravity was calculated using the formula given below.

Specific gravity at 27° C = A/B

A = Weight of 10 ml. of honey

B = Weight of equal volume of water

### 3.8.2 Moisture content

Twenty five gram of honey was taken in a clean and dry petridish. The petridish was then kept in a hot air oven maintained at 100°C overnight and cooled in a dessicator. The heating, cooling and weighing was continued until the honey attained a constant weight.

Moisture content was determined as follows

Initial weight of honey = A

Final weight of honey = B

∴ Per cent dry weight =  $B/A \times 100 = C$

∴ Moisture content (D) = 100 - C

### 3.8.3 Acidity

The acidity of honey was detrmind as follows. One gram of honey was dissolved in 75 ml of distilled water and mixed thoroughly. Four to six drops of phenolphthalein solution was added to the sample and was titrated against standard sodium hydroxide solution (0.1 N). Blank was determined using water, indicator and the standard sodium hydroxide solution.

Acidity was calculated as follows

$$\text{Acidity (as formic acid), per cent by weight} = \frac{0.46 \times v}{w}$$

v = Volume of sodium hydroxide required for titration

w = Weight in g of the sample taken for the test

### 3.8.4 pH

Ten gram of honey was dissolved in 100 ml double distilled water in magnetic stirrer for half an hour. The pH of the solution was determined with an ELICO pH meter having a combination electrode at 27<sup>0</sup> C.

### 3.8.5 Protein

One gram of honey was accurately weighed and digested in a kjeldahl flask. The mixture after digestion was cooled and then distilled using 40 per cent NaOH and distilled water. The ammonia evolved was trapped in a beaker containing four per cent boric acid and mixed indicator (methyl red and bromocresol green). It was then titrated against a standard acid (0.093 N H<sub>2</sub>SO<sub>4</sub>) until the colour changed from green to pink. Nitrogen content was calculated using the formula,

$$\frac{\text{Titre value} \times \text{Normality of acid} \times 0.014 \times 100}{\text{Weight of sample taken}}$$

The nitrogen content was multiplied with 6.25 to obtain the percentage of protein in the sample.

### 3.8.6 Ash

A silica crucible was thoroughly cleaned and dried and its empty weight was recorded. Ten gram of the honey sample was weighed in the crucible. It was carefully heated over a low flame until swelling ceased. The sample was then kept in a muffle furnace at 550<sup>0</sup> C and ignited till white ash was obtained. The process was repeated until a constant weight was obtained.

Ash per cent by weight was calculated as follows.

$$\text{Ash per cent} = \frac{100 (W_2 - W)}{W_1 - W}$$

W = Weight in g of the empty dish

W<sub>1</sub> = Weight in g of the dish with the sample

W<sub>2</sub> = Weight in g of the dish with the ash

### 3.8.7 Fructose

To find out standard fructose, to 2 ml of the solution containing 20, 40, 60 and 80 µg of fructose, 1 ml of resorcinol reagent was added followed by 7 ml of HCl solution. The mixture was stirred well and placed in water bath at 80°C exactly for 10 minutes. They were then removed and immersed in tap water for five minutes. Samples were read within 30 minutes after heating using a Spectrophotometer.

To find out fructose in the sample, 1 g of honey was dissolved in 100 ml of distilled water in thoroughly cleaned and dried Erlenmeyer flask. The solution was further diluted 100 times and 0.5 ml of this solution was taken for detection of fructose.

To 0.5 ml of this solution, 1.5 ml of distilled water, 1 ml of resorcinol reagent and 7 ml of HCl solution was added. The mixture was stirred well and placed in a water bath at 80°C exactly for 10 minutes. It was then removed and immersed in tap water for five minutes. The sample was read at 520 nm after heating. Fructose content was calculated from the standard graph.

Reading for 100  $\mu\text{g}$  of standard fructose = A

Reading for the diluted honey solution = B

Volume of aliquot taken = C

$$\therefore \text{Fructose per cent} = \frac{B}{A} \times \frac{100}{C} \times \frac{100 \times 100}{1000 \times 1000} \times 100$$

### 3.8.8 Glucose

To find out standard glucose to 1 ml of the solutions containing 20, 40, 60, 80  $\mu\text{g}$  of glucose, 1 ml of the reagent (25 parts of copper reagent A, one part of copper reagent B) were added and mixed properly. The tubes were heated in boiling water bath for 20 minutes. They were cooled in running tap water and 1 ml of arsenomolybdate reagent was added to each tube. The solutions were diluted to 25 ml and read at 520 nm within 30 minutes.

To find out glucose in the sample, one gram of honey was dissolved in 100 ml of distilled water in an Erlenmeyer flask, which was thoroughly cleaned and dried. The solution was further diluted 100 times and 0.5 ml of the solution was taken for detection.

To .05 ml of the solution 1.5 ml of distilled water and 1 ml of copper reagent was added. The mixture was stirred well and kept in boiling waterbath for 20 minutes. It was then removed and cooled in running tap water and 1 ml of arsenomolybdate was added to each tube. The sample was read at 520 nm after heating using a Spectrophotometer. Glucose content was calculated from the standard graph.

Reading for 100 per cent glucose = A

Reading for diluted honey sample = B

Volume of aliquot taken = C

$$\therefore \text{Glucose per cent} = \frac{B}{A} \times \frac{100}{C} \times \frac{100 \times 100}{1000 \times 1000} \times 100$$

### 3.9 Statistical analysis

The data generated from different experiments were subjected to statistical analysis by applying the technique of analysis of variance using CRD. Wherever significant differences were determined among treatments, CD were provided for pairwise comparison of treatments.



## **RESULTS**

## 4. RESULTS

The results obtained from different experiments are presented in this chapter.

### 4.1 Identification of the species of stingless bees

The bee samples collected from all the districts of Kerala were identified to be of the same species, viz., *Trigona iridipennis* Smith by Dr. Roubik, Smithsonian Tropical Research Institute, Apartado, 2082, Balboa, Republic of Panama, U.S.A.

### 4.2 General description of the colony

By observing various colonies the following general description was prepared.

The colony consisted of a queen, workers and few drones during the colony breeding season. The hive entrance was guarded by several bees (Plate 5). The building material used by *Trigona iridipennis* was cerumen (mixture of wax and resin). The nest cavity was sealed from outside world except for flight entrance. The entrance was made of wax and mud. The inner wall of nest cavity was thoroughly coated with resin (Plate 6).

This species arranged their brood in clusters and not as double sided combs as in *Apis*. Pillars of cerumen acted as foundation upon which the cluster of brood cells were arranged. Brood cells were made of cerumen and they were oval or elliptical in shape. There was no involucre (layers of waxy sheets) surrounding the brood chamber.

**Table 1. Average dimensions of brood cells of *T. iridipennis***

| Sl. No. | Parameter | Queen cell | Worker cell |
|---------|-----------|------------|-------------|
| 1.      | Height    | 5 mm       | 0.33 mm     |
| 2.      | Diameter  | 4 mm       | 0.22 mm     |

**Plate 5** Hive entrance guarded by bees

**Plate 6** Nest cavity coated with resin



Plate 5

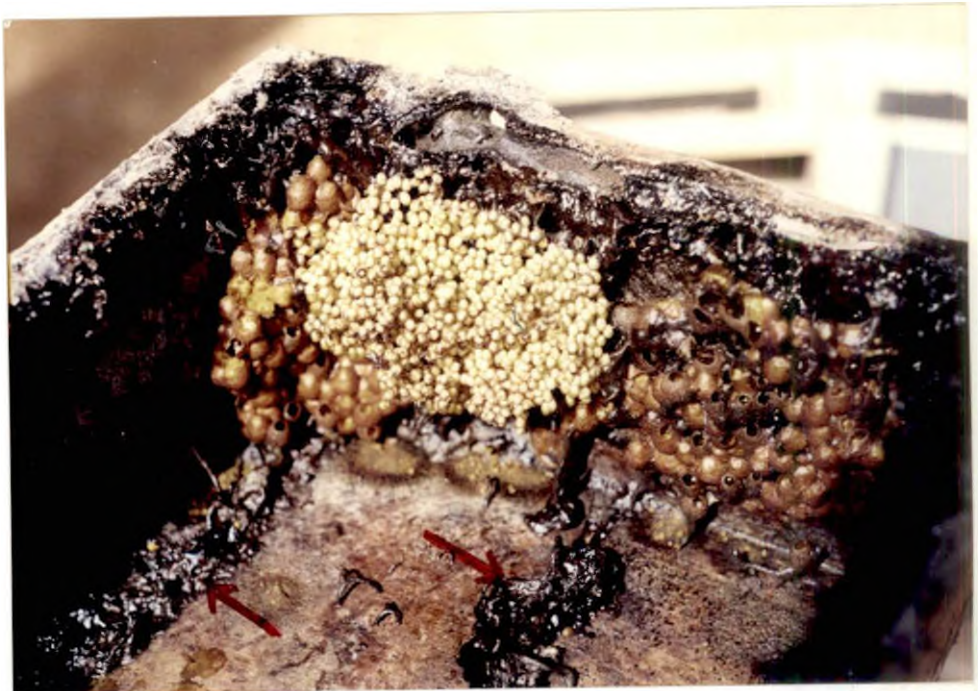


Plate 6

Worker brood cells had a mean height of 0.33 mm and a mean diameter of 0.22mm respectively (Table 1). Queen cells were larger in size than worker brood cells (Plate 7). They were found either intermixed with worker brood cells or at the periphery of the cluster. Queen cells were noticed in the hives during November to March and then in May. The average height of the queen cell was 5 mm with an average diameter of 4mm. Brood cells were dark brown in colour in the early stages and as the pupae matured, the cell walls were seen removed so that the cocoons were exposed and these were creamy in colour.

Food chamber of the species lay outside the brood nest unlike in *Apis*. The food chamber consisted of pollen and honey cells which were built with cerumen and normally had an oval shape. The storage pots were larger than brood cells. They were found to be fixed to the wall of the hive, surrounding the brood cells. Pollen and honey pots were found either separate or intermixed and they had almost the same size (Plates 8, 9).

The number of pollen pots in unit area and the measurements (height, perimeter, diameter and weight) of individual pollen pots were recorded. The data presented in Table 2 indicated that the mean perimeter of a pollen pot was 1.98 cm and the values ranged between 1.6 cm and 2.2 cm. The diameter of the pollen pots varied from 0.6 to 0.8 cm with a mean of 0.73 cm. Similarly the mean height was 0.97 cm with a range from 0.8 to 1.2 cm. Weight of individual pollen pot ranged from 0.15 to 0.37 g, with an average of 0.23 g. It was observed that an average of eight cells were present in an area of 2 cm<sup>2</sup>.

**Plate 7 Queen cells and worker brood cells**

**Plate 8 Pollen pots**

**Plate 9 Honey pots**

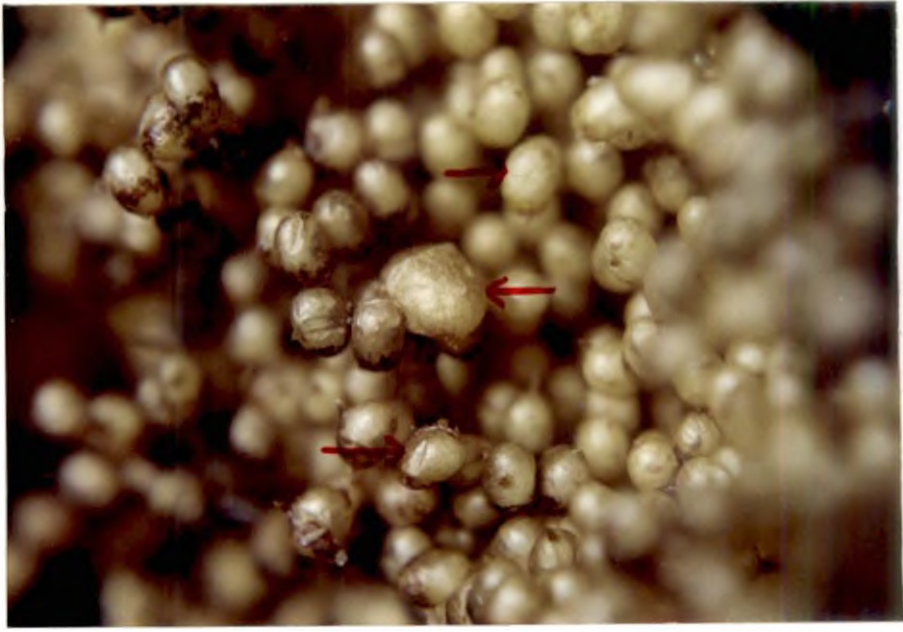


Plate 7



Plate 8



Plate 9



The number of honey pots in unit area and the measurements on height, diameter, perimeter and quantity of honey per pot were recorded for honey pots. The data are presented in Table 3. The perimeter of honey pot ranged from 1.9 to 3.0 with an average of 2.09 cm. The average diameter was 0.89 cm with values ranging from 0.6 cm to 1.1 cm. The height of the honey pots ranged from 0.5 cm to 1.1 cm with a mean value of 0.9 cm. 0.5 ml of honey was present in a single pot with values ranging from 0.5 ml to 0.6 ml. An average of 8 cells were present in and area of 2 cm<sup>2</sup>.

In the colony one or more piles of waste could be seen, situated at a distance from the brood. Piles of resin could also be observed in the colony.

#### **4.3 Hiving of feral colonies**

Observations on the acceptance of artificial domiciles by the stingless bees, *Trigona iridipennis* revealed that the bees accepted the wooden hive, bamboo hive and earthen pot equally.

The colonies were found establishing with the following activities. The worker bees constructed pillars of wax and connected the brood cells intact with the hive walls. The cells damaged during colony transfer, were seen repaired. Dead larvae and pupae were seen removed from the cells and from the hive. Resin was seen deposited in several places inside the hive. New brood cells were constructed and the queen laid eggs in it. Few honey and pollen pots were also constructed by the bees.

**Table 2. Average dimensions of pollen pots**

| Sl. No. | Parameter    | Range          | Mean value |
|---------|--------------|----------------|------------|
| 1.      | Perimeter    | 1.6 to 2.2 cm  | 1.98 cm    |
| 2.      | Diameter     | 0.6 to 0.8 cm  | 0.73 cm    |
| 3.      | Height       | 0.8 to 1.2 cm  | 0.97 cm    |
| 4.      | Weight / pot | 0.15 to 0.37 g | 0.23 g     |

**Table 3. Average dimensions of honey pots**

| Sl. No. | Parameter               | Range         | Mean value |
|---------|-------------------------|---------------|------------|
| 1.      | Perimeter               | 1.9 to 3.0 cm | 2.09 cm    |
| 2.      | Diameter                | 0.6 to 1.1 cm | 0.89 cm    |
| 3.      | Height                  | 0.5 to 1.1 cm | 0.96 cm    |
| 4.      | Quantity of honey / pot | 0.5 to 0.6 ml | 0.53 ml    |

## 4.4 Management of colonies

### 4.4.1 Division

Data on the mean brood development of stingless bees, *Trigona iridipennis* in the divided colonies maintained in wooden hives, bamboo hives and earthen pots are presented in Table 4 and illustrated in Fig 1.

Queen emerged in the newly divided colonies and they started laying eggs. There was development of brood in the colonies and they got established. Division of colonies was found to be successful.

### 4.4.2 Brood development

The mean volume of the brood transferred to wooden hives, bamboo hives and earthen pots were 144.33 cm<sup>3</sup>, 150.33 cm<sup>3</sup> and 144.5 cm<sup>3</sup> respectively. It was observed that the volume of the brood in the wooden hives showed a gradual increase from November 1998 to April 1999 with the maximum brood volume during February (275.33 cm<sup>3</sup>). The brood development in bamboo hive also showed a similar pattern of increase from November to April with the maximum volume of 255.16 cm<sup>3</sup> in February. The volume of brood in earthen pot too was maximum during February (243.33 cm<sup>3</sup>) showing an increasing trend from November to April.

Statistical analysis of the data showed that during November no significant difference was observed in the three different hives. However, by the month of December, wooden hive was found to be superior in brood development and it was on par with bamboo hive. During January, wooden

**Table 4) The mean brood development of *Trigona iridipennis* in different hives (cm<sup>3</sup>)**

| Sl. No. | Month                   | Wooden hives (cm <sup>3</sup> ) | Bamboo hive (cm <sup>3</sup> ) | Earthen pot (cm <sup>3</sup> ) | Mean (cm <sup>3</sup> ) |
|---------|-------------------------|---------------------------------|--------------------------------|--------------------------------|-------------------------|
| 1.      | November                | 144.33                          | 150.33                         | 145.50                         | 146.72                  |
| 2.      | December                | 170.50                          | 165.66                         | 158.16                         | 164.78                  |
| 3.      | January                 | 195.66                          | 179.33                         | 169.83                         | 181.61                  |
| 4.      | February                | 275.33                          | 255.16                         | 243.33                         | 257.94                  |
| 5.      | March                   | 264.16                          | 247.66                         | 230.16                         | 247.33                  |
| 6.      | April                   | 247.16                          | 229.16                         | 199.00                         | 225.11                  |
|         | Mean (cm <sup>3</sup> ) | 216.19                          | 204.56                         | 191.00                         |                         |

F 17.079\*\* (Hives) 360.54\*\* (Months) 4.653\*\* (Interaction)

CD 9.195 ( " ) 6.844 ( " ) 11.854 ( " )

\*\* significant at 1 per cent level

**Table 5) Honey yield of *Trigona iridipennis* maintained in different hives**

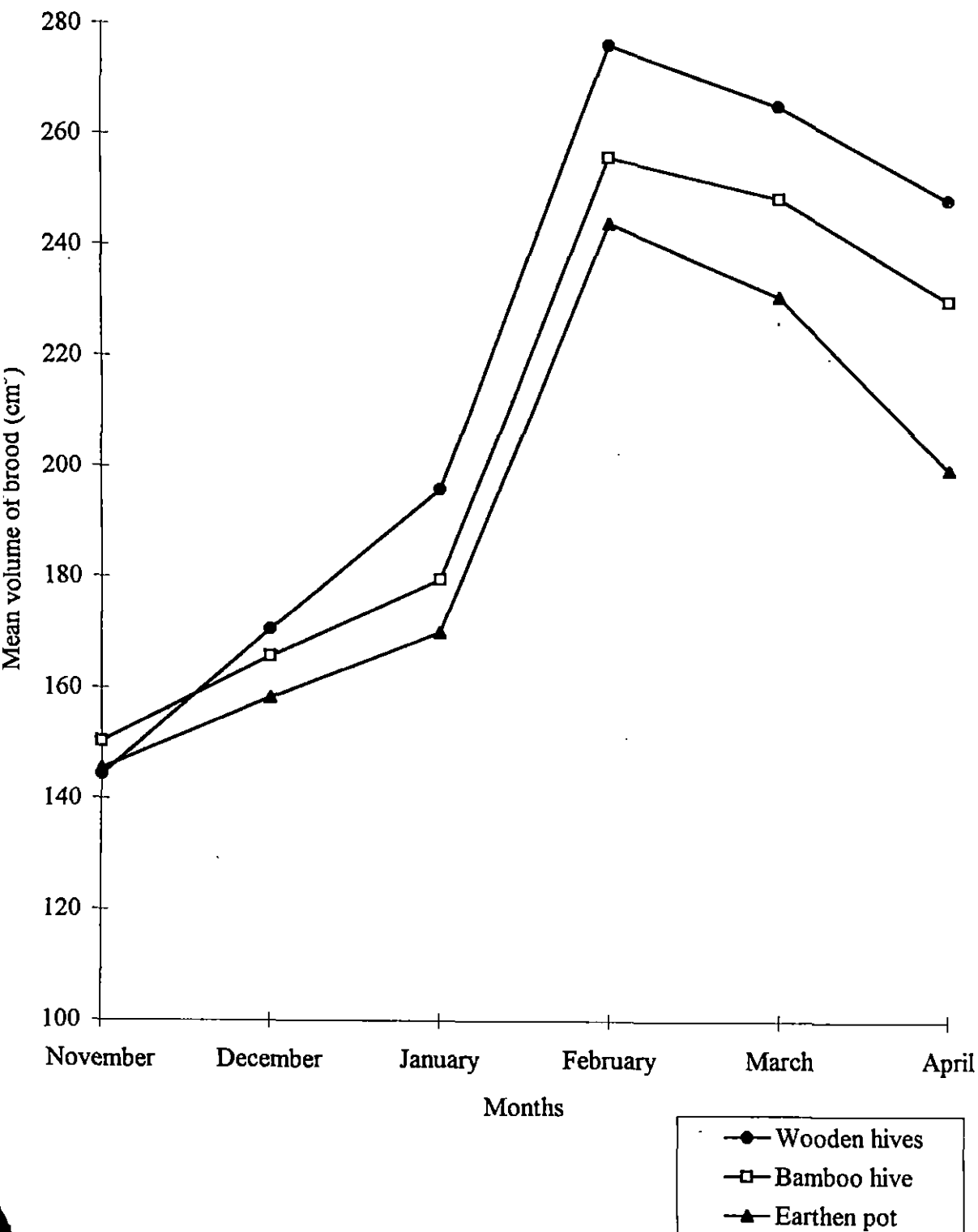
| Sl. No. | Hive        | Yield (ml) |
|---------|-------------|------------|
| 1.      | Wooden hive | 44.25      |
| 2.      | Bamboo hive | 26.5       |
| 3.      | Earthen pot | 19.6       |

F 6.112\*

CD 6.876

\* significant at 5 per cent level

**Fig. 1. Mean brood development of stingless bees in different hives**



hive was superior to bamboo hive and earthen pot which were on par. In February, March and April the brood development in the wooden hive was superior to bamboo hive which was superior to earthen pot. The overall rate of brood development in wooden hive was found to be the best.

#### **4.4.3 Method of extraction of honey**

It was possible to extract the honey by the three different methods. Squeezing the honey pots was found to be easy, but the honey extracted by this method contained large amount of extraneous matter.

By keeping the storage pots in sunlight, clear honey could be collected. This was relatively a simple method.

Extraction of honey with syringe was difficult. The needle got clogged with wax and use of syringe was difficult as the storage pots were in clusters.

#### **4.4.4 Honey yield**

The data on the honey yield is presented in Table 5. The yield of honey ranged from 9 ml to 65 ml with an average yield of 44.25 ml in wooden hive followed by an average of 26.5 ml in bamboo hive and 19.6 ml in earthen pot. Of the three hives tried, bees hived in wooden box stored maximum honey followed by bamboo hive. Earthen pot recorded the lowest honey yield (19.6 ml). Statistical analysis of the data showed that honey storage in wooden hives was significantly superior to those in bamboo hive and earthen pot which were on par.

## **4.5 Behaviour and biology of stingless bees**

### **4.5.1 Behaviour**

The worker bees transferred to observation hives were seen putting wax irregularly over the inner surface of glass as well as in different places inside the hive. In most cases the lids were seen tightly sealed to the hive using cerumen.

#### **4.5.1.1 Mode of construction of cells**

Worker bees at first constructed pillars of wax that provided a base or foundation to the brood cells (Plate 10). On these pillars, brood cells were constructed in clusters (Plate 11a). The brood cells were made of wax and were oval or elliptical in shape. Each cell was built by the summation of activities of several workers. Cell construction started from the bottom by accumulating cerumen on the pillars. The cells were seen growing through successive and intermittent contributions of workers. After some time the cell orifice reached the required height and was slightly narrowed; its margin appeared knife edged and this "collared" cell attracted more workers for provisioning. At a time, a batch of cells were usually constructed by several workers. Once the first set of brood cells were provisioned and oviposited, newer cells were constructed above it. Construction of the brood cells was in an upward direction. Cells were also constructed by accumulating cerumen on the side walls of another cell. Within a set of brood cells, some of them were connected to each other by means of wax connectives while others were not (Plate 11b). As one layer of brood got old, another layer of brood was constructed over it. Cells of various stages of

**Plate 10 Pillars of wax acting as a foundation for brood cells**

**Plate 11.a. Brood cells *T. iridipennis* constructed in clusters**

**Plate 11b. Wax connectives between brood cells of *T. iridipennis***

**Plate 12 Brood cells at various stages of construction**



Plate 10

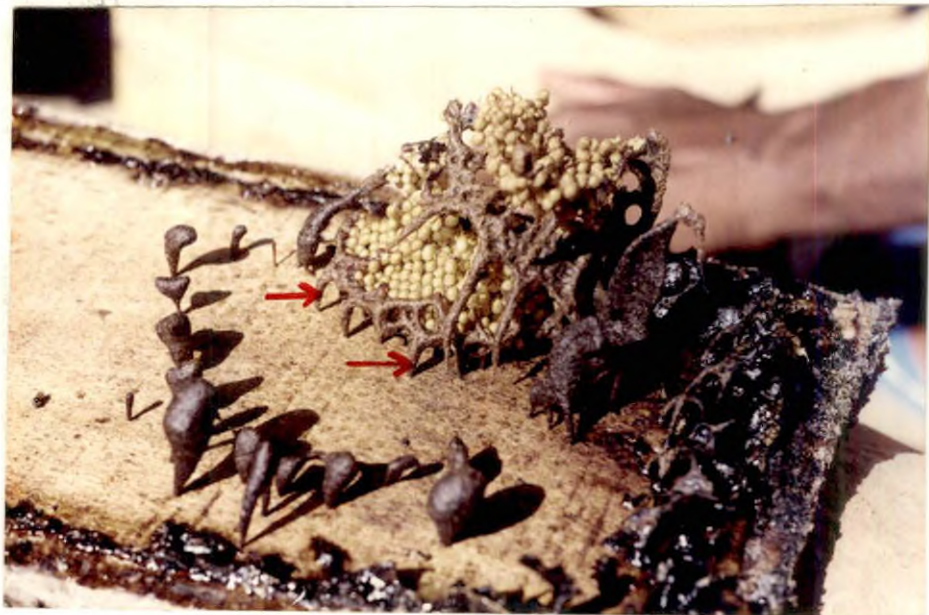


Plate 11

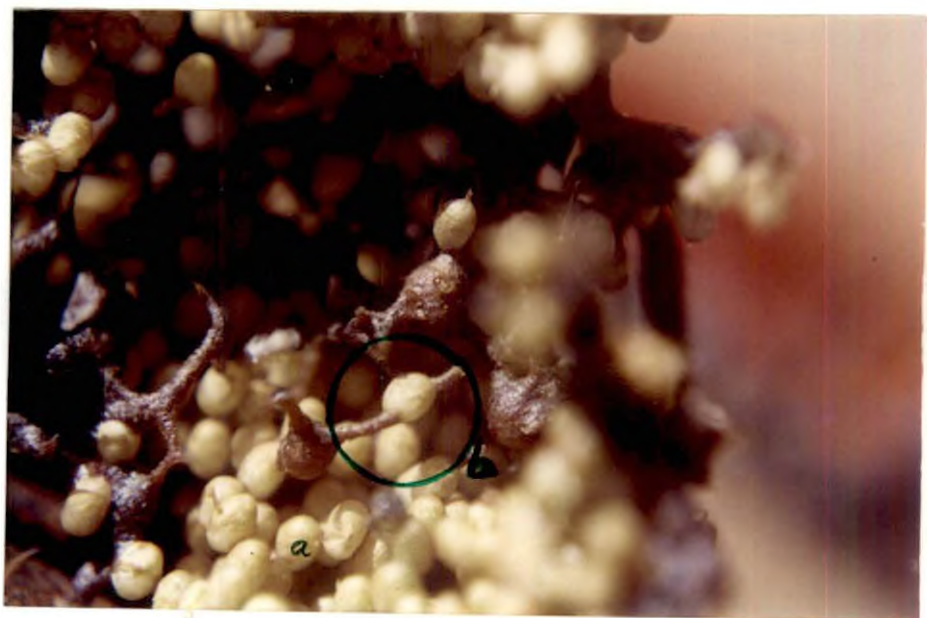
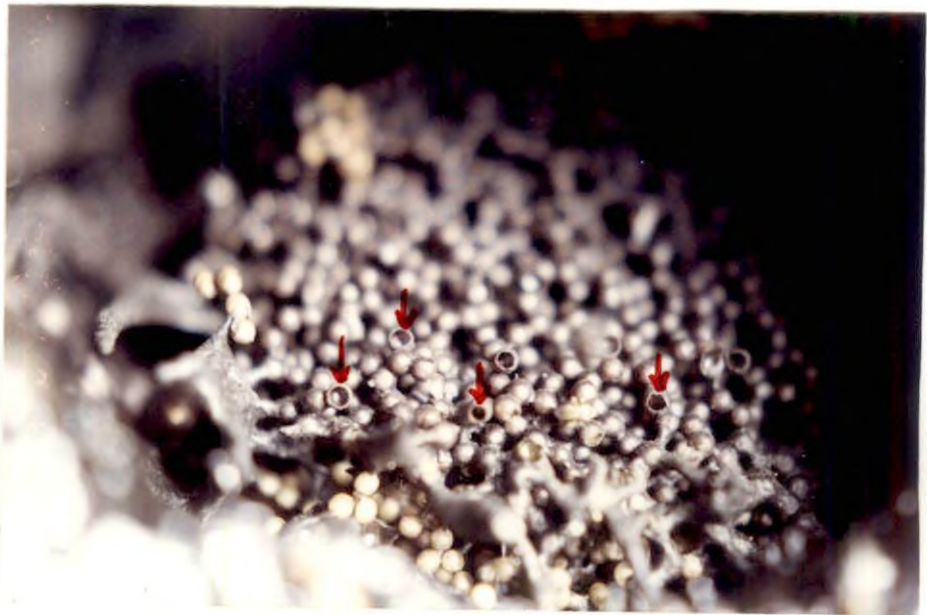


Plate 12



construction could be seen at a time in a colony (Plate 12). Brood cells were seen used only once by these bees.

#### **4.5.1.2 Provisioning of cells**

About 3-5 workers were involved in provisioning a cell. Unlike honeybees, mass-provisioning was followed in stingless bees. Before provisioning, the queen made several walkings over the brood comb and inspected the cells. After these walkings she settled down near a cell that has been collared. Several workers were seen around the queen during this time. The workers then hurriedly filled the cell with food.

#### **4.5.1.3 Ovipositioning of cells**

After the workers provisioned the cell, the queen inserted her abdomen into the cell and laid the egg in it. The egg was inserted in the centre of the liquid food in a perpendicular direction. The queen after oviposition left the cell immediately.

#### **4.5.1.4 Capping of cells**

The workers that were engaged with provisioning also left the oviposited cell. Usually a single worker was involved in closing the cell that was oviposited. The worker closed the cell orifice by sitting on the cell, inserting her abdomen into it and rotating the body. After the orifice becomes sufficiently small the worker stopped rotation and continued to close the cell while sitting by the cell.

#### **4.5.1.5 Waste and resin dumps**

In many hives, piles of yellowish brown coloured matter (Plate 13) could be seen at a distance from the brood cells. Bees were found flying out with small cakes of this material. These waste dumps were seen to contain dead bees or parts of them, remains of brood and cocoons as well as faecal material.

Resin in the form of creamy droplets could also be observed in the hive. Small lumps of resin were also found to be attached near to the hive entrance and to other parts of the hive. When a hive was disturbed the bees were seen depositing resin at several parts of the hive, brood cells etc.

#### **4.5.1.6 Defence**

The stingless bees did not sting, but they could bite as a means of defence. They attacked the intruder by biting the skin and ejecting a caustic fluid in very small amount. The bees entangled themselves into the hairs and got into the nose and ears of the intruder.

#### **4.5.2 Biology**

After the brood cell was constructed and provisioned by the workers, the queen laid eggs in the centre of the cell on the food. The eggs when oviposited were perpendicular in position (Plate 14). The mean length and breadth of the egg were 1.07 and 0.39 mm respectively. The egg hatched in a mean period of 4.7 days (Table 6).

**Table 6. Duration of developmental stages of workers of *T. iridipennis***

| Sl. No. | Stage | Days.(mean) |
|---------|-------|-------------|
| 1.      | Egg   | 4.7         |
| 2.      | Larva | 18.6        |
| 3.      | Pupa  | 21.8        |

(Total duration = 45.1 days)

**Plate 13** Waste dumps found in a colony of *T. iridipennis*

**Plate 14** Eggs of *T. iridipennis*

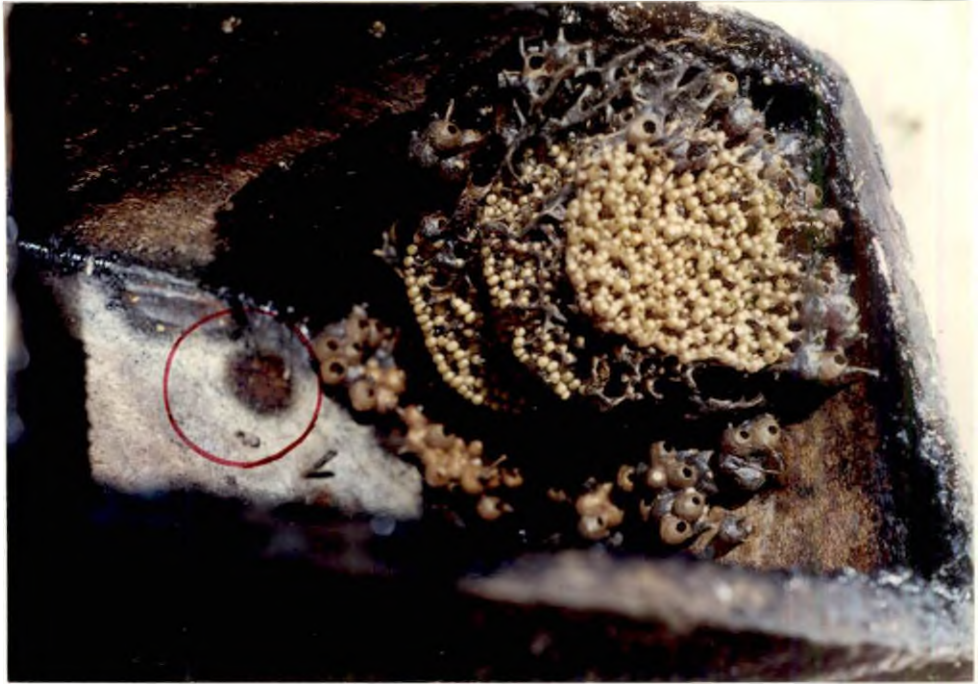


Plate 13

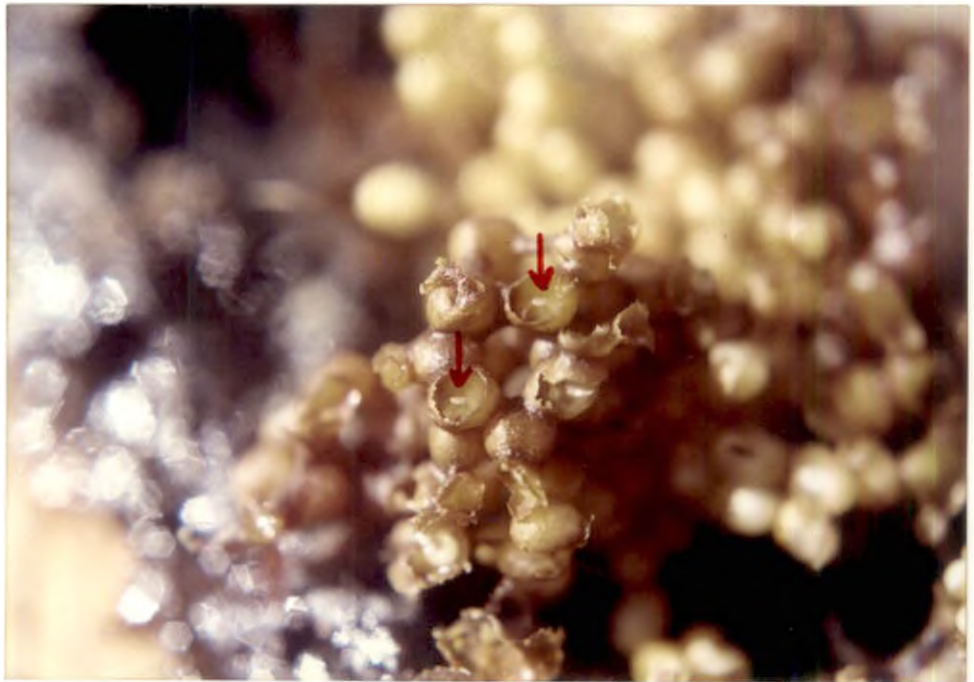


Plate 14

On hatching, the larva assumed a horizontal position in the cell. During the later stages, the 'C' shape of the larva was obvious. On an average, the larva took 18.6 days to pupate.

The pupa remained in the cocoon with head directed upwards. About one week prior to the emergence of adults, black eyes of the pupa could be seen through the pupal case. On an average the pupa took 21.8 days to emerge as adult. Thus the average time required from egg to adult in *Trigona iridipennis* was 45.1 days.

During the egg stage the brood cells were dark brown in colour as they were made of wax. Towards the late larval stages cerumen was seen to be gradually removed from the cells. By the time the larva pupated, the entire wax was removed and the pupal case were visible with yellowish cream or straw colour (Plate 15).

### 4.5.3 Morphological features

The data on the measurements of the body parts of queen and workers are presented in Tables 7,8. Queen could be easily distinguished from the workers due to its large size (Plate 16). The queen had a golden brown colour and pointed abdomen. It had a mean body length of 10.08 mm, while the diameter of abdominal region was 4 mm.

Total length of the antenna was 3.73 mm and the flagellum had 10 segments. The length and breadth of forewing was 3.55 mm and 1.3 mm respectively. The hindwing had a length of 2.26 mm and a breadth of 0.65 mm. The number of hamuli on the hindwing was 5. The fore, middle and

Table 7- Morphometric characters of the queen of *Trigona iridipennis*

| Sl. No.         | Characters                     | Mean            |                    |                 |
|-----------------|--------------------------------|-----------------|--------------------|-----------------|
| <b>Head</b>     |                                |                 |                    |                 |
| 1.              | Length of proboscis            | 1.3 mm          |                    |                 |
| 2.              | Length of scape                | 0.72 "          |                    |                 |
| 3.              | Length of pedicel              | 1.62 "          |                    |                 |
| 4.              | Length of flagellum            | 1.39 "          |                    |                 |
| 5.              | Number of segment of flagellum | 10 Nos          |                    |                 |
| 6.              | Size of mandible               | 0.624 mm        |                    |                 |
| <b>Forewing</b> |                                |                 |                    |                 |
| 7.              | Length of forewing             | 3.55 mm         |                    |                 |
| 8.              | Breadth of forewing            | 1.30 "          |                    |                 |
| <b>Hindwing</b> |                                |                 |                    |                 |
| 9.              | Length of hindwing             | 2.26 mm         |                    |                 |
| 10.             | Breadth of hindwing            | 0.65 "          |                    |                 |
| 11.             | Number of hamuli               | 5 Nos           |                    |                 |
| 12.             | Extent of hamuli               | 0.24 mm         |                    |                 |
| <b>Leg</b>      |                                | Foreleg<br>(mm) | Middle leg<br>(mm) | Hindleg<br>(mm) |
| 13.             | Length of coxa                 | 0.43            | 0.672              | 0.72            |
| 14.             | Length of trochanter           | 0.48            | 0.48               | 0.53            |
| 15.             | Length of femur                | 0.96            | 1.032              | 1.25            |
| 16.             | Length of tibia                | 0.912           | 0.96               | 1.25            |
| 17.             | Length of tarsus               | 1.25            | 1.44               | 1.46            |
| 18.             | Total length of body           | 10.08 mm        |                    |                 |



**Table 8. Morphometric characters of the worker of *Trigona iridipennis***

| Sl. No. | Characters                     | Mean         |                 |              |
|---------|--------------------------------|--------------|-----------------|--------------|
|         | <b>Head</b>                    |              |                 |              |
| 1.      | Length of proboscis            | 1.38 mm      |                 |              |
| 2.      | Length of scape                | 0.547 "      |                 |              |
| 3.      | Length of pedicel              | 0.13 "       |                 |              |
| 4.      | Length of flagellum            | 1.9 "        |                 |              |
| 5.      | Number of segment of flagellum | 10 Nos.      |                 |              |
| 6.      | Size of mandible               | 0.6 mm       |                 |              |
|         | <b>Forewing</b>                |              |                 |              |
| 7.      | Length of forewing             | 3.6 mm       |                 |              |
| 8.      | Breadth of forewing            | 1.36 "       |                 |              |
|         | <b>Hindwing</b>                |              |                 |              |
| 9.      | Length of hindwing             | 2.47 mm      |                 |              |
| 10.     | Breadth of hindwing            | 0.63 "       |                 |              |
| 11.     | Number of hamuli               | 5 Nos.       |                 |              |
| 12.     | Extent of hamuli               | 0.22 mm      |                 |              |
|         | <b>Leg</b>                     | Foreleg (mm) | Middle leg (mm) | Hindleg (mm) |
| 13.     | Length of coxa                 | 0.35         | 0.53            | 0.53         |
| 14.     | Length of trochanter           | 0.29         | 0.32            | 0.36         |
| 15.     | Length of femur                | 0.75         | 0.86            | 1.03         |
| 16.     | Length of tibia                | 0.72         | 0.91            | 1.43         |
| 17.     | Length of tarsus               | 0.95         | 1.12            | 1.20         |
| 18.     | Total length of body           | 4.068 mm     |                 |              |

**Plate 15 Brood cells ready for the emergence of adult bees**

**Plate 16 Stingless bee queen over the brood cells**



Plate 15



Plate 16

hind leg had a length of 4.032, 4.584 and 5.21 mm respectively.

The workers were small in size. They had an average body length of 4.068 mm. The colour of the abdomen varied in different workers. Very young bees were pale in colour. Other bees had yellow coloured abdomen, while the foragers and guard bees had fully pigmented i.e., black colour of abdomen.

The workers had a proboscis length of 1.38 mm while that of antenna was 2.577 mm (Plate 17). Flagellum was ten segmented. Mandibles were modified (Plate 18) and differed from the mandibles of other *Apis* bees.

Fore wing had a length and breadth of 3.6 mm and 1.36 mm respectively (Plate 19). Wing venation was found to be reduced in this species.

Hind wing had 2.47 mm length and 0.63 mm breadth (Plate 20). The average number of hamuli was 5 and the extend of hamuli was 0.22 mm. The fore leg, middle leg and hind leg had a total length of 3.66 mm, 3.74 mm and 4.55 mm respectively (Plates 21, 22, 23).

Hind leg had a pollen basket and also a row of stiff spine like setae arising at the inner apical margin of tibia (Plate 24). Queen did not have such spine like structures.

#### **4.5.4 Foraging behaviour**

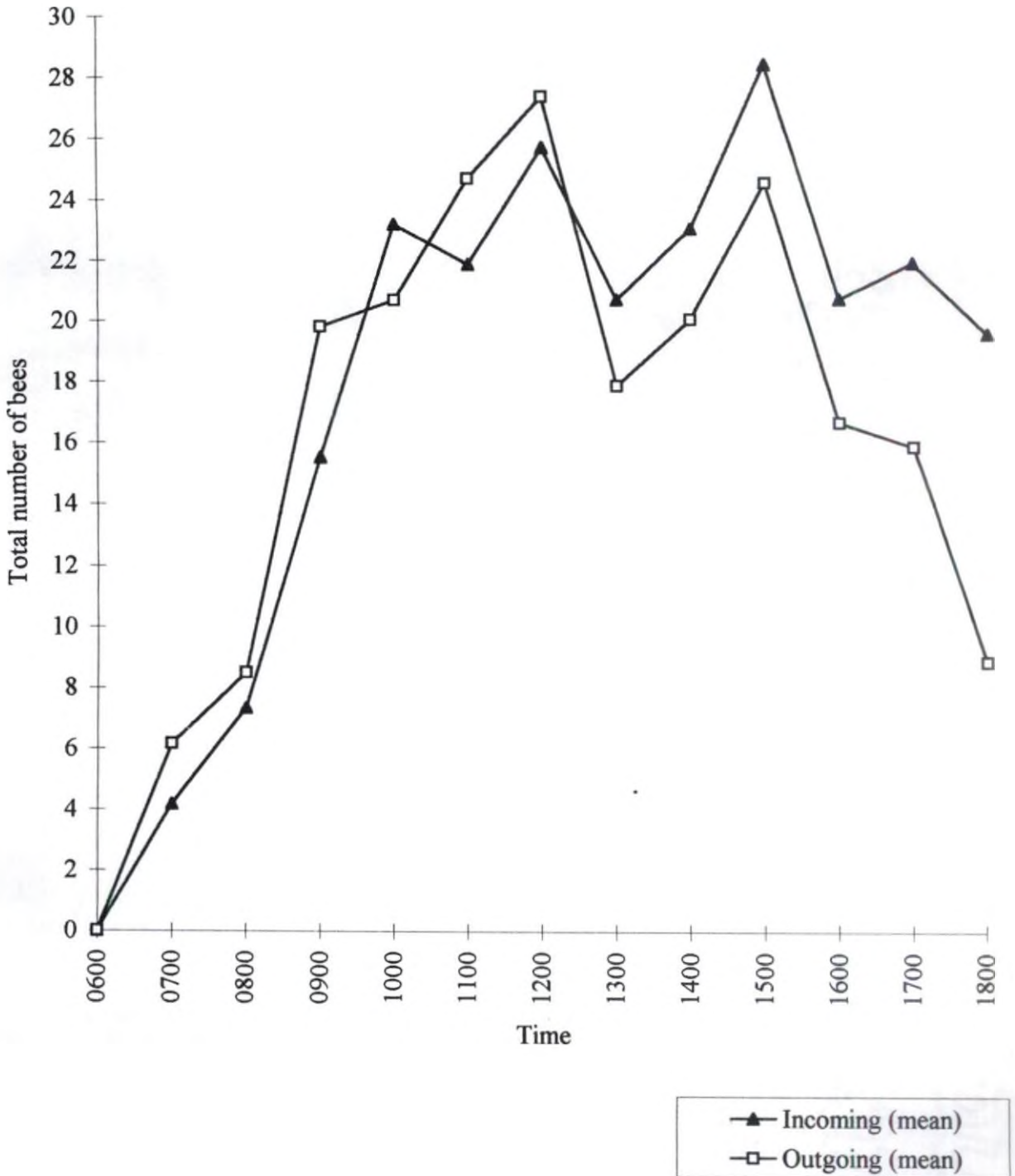
The data pertaining to the diurnal variations in the foraging behaviour is presented in Table 9 and illustrated in Fig. 2. Foraging activity was noticed from 0070 h in the morning till 1800 h in the evening. Lower activity was noticed till 0800 h and the activity began to rise gradually reaching the first peak at 1200 h. A decline in activity was observed at 1300 h. The activity

**Table 9. Diurnal variations in the foraging activity (nectar and pollen) of *Trigona iridipennis***

| Time | Mean number of foragers per colony |                 |
|------|------------------------------------|-----------------|
|      | Incoming (mean)                    | Outgoing (mean) |
| 0600 | 0                                  | 0               |
| 0700 | 4.16                               | 6.16            |
| 0800 | 7.33                               | 8.5             |
| 0900 | 15.5                               | 19.8            |
| 1000 | 23.16                              | 20.66           |
| 1100 | 21.83                              | 24.66           |
| 1200 | 25.66*                             | 27.33*          |
| 1300 | 20.66                              | 17.83           |
| 1400 | 23.00                              | 20.00           |
| 1500 | 28.33*                             | 24.5*           |
| 1600 | 20.66                              | 16.6            |
| 1700 | 21.83                              | 15.8            |
| 1800 | 19.5                               | 8.83            |

\* peak periods of activity

**Fig. 2. Foraging activity of *Trigona iridipennis* during different hours of the day**



**Plate 17** Antenna of *T. iridipennis* worker

**Plate 18** Mandible of *T. iridipennis* worker

**Plate 19** Fore wing of *T. iridipennis* worker

**Plate 20** Hind wing of *T. iridipennis* worker

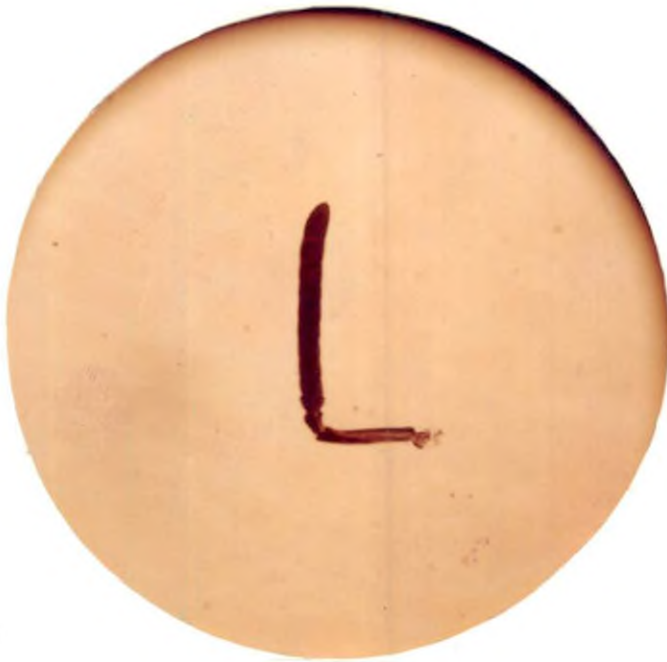


Plate 17



Plate 18

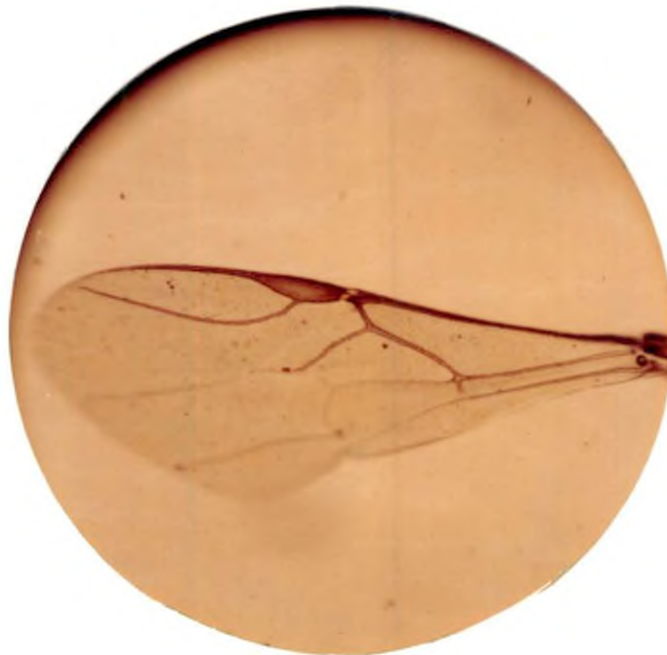


Plate 19



Plate 20



**Plate 21** Fore leg of *T. iridipennis* worker

**Plate 22** Middle leg of *T. iridipennis* worker

**Plate 23** Hind leg of *T. iridipennis* worker

**Plate 24** Hind leg and metatarsus of *T. iridipennis* showing *Pencillium*



Plate 21



Plate 22



Plate 23



Plate 24

again increased gradually showing another peak at 1500 h and then declined thereafter.

#### 4.6 Flora visited by bees

The flora visited by *T. iridipennis* bees are presented in Table 10. It was observed that 27 plants were visited by the stingless bees for nectar or pollen or both (Plates 25 to 36). Of the 27 plants recorded, twelve plants (rubber, cashew, coconut, chilly, cannon ball tree, coral creeper, drumstick, guava, rose, birds cherry and tamarind) provided both nectar and pollen. Eight plants (anthurium, bajra, sponge gourd, cinnamon, ixora, mango, touch-me-not and sunflower) noted, were sources of pollen while seven plants (balsam, banana, duranta, euphorbia, hamelia, marigold and phyllanthus) provided nectar only.

#### 4.7 Natural enemies and diseases

Ants (*Solenopsis geminata*) were seen entering the hives with weak colonies and robbing pollen stock. Two species of (unidentified) spiders were found to predate on the bees (Plates 37, 38). They were found catching the bees at the hive entrance or in the foraging sites. A species of megachilid bee was found to snatch the wax from the hive entrance. But it did not attack or harm the bees. No instance of disease occurrence could be noticed during the period of study.

Table 10. Flora visited by *Trigona iridipennis*

| Sl. No. | Common name     | Scientific name                                    | Family          | Source of N or P |
|---------|-----------------|--|-----------------|------------------|
| 1.      | Rubber          | <i>Hevea brasiliensis</i>                          | Euphorbiaceae   | N & P            |
| 2.      | Coconut         | <i>Cocos nucifera</i>                              | Palmaceae       | N & P            |
| 3.      | Cashew          | <i>Anacardium occidentale</i>                      | Anacardiaceae   | N & P            |
| 4.      | Mango           | <i>Mangifera indica</i>                            | Anacardiaceae   | P                |
| 5.      | Tapioca         | <i>Manihot esculenta</i>                           | Euphorbiaceae   | N & P            |
| 6.      | Chilli          | <i>Capsicum annum</i>                              | Solanaceae      | N & P            |
| 7.      | Coral creeper   | <i>Antigonon leptopus</i>                          | Polygonaceae    | N & P            |
| 8.      | Touch-me-not    | <i>Mimosa pudica</i>                               | Mimosaceae      | P                |
| 9.      | Drumstick       | <i>Moringa oleifera</i>                            | Moringaceae     | N & P            |
| 10.     | Guava           | <i>Psidium guajava</i>                             | Myrtaceae       | N & P            |
| 11.     | Sunflower       | <i>Helianthus annus</i> L.                         | Compositae      | P                |
| 12.     | Bajra           | <i>Pennisetum typhoides</i><br>(Burm. f.) & S & H) | Poaceae         | P                |
| 13.     | Sponge gourd    | <i>Luffa cylindrica</i>                            | Cucurbitaceae   | P                |
| 14.     | Marigold        | <i>Tagetes erecta</i> L.                           | Compositae      | N                |
| 15.     | Anthurium       | <i>Anthurium andreaenum</i>                        | Areaceae        | P                |
| 16.     | Balsam          | <i>Impatiens balsaminae</i>                        | Balsammaceae    | N                |
| 17.     | Hamelia         | <i>Hamelia patens</i>                              | Rubiaceae       | N                |
| 18.     | Rose            | <i>Rosa</i> sp.                                    | Rosaceae        | N & P            |
| 19.     | Birds cherry    | <i>Mundingia carabura</i>                          | Verbenaceae     | N & P            |
| 20.     | Cinnamon        | <i>Cinnamomum zeylanicum</i>                       | Lauraceae       | P                |
| 21.     | Banana          | <i>Musa</i> sp.                                    | Musaceae        | N                |
| 22.     | Duranta         | <i>Duranta goldiana</i>                            | Verbenaceae     | N                |
| 23.     | Cannonball tree | <i>Cauiropita guinensis</i>                        | Apocyanaceae    | N & P            |
| 24.     | Tamarind        | <i>Tamarindus indica</i>                           | Caesalpiniaceae | N & P            |
| 25.     | Ixora           | <i>Ixora</i> sp.                                   | Rubiaceae       | P                |
| 26.     | Phyllanthus     | <i>Phyllanthus niruri</i>                          | Euphorbiaceae   | N                |
| 27.     | Euphorbia       | <i>Euphorbia hirta</i>                             | Euphorbiaceae   | N                |

N = Nectar

P = Pollen

**Plate 25 Bee flora - *Ixora* - *Ixora* sp.**

**Plate 26 Bee flora - Cannon ball tree - *Cauropita guinensis***

**Plate 27 Bee flora - Drum stick - *Muringa oleifera***

Plate 25



Plate 26



Plate 27



**Plate 28** Bee flora - Birds cherry - *Mundinia calabura*

**Plate 29** Bee flora - Cinnamon - *Cinnamomum zeylanicum*

**Plate 30** Bee flora - Bajra - *Pennisetum typhoides*



Plate 28



Plate 29



Plate 30



**Plate 31 Bee flora - Sunflower - *Helianthus annuus***

**Plate 32 Bee flora - Sponge gourd - *Luffa cylindrica***

**Plate 33 Bee flora - Marigold - *Tagetes erecta***



Plate 31



Plate 32



Plate 33

**Plate 34 Bee flora - Balsam - *Impatiens balsaminae***

**Plate 35 Bee flora - Coral creeper - *Antigonon leptopus***

**Plate 36 Bee flora - Hamelia - *Hamelia patens***



Plate 34



Plate 35



Plate 36

**Plate 37 Spider - Predator on *T. iridipennis* (unidentified) waiting over the hive**

**Plate 38 A spider predating a stingless bee in the field**



Plate 37



Plate 38

- 171586

#### 4.8 Characteristics of honey

The honey was slightly acidic to taste and had a dark brown colour. The honey had a specific gravity of 1.394 g/cc. Acidity was found to be 0.294 and pH as 3.98. Results of analysis done on the composition of honey is given below.

|                  |         |
|------------------|---------|
| Moisture content | 20.70 % |
| Fructose         | 41.60 % |
| Glucose          | 37.10 % |
| L/D ratio        | 1.12    |
| Protein          | 1.49 %  |
| Ash              | 1.10 %  |

# **DISCUSSION**



## 5. DISCUSSION

The stingless bees (Meliponinae) so called because of their vestigial sting are distributed exclusively in tropical regions and they have a larger biodiversity than honey bees (Sommiejer, 1993). Mishra (1995) observed that stingless bees are found in tropical parts of India. Kerala also has a rich wealth of stingless bee colonies and the feral colonies are found in the hollow of tree trunks, crevices of masonry walls, rocks and similar situations. These bees are major pollinators of tropical flora. The honey they produce, though limited in quantity is highly valued for its medicinal properties. However, bee keeping with stingless bees (Meliponiculture) has not received enough attention in the state. This may be due to its peculiar nesting habits and their honey storage cells which are in amorphous groups unsuited to mechanical handling. Further they are not easily amenable for successful domestication.

In India, especially in Kerala, scientific studies on stingless bees and its domestication are very limited. The species of stingless bees existing in Kerala had not been correctly identified. The present studies were hence taken up to identify the stingless bees in Kerala and to gather basic information on their biology, behaviour and adaptability to artificial hives. The findings are discussed below.

### 5.1 Identification of bee samples

The stingless bees collected from all districts of Kerala were identified as *Trigona iridipennis* Smith. by Dr. Roubik, Smithsonian Tropical Research

Institute, Apartado, 2082, Balboa, Republic of Panama, U.S.A.

Lindauer (1957) reported that *Trigona iridipennis* and *T. ventralis* were native to Ceylon. Michener (1961) observed *T. iridipennis* to be widespread in Indo-Malayan region. Kshirsagar and Chauhan (1977) reported that *Trigona* (Tetragona) *iridipennis* Smith, the Indian stingless bee, was widely spread in India. Sakagami (1978) also observed that *T. iridipennis* was confined to India and Srilanka. According to Biesmeijer (1993) all Asian and African stingless bee species belonged to the tribe *Trigonini*.

It is evident from the study that *Trigona iridipennis* is the species of stingless bee commonly found in Kerala.

## 5.2 Hiving of feral colonies

Crane (1992) opined that stingless bees could be kept in hives like *Apis mellifera* and *A. cerana*. Mishra (1995) also asserted that *Trigona* could be kept in hives. Stingless bee nests were transferred into hollow tree trunks, logs and wooden boxes (Bruijin, 1996) and he observed that these hives could be used for meliponiculture. In the present study, feral colonies were kept in three different hives *viz.*, wooden hives, bamboo hives and earthen pots. It was found that the colonies established in these hives and there was considerable brood development in them. The development of stingless bees in different hives show that the bees can be hived in artificial domiciles which is the first satisfactory step towards domestication.

### 5.3 Management of colonies

In the present investigation, division of stingless bee colony was carried out in colonies having queen cells. About half of the brood cells along with pollen and honey and the queen cell were transferred to a new hive. New queen emerged from the queen cells and got mated and started laying eggs with a gradual increase in the brood volume. The mother colony in which the original queen was retained also showed an increase in brood volume. The result that the stingless bee colony could be successfully divided is in conformity with the results obtained by Percy (1989), but there was no need to supply a new queen to the daughter colony as done by Percy (1989) since the queen emerged from the queen cells and the colony got established. This is the first successful record of the division of colony without providing a queen to the new colony.

In the division studies, the mother colony with queen was removed from the original site in the apiary and kept away in a new site. The daughter colony was retained in the original place where mother colony was kept. Thus the mother colony was intact with old queen and young bees enabling its proper development. By keeping the daughter colony in the original site, the foragers could return to the new hive and hence the colony established easily by the working of large number of worker bees. The new colony, otherwise would have only lesser number of workers and hence may take more time to establish. By this method both the colonies had almost equal number of workers.

There are no earlier records regarding the brood development of stingless bees in different types of hives. In the present study it was seen that the bees established in the three types of hives suggesting that *T. iridipennis* could be maintained in wooden hives, bamboo hives and earthen pots. Statistical analysis of data show that there was maximum brood development in wooden boxes compared to bamboo and earthen hives.

Wooden hives used in the present study was found to be larger for the bees. The colonies occupied only half a portion of the hive. It seems that the space was larger than needed. So a smaller size of the wooden hive may be suitable. This will require further scientific studies in this direction. In the earthen pots also there was additional space left. Storage pots were found separate.

The present studies on the method of extraction of honey indicated the feasibility of collecting honey by keeping the storage pots in trays under sunlight. This method gave clear honey and it was relatively a simple method.

Yield of honey was found to be comparatively low in the present study. This may be due to the lesser availability of suitable flora near the experimental site to provide nectar. Again the species is said to be a poor yielder. Detailed studies with more number of colonies and longer duration are required to gather precise information on the actual honey yield. In the present studies however, honey yield was found to be higher in wooden hives.

The above studies on brood development and honey yield in different types of hives indicated that the wooden hives were better compared to bamboo hives and earthen pots.

#### 5.4 Behaviour and biology of stingless bees

The building material used in the nest of *T. iridipennis* was cerumen as in most of the stingless bee species. The nest entrance had been reported to continue as an elaborate tube (tunnel) into the nest cavity in many species such as *Friesella schrottkyi* and *Trigona spinipes* (Sakagami, 1975) as quoted by Sakagami (1982). But in the present studies no such tunnel was noticed.

In the present studies an involucre was not found surrounding the brood chamber as was observed by Michener (1961) in *T. iridipennis*. However, Bruijin (1996) reported the presence of involucre in many species of stingless bees.

*T. iridipennis* arranged their brood in cluster and not in combs. Willie (1983) observed a wide variation in the arrangement of brood in different species. He observed spiral patterns of combs in *T. tataira*, irregular combs in *T. fulviventris*, irregular horizontal comb type in *Trigona (Plebeia) schrottkyi*, specialized cluster type in *Trigona (Plebeia) tica* and vertical combs in African species *Dactylurina staudingeri*. However, Michener (1961) and Kshirsagar and Chauhan (1977) reported that *T. iridipennis* built its brood cells in clusters.

George (1934) opined that the queen cells of *T. iridipennis* were larger than that of worker cells. Kshirsagar and Chauhan (1977) reported that height and diameter of brood cells as 3.12 mm and 4.086 mm. The results obtained in the measurements of queen and worker brood cells are in conformity with the results of earlier workers (Kshirsagar and Chauhan, 1977).

The cells are built one upon the other after the first batch has been

provisioned, the cells filled with liquid food, egg laid and closed straight away. The mode of construction of cells by the worker bees observed in the present studies is consistent with the findings of Sakagami (1982). It was also found that the brood cells were used only once unlike in honey bees. According to Darchan (1972) the uneconomical usage seems to be compensated by efficient application of removed cerumen for other purposes. In *Schwarziana* and *Scaptotrigona* cells of various stages could be seen at a given moment (Sakagami, 1982) as observed in the present study.

The mass provisioning of brood cells as noticed in the present instance is a general character of stingless bees as explained by Lindauer (1957), Sakagami (1982), Willie (1983), Crane (1992). Kshirsagar and Chauhan (1977) noticed similar pattern of bee behaviour with regard to provisioning of brood cells in *T. iridipennis*, as observed in the present study.

The behaviour of queen and workers in the process of oviposition observed in the present studies are in conformity with those of earlier workers in stingless bees (Sakagami *et al.*, 1965; Kshirsagar and Chauhan, 1977).

The food chamber of *T. iridipennis* lay outside the brood nest and they were larger than the brood cells. This was the first attempt to record the weight of a single pollen pot and the quantity of honey per pot. No other work has been found so far in this regard. The observations recorded in the present study on size, shape, measurements and arrangements of storage pots agree with the observations of Lindauer (1957) and Bruijn (1996).

In the present investigation, piles of yellow brown material in the form of cakes could be seen in the bee hives. Bees were found to carry the material

outside the hive. Bruijn (1993) reported that these waste dumps were an important architecture of stingless bee hives and they contained the remains of the brood and the cocoons from which adult bees emerged. They also contained dead bees or parts of them.

Piles of resin as well as scattered droplets of resin could be seen in different parts of the hive and also near the hive entrance, in the present investigation. This observation is in conformity to the observations of earlier workers (Friese, 1914; Lindauer, 1957). Probably the resinous matter helped the stingless bees in defending their nests. Resin applied in the crevices may have helped the bees to prevent the entry of intruders. Silva (1972) as quoted by Sakagami (1982) observed that the stingless bees were more resistant than social wasps to the attack of army ants and this depended on their ability to manipulate resin.

In the present study it was found that stingless bees attacked the intruder by biting the skin, ejecting a caustic fluid and entangling themselves into the hairs of the intruder and getting into nose and ears. Similar findings were reported by Lindauer (1957), Willie (1983) and Crane (1992). In stingless bees, the sting is poorly developed and they use their mandibles for their defence from enemies.

## **Biology**

The eggs when oviposited were perpendicular in position. This is in conformity to the observation of George (1934) and Michener (1961) in *T. iridipennis*. The mean length and breadth of egg was found to be 1.07 and

0.39 mm respectively, in the present study. This is the first observation recorded in this line.

The present investigation on the life cycle of *Trigona iridipennis* revealed that it took a period of 45.1 days for total development with an egg period of 4.7 days, larval period 18.6 days and pupal period 21.8 days.

George (1934) observed a period of 21 to 25 days for the development of *T. iridipennis* from egg to adult in Karnataka. However, Salmah *et al.* (1996) observed a period of 46.5 days for the development of *T. itama*.

In the present study, it was observed that during the egg stage, brood cells were dark brown in colour and in pupal stages creamish yellow in colour. These results are confirmed by the observations of various workers (Friese, 1914; Darchan, 1972 ; Kshirsagar and Chauhan, 1977). This change of colour was due to the fact that the bees removed the cerumen from the brood cells with the advancement of age and in the pupal stage, cerumen was wholly removed making visible the pupal case.

### **Morphological features**

The stingless bee queen was larger in size than the workers as in other *Apis* species (Sakagami, 1982). But no information is available on the size of the queen. An attempt was hence made in the present study to measure the body length of the queen and it was found to be 10.08 mm. The result that the hindleg of stingless bee queen was not corbiculated as in the case of workers is in conformity with the observation of Sakagami (1982).

In the present study it was found that the workers had a body length of



4.068 mm. Crane (1992) reported that *Trigona* bees had a body length from 2 mm upwards. Mandibles were modified and had a length of 0.6 mm. Unlike honeybees, mandibles were modified for performing the function of defence since the general defence organ, the sting is reduced in this species. Wing venation was reduced in this species.

The hind leg of worker bees were corbiculated and also had a row of stiff spine like setae arising at the inner apical margin of tibia. This structure corresponds to 'pencilium' which is a unique character of Meliponinae (Willie, 1983; Prentice, 1991). Willie (1983) also observed that pencilium was absent or much reduced and soft, however in the subgenus *Hypotrigona* and the parasitic genera *Lestrimellita* and *Cleptotrigona*.

### **Foraging behaviour**

Only limited information is available on the foraging behaviour of this species.

Gilbert (1973) found two peak periods of activity, one at 1000 h and the other at 1700 h for *T. fulviventris* while Beismeyer (1993) found a first peak at 0915 h and a second peak between 1300 h and 1400 h in *T. biroi*. The present investigations showed two peak periods of activity of *T. iridipennis* at 1200 h and 1500 h respectively. Bee activity is regulated by the availability of flora in the surroundings. The variation in the peak periods of activity in the present investigation might be due to the fact that maximum availability of nectar and pollen in the plants in the experimental location was during these periods.

## 5.5 Flora visited by bees

*T. iridipennis* is an indigenous pollinator. Its pollen and nectar sources have not been fully investigated. The present study was aimed at recognising these sources.

Twenty seven plants were recorded as flora in the present study. Out of these, sunflower (Goel and Kumar, 1981; Rao *et al.*, 1995; Sharma and Bichoo, 1996), coconut (Ramanujan *et al.*, 1993), coral creeper (Ramanujan *et al.*, 1993) have already been reported as bee flora for *T. iridipennis*. The rest of the plants viz., rubber, cashew, mango, chilly, cannon ball tree, drumstick, guava, rose, birds cherry, tamarind, anthurium, bajra, sponge gourd, cinnamon, ixora, touch-me-not, balsam, banana, duranta, euphorbia, hamelia, marigold and phyllanthus are being recorded as new sources for *T. iridipennis*. The knowledge about bee flora may be of help in utilising these bees in the pollination requirement of these crops and also in judging suitable areas for bee culture with *T. iridipennis*.

## 5.6 Natural enemies and diseases

During the course of the investigation, spiders were found to predate stingless bees which were not reported earlier by other workers. Ants (*Solenopsis geminata*) were also found to attack weak colonies of stingless bees as reported by Roubik (1995). No other enemies reported by earlier workers (Simoes *et al.*, 1980; Baker and Baker 1985; Fain and Flechtman, 1985 ; Baker and Baker, 1988; Delfinado-Baker *et al.* 1989, Roubik, 1995) have been noticed in the present studies.

## 5.7 Honey

The chemical analysis of stingless bee honey was attempted by various workers (Phadke, 1968; Wakhle and Desai, 1991; Crane, 1992; Ramanujan *et al.*, 1993). The stingless bee honey is believed to have medicinal properties. This may be due to the fact that the bees could forage on nectaries of medicinal herbs with small flowers that are not visited by other larger species.

The present investigation showed that the specific gravity of the honey of stingless bee was 1.39 which is in conformity with the earlier finding (Phadke, 1967). Moisture content was found to be 20.7 per cent. In previous studies Phadke (1968) reported it as 24.05 per cent and Wakhle and Desai (1991) as 22.75 per cent. Phadke (1968) also observed 0.228 per cent acidity in stingless bee honey while in the present investigation it was found to be 0.2994 per cent. pH observed in the present study was 3.98 while Wakhle and Desai (1991) reported the pH as 4.15. Fructose and glucose were found to be 32.34 per cent and 20.05 per cent by Phadke (1968), while Ramanujan *et al.* (1993) recorded 43.4 per cent and 32.189 per cent fructose and glucose respectively. In the present investigation the percentages of fructose and glucose were found to be 41.6 and 37.1 respectively. The L/D ratio was 1.12. The high L/D ratio is due to the low dextrose in the honey. There are earlier reports that due to the high L/D ratio, the stingless bee honey was not found to granulate even on prolonged storage Wakhle and Desai, 1991 ; Phadke, 1968).

specific gravity was 1.394 g/cc. It had a moisture content of 20.7 per cent. Glucose and fructose were 37.1 per cent and 41.6 per cent respectively. The L/D ratio was 1.12. The honey had a higher protein content of 1.49 per cent and ash content of 1.1 per cent.

# **SUMMARY**

## 6. SUMMARY

The present studies on bio-ecology and domestication of stingless bees were conducted in the apiary of College of Agriculture, Vellayani with the following objectives.

1. Identification of the species present in Kerala
2. Study of biology, behaviour and management of the identified species
3. Documentation of stingless bee flora in Kerala
4. Composition of stingless bee honey

The salient results of the study are presented below:

The bee samples collected from all the districts of Kerala were identified to be of the same species, viz., *Trigona iridipennis* Smith.

The colony consisted of a queen, workers and few drones during the colony breeding season. The inner wall of the nest cavity was thoroughly coated with resin. The building material used by *T. iridipennis* was cerumen. The species arranged their brood in clusters. Brood cells were made of cerumen and were oval or elliptical in shape. Queen cells were found either intermixed with brood cells or at the periphery of the cluster.

Food chamber of the species lay outside the brood nest unlike in *Apis*. The food chamber consisted of pollen and honey pots built of cerumen and were oval in shape. They were larger than worker brood cells. Pollen and honey pots were found either intermixed or separate and had almost the same size.

In order to assess the acceptance of bees to artificial domiciles, feral colonies found in rock walls were transferred to three different types of hives

*viz.*, wooden hive, bamboo hive and earthen pot. The development of the bees in these hives showed that the bees could be hived in artificial domiciles which was a satisfactory step towards domestication.

Division was carried out successfully by transferring half the amount of brood cells, pollen and honey pots along with queen cell to the daughter colony.

Brood development in wooden hives were superior to bamboo hives and earthen pots.

The studies on the method of extraction of honey indicated the feasibility of collecting honey by keeping the storage pots in trays under sunlight and collecting the drained honey.

Honey yield was found to be higher in wooden hives compared to that in bamboo hives and earthen pots. Wooden hives were thus found superior to bamboo hives and earthen pots with regard to brood development and honey yield.

For making the brood cells, worker bees first constructed pillars of wax which provided a foundation for the cells. Each cell was built by the summation of activities of several workers. Construction of brood cells was in an upward direction. Unlike honey bees, mass provisioning was noticed in stingless bees. Queen laid egg in the cell provisioned by workers. After the oviposition by the queen, the workers closed the cell. Brood cells were seen used only once.

Waste dumps were seen at several places in a colony. It contained dead bees or parts of them, remains of brood and cocoons as well as faecal material.

As a defence mechanism stingless bees were seen to attack the intruder by biting the skin and ejecting a caustic fluid in very small amount. The bees

entangled themselves into the hairs and got into the nose and ears of the intruder.

The average egg, larval and pupal periods was found to be 4.7, 18.6 and 21.8 days respectively with a total developmental period of 45.1 days. The bees removed the cerumen from the brood cells with the advancement of age and in the pupal stage cerumen was wholly removed making visible the pupal case.

The species had reduced wing venation. The mandibles were modified. The hindleg of worker bees had 'pencilium', a row of stiff spine like setae arising at the inner apical margin of tibia. The queen was larger in size than the workers and had a body length of 10.08 mm while it was 4.06 mm for workers.

Investigation on the foraging behaviour of *T. iridipennis* showed two peak periods of activity, one at 1200 h and the other at 1500 h.

Twenty seven plants were recorded as flora for *T. iridipennis*. Of the 27 plants recorded, twelve plants (rubber, cashew, coconut, chilly, cannon ball tree, coral creeper, drumstick, guava, rose, birds cherry and tamarind) provided both nectar and pollen. Eight plants (anthurium, bajra, sponge gourd, cinnamon, ixora, mango, touch-me-not and sunflower) noted, were sources of pollen while seven plants (balsam, banana, duranta, euphorbia, hamelia, marigold and phyllanthus) provided nectar only.

During the period of study two species of spiders (unidentified) were found to predate on the bees. Ants, *Solenopsis geminata*, were also found to attack the weak colonies. No disease incidence was noticed.

Stingless bee honey was slightly acidic to taste and had a dark brown colour with the acidity and pH values being 0.294 and 3.98 respectively. The



specific gravity was 1.394 g/cc. It had a moisture content of 20.7 per cent. Glucose and fructose were 37.1 per cent and 41.06 per cent respectively. The L/D ratio was 1.112. The honey had a higher protein content of 1.49 per cent and ash content of 1.1 per cent.

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\* Original not seen

**BIO-ECOLOGY AND MANAGEMENT OF  
STINGLESS BEES (Apidae : Meliponinae)**

**By**

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**ABSTRACT OF THE THESIS  
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## ABSTRACT

With the objective of studying the bio-ecology and management of stingless bees, a study was conducted at the College of Agriculture, Vellayani during 1997-99.

The bee samples collected from all districts of Kerala were identified to be of the same species *viz.*, *Trigona iridipennis* Smith.

The colony consisted of a queen, workers and few drones during the colony breeding season. The building material used by *T. iridipennis* was cerumen. The species arranged their brood in clusters. Queen cells were larger than worker brood cells. Food chamber of the species lay outside the brood nest. The food chamber consisted of honey and pollen pots which were larger than brood cells.

Feral colonies which were transferred to different types of hives established and this showed that the bees could be hived in artificial domiciles which was a satisfactory step towards domestication.

Different management practices were tried. Division of the bee colony could be successfully carried out by transferring half the amount of brood cells, pollen and honey pots along a queen cell to the daughter colony. Wooden hives were found to be more suitable for hiving *T. iridipennis* since the brood development and honey yield were superior in this type of hive. The studies on the method of extraction of honey indicated the feasibility of collecting honey by keeping the storage pots in trays under sunlight.

Observations on the behaviour of *T. iridipennis* showed that each

brood cell was constructed by the summation of activities of several workers. Mass provisioning of the brood cells was noticed. The brood cells were seen used only once. Queen laid egg in the provisioned cell and after the oviposition a worker closed the cell. The bees used their mandibles as a means of defence.

The average egg, larval and pupal period of *T. iridipennis* was found to be 4.7, 18.6 and 21.8 days respectively with a total developmental period of 45.1 days.

The species had reduced wing venation. The mandibles were modified. The hind leg of the worker bees had the 'pencilium'. The stingless bee queens were larger than workers

Investigations on the foraging behaviour showed two peak periods of activity one at 1200 h and the other at 1500 h. Twenty seven plants were recorded as bee flora. Of the 27 plants recorded, twelve plants (rubber, cashew, coconut, chilly, cannon ball tree, coral creeper, drumstick, guava, rose, birds cherry and tamarind) provided both nectar and pollen. Eight plants (anthurium, bajra, sponge gourd, cinnamon, ixora, mango, touch-me-not and sunflower) noted, were sources of pollen while seven plants (balsam, banana, duranta, euphorbia, hamelia, marigold and phyllanthus) provided nectar only.

Two species of spiders (unidentified) and ants (*Solenopsis geminata*) were found as enemies of *T. iridipennis*.

Stingless bee honey was slightly acidic to taste and had a dark brown colour with the acidity and pH values being 0.294 and 3.98 respectively. The specific gravity was 1.394 g/cc. It had a moisture content of 20.7 per cent. Glucose and fructose were 37.1 per cent and 41.6 per cent respectively. The honey had a higher protein content of 1.49 per cent and ash content of 1.1 per cent.